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Dr. K. P. Sharma (Honorary Editor)

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Jaipur-302004
Rajasthan, India

Executive Editor: Ms. Apurva Goel (Bachelor of Engineering; Masters in Environment) C-102, Building No.12, Swarna CGHS, Beverly Park, Kanakia, Mira Road (E) (Thane) Mumbai-401107, Maharashtra, India
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Evaluation of Phytoremediation Potential of Vetiver Grass (*Chrysopogon zizanioides* L.) Grown on Contaminated Soils in Bulgaria

Violina Angelova† 

Agricultural University, Plovdiv, 12 Mendelev Str., 4000 Plovdiv, Bulgaria

†Corresponding author: Violina Angelova; vileriz@abv.bg

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ABSTRACT

This research investigated the potential for using vetiver grass (*Chrysopogon zizanioides* L.) to remediate metal-polluted sites in Bulgaria. In the second year of the experiment, the vetiver grass was gathered. The heavy metal (Pb, Cd, Hg), micro (Fe, Cu, Zn, Mn), and macro (P, K, Ca, Mg) element contents in vetiver roots and shoots were determined by ICP after microwave mineralization. The essential oil of the ground vetiver roots was obtained by steam distillation in laboratory conditions. Gas chromatography-mass spectrometry identified fifteen compounds in the oil, mainly sesquiterpenes. The vetiver grass is tolerant to heavy metals with no signs of toxicity (chlorosis and necrosis) and can be grown on heavy metal polluted soils (37.7 mg.kg⁻¹ Cd, 1238.7 mg.kg⁻¹ Pb and 1676.4 mg.kg⁻¹ Zn). Bioaccumulation factor and translocation factor values (BAF and TF < 1) were less than one, suggesting low accumulation in the shoot. This crop can be referred to as a non-accumulating plant for Pb, Cd, and Zn and can be used for the phytostabilisation of contaminated soils in situ. Cultivating vetiver on soils contaminated with heavy metals has a beneficial effect on the yield and production of oil of high commercial value (high in khusimol).

INTRODUCTION

Vetiver (*Chrysopogon zizanioides*, (Family Poaceae) is a perennial herbaceous plant that is mainly cultivated in tropical and subtropical countries (India, Indonesia, Haiti, China, Japan, Brazil, and the Reunion Islands) (Chomchalow 2000, Maffei 2002, Pandey & Tiwari 2024). Vetiver is cultivated mainly to produce essential oil obtained by steam distillation from the plant's roots (Massardo et al. 2006). The oil is amber, brown, or dark golden, with high viscosity and extremely low volatility, with a strong and persistent woody odor characterized by different nuances depending on the geographical origin, cultivation, and extraction process. The oil is used in medicine, aromatherapy, and perfumery (Maffei 2002, Massardo et al. 2006). The oil balances the hormonal system, nourishes and moisturizes the skin, and promotes faster wound healing. The roots can be used to heal burns, improve blood circulation, as an immunity booster (Duke 2002), the shoots are used to remove parasitic infections in animals (Pareek & Kumar 2013), and the whole plant is used as a painkiller for rheumatoid arthritis, lumbago, and sprains (Grover et al. 2021). Vetiver can be used to construct green belts to reduce erosion risks, landslides, and flooding (Kim et al. 2022).

Phytoremediation is an inexpensive and environment-friendly method used for cleaning up heavy metal-contaminated soils and waters (McGrath et al. 2002, Khoiriyah et al. 2024, Hemani et al. 2024). This technology uses plants to extract contaminants from the environment or to reduce their toxicity. The plants are planted in metal-contaminated soils and grown according to established agronomic practices. Being a plant-based technology, the success of phytoextraction will depend on the extent of uptake of metals by the root system, shrinkage, and



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accumulation in the shoots. Factors such as short growing cycles, rapid growth, large biomass production, disease resistance, and tolerance to heavy metals are also essential in plant selection (Baker et al. 1994). Plants used for phytoextraction must be tolerant of metals, translocate them from the roots to the aerial parts (Reeves & Baker 2000), tolerate difficult soil conditions (i.e., soil pH, soil salinity, soil texture, water content), form a dense root system, and be easy to grow.

Vetiver is a perennial crop (can be grown for 50 years) and has an advantage over annual plants that require annual planting, significantly reducing farmers' costs. Vetiver can be used for remediation and rehabilitation due to its extraordinary and unique morphological and physiological characteristics. The time to extract metals from the soil will depend on the root system's depth and the medium's degree of contamination (Truong et al. 2008, Danh et al. 2009). In contrast to grasses, which are characterized by a horizontal root system, vetiver has a deeply penetrating root system (up to 3-4 m deep), making it a suitable plant species for erosion control and rice terrace stabilization (Truong 2000, Danh et al. 2009). The plant is highly tolerant to elevated levels of heavy metals such as As, Cd, Cu, Cr, Pb, Hg, Ni, Se, and Zn in the soil without affecting its growth (Truong et al. 2008, Roongtanakiat et al. 2008, Danh et al. 2009). Vetiver has excellent potential for nutrient (nitrogen and phosphorous) and organic pollutant uptake (Truong 2000, Truong et al. 2010). Furthermore, vetiver has a high tolerance and adaptability to extreme conditions such as prolonged drought, flooding, temperature extremes (-14 to 55°C), high soil acidity and alkalinity (pH 3.3 to 9.5), saline soils (electrical conductivity up to 4.0 (Danh et al. 2009) and aluminum and Mn toxicity (Truong et al. 2010).

In recent years, interest in vetiver has been steadily increasing, as has research related to vetiver's potential to prevent soil erosion (Oshunsanya et al. 2017), heavy metal soil cleaning (Roongtanakiat & Chairojy 2001, Chen et al. 2004, Antiochia et al. 2007), wastewater (Roongtanakiat et al. 2008) and tailings (Yang et al. 2003, Roongtanakiat et al. 2008). Vetiver tolerates high concentrations of individual heavy metals in soils and combinations of several heavy metals. Typically, highly contaminated soils are contaminated with more than one element (polymetal contamination) with different dominance of the various metals (Ernst et al. 2000, Walker & Bernal 2004). The pot experiments showed that vetiver could survive and grow well on contaminated soils with Pb contents ranging from 1155 to 3281.6 mg.kg⁻¹, Zn from 118.3 to 1583 mg.kg⁻¹, and Cu from 68 to 1761.8 mg.kg⁻¹ (Wide et al. 2005, Chiu et al. 2006). Under field conditions, it has been found that vetiver can grow on soils containing Pb from 2078 to 4164 mg.kg⁻¹,

Zn, from 2472 to 4377 mg.kg⁻¹, Cu from 35 to 174 mg.kg⁻¹ and Cd from 7 to 32 mg.kg⁻¹ (Shu et al. 2002, Yang et al. 2003, Shu et al. 2004).

Vetiver cultivation is minimal in Europe due to less favorable growing conditions (cold winters and long, hot summers) than in tropical and subtropical regions. The first field study of Vetiver in Europe was carried out in 1994 in southern Spain to determine the possibilities for growing vetiver under Mediterranean conditions. In 1996, trials were carried out in Portugal with vetiver to protect dam walls and to stabilize the banks of a heavily eroded watercourse that flows down a cultivated valley. In 1998, studies were carried out in Italy on biomass production and the tolerance of vetiver to salinity, and a private nursery was established near Milan, which was later moved to near Pisa, where the climatic conditions are much more favorable for vetiver production. In Albania, an attempt was made in 1997 to grow vetiver to address erosion problems in small plots. Still, the climatic conditions and low temperatures in winter did not allow satisfactory results. Vetiver cultivation is possible in southern Europe and Mediterranean countries such as Greece, Syria, Turkey, Morocco, Portugal, and Spain (Pease 2000). Vetiver is not cultivated in Bulgaria. Climate change and global warming are the reasons for growing non-traditional, more heat-loving crops in Bulgaria. Due to climate change and rising winter temperatures, it has become possible to grow vetiver under field conditions as a perennial crop without the danger of frost. The present study is the first attempt to establish vetiver cultivation possibilities in Bulgaria's industrially polluted areas.

In Bulgaria, about 61% of the areas contaminated with heavy metals above the maximum permissible content of harmful substances in soil are located in the areas of metallurgical plants. In the regions of smelters, combined contamination of plants is observed - (i) uptake of accumulated heavy metals in the soil by the root system of plants and (ii) by aerosols from the atmosphere on the leaf surface (Kabata-Pendias & Pendias 2001). The aerosols' concentration varies with distance from the source, weather conditions, and particle size. Larger particles settle quickly to the ground surface, in contrast to smaller particles, which can be lifted by air currents to high altitudes and disperse over considerable distances. The height of smelter stacks, wind speed, rainfall, and the terrain's character also influence. Aerosols cling to plant shoots, and trace elements may be absorbed. Pb remains mainly as a surface deposit, while Zn and Cd penetrate partially into the shoots (Kabata-Pendias & Pendias 2001).

The area around a Non-Ferrous Metal Works-Plovdiv (NFMW) is one of the "hot" ecological spots of the country. The plant's production activities are associated with the

emission of industrial gases and dust (aerosols), the region's primary pollutants. These emissions are dominated by aerosols of Pb and Zn, accompanied by Cd and Hg. The metals in the emissions are predominantly in the form of oxides. In recent years, there has been a downward trend in heavy metal and sulfur oxide emissions to the air due to improvements in Pb and Zn production technology at NFMW. However, accumulated heavy metals from previous years remain in the surface soil layer (Dimitrov et al. 2019). Finding suitable plant species that can be grown on these contaminated soils is necessary.

The present work aimed to conduct a comparative study to determine the heavy metal (Pb, Cd, Hg), micro (Fe, Cu, Zn, Mn), and macro (P, K, Ca, Mg) element contents in vetiver, the composition of vetiver oil, as well as the possibilities to use the vetiver for phytoremediation of heavy metal contaminated soils.

MATERIALS AND METHODS

Plant Material

The study was conducted with a vetiver test plant. Vetiver seedlings were purchased from Spain. The seedlings were planted in areas spaced at different distances from the pollution source NFMW - Plovdiv (0.1 km, 3.5 km, and 15 km). The study area is located in southern Bulgaria and falls within a transitional-continental climatic region. The winters are relatively mild, and the summers are long and hot. Summers are hot, autumns warm and long, and spring cool, with frequent frosts. The average daily maximum is 36°C in July and August, the average daily minimum is 0°C in January, and rainfall is 56 mm. Nearly 90% of the precipitation falls during the winter months from December to April.

The field trials were laid out using the block method in four replications with an experimental plot size of 100 m². Vetiver seedlings were planted in spring on the previously prepared plots at each sampling location. The plots were plowed to a depth of 40 cm to break up the soil and eradicate the rhizomes of perennial weeds. After the initial tillage, the land was disked, and planting holes were dug. Vetiver seedlings were planted in a 50 x 50 cm scheme. After planting, the plants were watered regularly, and in the second year, they were watered mainly during the hot months of the year. In the second year after planting, samples of plant material (roots and above-ground mass) of vetiver were taken for analysis. For this purpose, plants were removed with the roots from each experimental plot in October. The roots were separated from the above-ground mass. Vetiver oil was extracted from the roots in the 19th month of planting.

The roots were washed, dried, cut, and soaked in water. The oil was obtained under laboratory conditions by steam distillation for 16 h using a Clevenger-type apparatus.

Roots and shoots from each experimental plot were analyzed to determine the vetiver's heavy metal and nutrient contents. The analysis was performed on pre-dried at 75°C root and leaf samples.

Soil Samples

Soil samples were also collected from individual plots before vetiver seedlings were planted. Soil sample 1 (S1) is located at the 0.5 distance from NFMW-Plovdiv (0.5 km). Sample 2 (S2) was taken at a distance of 3.5 km from NFMW - Plovdiv (from the experimental field of the Agricultural University-Plovdiv in Brestnik village), and sample 3 (S3) from the experimental field of the Agricultural University-Plovdiv (15 km from NFMW - Plovdiv).

Soil samples were collected using a hand probe at 0 - 30 cm depth. One average sample was taken from each plot, formed from 10 stitches taken diagonally. Soil from all stitches from each plot was homogenized, air-dried, and sieved through a 2 mm sieve.

A soil subsample was characterized for soil pH (H₂O) in deionized water suspension of 1:5 (w/v); total nitrogen by the Kjeldahl method (N Kjeldahl); total oxidizable organic carbon according to the Tube digestion method (with titration) (Sparks 1996).

Heavy Metals Analysis

The total and DTPA-extractable concentrations of metals in the soils were determined. The total content of metals in soils was determined by ISO 11466. 3 g of air-dry soil to the nearest 0.001 g was weighed and moistened with 1 mL H₂O. Add 28 mL of a mixture of HCl: HNO₃ (3:1). The sample is left for 16 h at room temperature, then placed in a sand bath and heated for 2 h at 180 to 200°C. The residue was quantitatively transferred to a volumetric flask by filtration through a blue band filter, and the volume was made up to 100 mL.

The available (mobile) metals (Pb, Zn, Cd, Hg) contents were extracted by a solution of DTPA (ISO 14870). To prepare 1 L of DTPA extraction solution, 14.91 g of TEA, 1.957 g of DTPA, and 1.47 g of CaCl₂ were used and dissolved in 1 L of deionized water. The solution was adjusted to pH 7.3 by the addition of 6 mol.L⁻¹ HCl. 10 g of soil (sieved through a 2-mm sieve) was weighed into a 100 mL container and added 20 mL of DTPA solution. The samples were placed on a shaker and shaken for 120 minutes, then centrifuged, decanted, and filtered through a pleated

filter. Before measuring elemental content by ICP-OES, samples were diluted 1:25 with 2% HNO₃.

The concentrations of heavy metals and nutrients (Pb, Cd, Hg, Fe, Cu, Zn, Mn, P, K, Ca, Mg) in roots, shoots, and oils were determined by the method of microwave mineralization. A milestone 1200 MEGA microwave system with 10 MRD 300 rotors (10 positions) was used. 0.2 g of the dried and well-homogenized sample was weighed to the nearest 0.001 and transferred to Teflon bombs, and 5 mL HNO₃ and 1 mL H₂O₂ were added to each sample. The mineralization program includes three stages: (i) 5 minutes of 250 W non-pulsed irradiation, (ii) 5 minutes of 400 W pulsed irradiation; and (iii) 5 minutes of 600 W pulsed irradiation. After ventilation for 1 minute, the sample was cooled and transferred quantitatively to a 25 mL volumetric flask.

An inductively coupled emission spectrometer (Jobin Yvon Emission - JY 38 S, Paris, France) was used to determine the element content in the samples. The mercury (Hg) content of the soil and plant samples was determined without prior sample preparation using an MA-3000 mercury analyzer (Nippon Instruments Corporation). The quality and analytical performance of the ICP and the Hg analyzer were validated using standard reference materials from contaminated brickyard soil, ERM CC135a, and apple leaves (SRM 1515, National Institute of Standards and Technology, NIST). The results showed acceptable agreement between the established and certified values for the elements.

Evaluation of the Phytoextraction Ability of Vetiver Plants

The phytoremediation ability of the plants was evaluated by calculating the translocation factor (TF) and the bioconcentration factor (BCF).

The translocation factor (TF), which is the ability of a plant to translocate metals from its roots to shoot, was calculated by the following formula.

$$BCF = \frac{C_{\text{plant parts}}}{C_{\text{soil}}}$$

Where Cshoots represent the element contents (in mg.kg⁻¹) in the plant shoots, and Croots shows the element concentration in the roots (in mg.kg⁻¹).

Bioconcentration factor (BCF) is also used to calculate metals transfer from soil to various plant parts. BCF was calculated by the following formula.

$$BCF = \frac{C_{\text{plant parts}}}{C_{\text{soil}}}$$

Where Cplant parts represent the element contents (in mg.kg⁻¹) in the plant organs (roots, shoot), and Csoil (in mg.kg⁻¹) shows the element concentration in the corresponding soils.

Essential Oil Extraction

Vetiver essential oil is produced in secretory cells located in the first cortical layer outside the endodermis of mature vetiver roots, and vetiver oil is distilled from the roots of this monocotyledonous plant. Vetiver roots do not readily release the oil because the essential oils are located in hard-to-reach root tissues. To be extracted, these oils must diffuse (a relatively slow physical process) from the interior of the fibrous tissues outward to the surface. In addition, vetiver oil consists of a high percentage of sesquiterpenes (which have high molecular weights with low vapor pressures), contributing to the long extraction time required. The most valuable fractions of vetiver oil have the highest boiling points and constitute the high-specific-gravity oil fraction. They are separated in the most significant volume at the end of distillation. These fractions are rich in vetivones and vetiverol (Chomchalow 2001).

Essential oils from the roots of plants were obtained in laboratory conditions by steam distillation for 16 h in a Clevenger-type apparatus. Before distillation, air-dried roots were blended to a fine powder and soaked overnight in water. The oils were dried over anhydrous sodium sulfate and, after filtration, stored in a freezer at -20°C until tested and analyzed.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Essential Oils

GC-MS analysis was carried out on a 7890A gas chromatograph interfaced with a 5975 C mass selective detector (Agilent Technologies). Separations were performed using a 30 m × 0.25 mm (i.d.) DB-5ms silica-fused capillary column coated with 0.25 µm film of poly (dimethylsiloxane) as the stationary phase. The flow rate of carrier gas (helium) was maintained at 1.0 ml.min⁻¹. The injector and the transfer line temperature were kept at 250°C. The oven temperature program used was 100°C for 2 min, then 15°C.min⁻¹ to 180°C for 1 min, then 5°C.min⁻¹ to 300°C for 10 min. The injection volume was 1 µl. The injections were carried out in a split mode 20:1. The mass spectrometer was scanned from 50 to 550 m/z. All mass spectra were acquired in electron impact (EI) mode with 70 eV.

A mixture of aliphatic hydrocarbons (C8-C40) (Sigma) was injected into the system under the above temperature program to calculate the relative retention index of each compound. Identification of components was obtained by

Table 1: Soil characteristics of the study areas.

Parameter	S1 [0.1 km] x±sd	S2 [3.5 km] x±sd	S3 [15 km] x±sd
pH	7.5	7.8	8.0
Organic C, %	1.56	1.52	1.54
N Kjeldal, %	0.34	0.22	0.13
Pb, mg.kg ⁻¹	1238.7±14.5	214.4±2.1	33.3±0.5
Cd, mg.kg ⁻¹	37.7±0.5	3.1±0.2	0.5±0.05
Hg, mg.kg ⁻¹	2.2±0.1	0.5±0.01	0.06±0.01
Zn, mg.kg ⁻¹	1676.4±13.5	329.8±2.3	70.0±0.8
Cu, mg.kg ⁻¹	222.1±2.1	78.8±1.1	7.6±0.3
Fe, mg.kg ⁻¹	27388±16.0	50329±20.0	15566±14.5
Mn, mg.kg ⁻¹	1045.3±5.2	1683.0±6.3	884.2±2.4
K, mg.kg ⁻¹	8029.5±17.9	10555±20	6780.0±16.4
Ca, mg.kg ⁻¹	243556±20	13152±18	10061±17
Mg, mg.kg ⁻¹	12574±18	8412.3±16	7926.8±16
P, mg.kg ⁻¹	607.2±8.8	783.9±9.1	604.8±8.9

x- average value(mg.kg⁻¹) from 5 repetitions; sd - mean standard deviation
 MAC (pH>7.4) – Pb -100 mg.kg⁻¹, Cd - 3.0 mg.kg⁻¹, Zn -400 mg.kg⁻¹,
 Hg -1.5 mg.kg⁻¹

comparing the RI and the spectral data from the NIST'08 (National Institute of Standards and Technology, USA).

Statistical Analysis

The SPSS for Windows program was used in the statistical processing of the data.

RESULTS

Content of Heavy Metals in Soils

To elucidate the extent of soil contamination with heavy metals and their localization in the vegetative organs of vetiver, soil samples were collected from the areas located at different distances from MFMW (0.5, 3.5 and 15 km). The physical and chemical properties of the soil samples are presented in Table 1. The soils are characterized by a slightly alkaline reaction, medium organic

carbon content, and medium to high nutrient (N, P, K) availability.

The results presented in Table 1 show that with distance from NFMW - Plovdiv, there is a well-defined tendency for a decrease in the total content of heavy metals in the soil. In soil samples collected from the area 0.1 km and 3.5 km away from NFMW, values for Pb exceeding the maximum permissible concentrations (MAC) (100 mg.kg⁻¹) and the accepted values of 100 mg.kg⁻¹ for toxic effect on plants were recorded (Kabata-Pendias & Pendias 2001).

In the samples taken from S1 (0.1 km away), the Pb values reached 1238.7 mg.kg⁻¹. The Pb content in S2 (3.5 km away from the NFMW) reached 214.4 mg.kg⁻¹, while in the area 15 km away (S3), the Pb content decreased significantly to 33.3 mg.kg⁻¹. Similar results were obtained for Cd, Zn, and Hg. At S1, 1676.4 mg.kg⁻¹ Zn, 37.7 mg.kg⁻¹ Cd, and 2.1 mg.kg⁻¹ Hg were recorded. At S2 – 329.8 mg.kg⁻¹ Zn, 3.1 mg.kg⁻¹ Cd and 0.5 mg.kg⁻¹ Hg, and at the more remote area (15 km from NFMW) 70.0 mg.kg⁻¹ Zn, 0.5 mg.kg⁻¹ Cd and 0.06 mg.kg⁻¹ Hg were detected (Table 1).

Table 2 presents the results for the mobile forms of Pb, Zn, Cd, and Hg in the investigated soils. The table also gives the percentage of mobile forms relative to the total amount of the elements in the soil.

The results for the mobile forms of the metals determined by DTPA show that the mobile forms of Cd in the contaminated soils are the most significant fraction of its total content, followed by Pb, Zn, and Hg. In terms of Cd, they ranged from 47.6% (S1) to 51.6% (S2). Similar results were obtained for Pb and Zn. The mobile forms of Pb ranged from 9.2% (S2) to 49.9% (S1), Zn from 7.0% to 13.3% and Hg from 1.3 to 2.5%. In uncontaminated soils, again, mobile forms of Cd accounted for the largest proportion of the total content and reached up to 40%, followed by Pb with 15.1%, Zn with 7%, and Hg with 3.4%.

Content of Heavy Metals and Nutrients in Vetiver Roots and Shoots

Soil conditions and plant type influence the uptake of

Table 2: Mobile forms (DTPA- extractable) of Pb, Zn, Cd (mg/kg), and Hg (ng/g) in soils from the study area.

Distance from NFMW, km	Pb, mg.kg ⁻¹ x±sd	Zn, mg.kg ⁻¹ x±sd	Cd, mg.kg ⁻¹ x±sd	Hg, µg.kg ⁻¹ x±sd
S1 (0.1 km)	617.9± 3.2 49.9%*	223.2± 1.0 13.3%	21.8± 0.5 47.6%	24.0± 0.1 1.3%
S2 (3.5 km)	33.3± 1.5 9.2%	23.8± 0.4 7.2%	1.6 ±0.3 51.6%	12.6 ±0.1 2.5%
S3(15 km)	5.1± 0.5 15.1%	4.6± 0.2 7.0%	0.2 ±0.05 40.0%	2.1 ±0.01 3.4%

*%DTPA -extractable/total content

Table 3: Content of the heavy metal (Pb, Cd, Hg), micro (Fe, Cu, Zn, Mn), and macro (P, K, Ca, Mg) elements in vetiver roots and shoots.

Element	Roots (S1)	Leaves (S1)	Roots(S2)	Leaves (S2)	Roots (S3)	Leaves (S3)
Pb, mg.kg ⁻¹	709.6±5.3	190.3±2.4	19.4±0.5	3.8±0.2	5.6±0.2	2.6±0.1
Cd,mg.kg ⁻¹	36.0±1.0	5.8±0.2	2.3±0.1	1.7±0.1	0.35±0.05	0.21±0.05
Hg, µg.kg ⁻¹	2870±10	696.2±2	915.7±2	235.0±1	223.6±1	66.4±1
Zn, mg.kg ⁻¹	442.0±1.2	113.9±0.7	28.0±0.3	7.7±0.2	15.1±0.3	6.7±0.2
Cu,mg.kg ⁻¹	55.0±0.5	17.5±0.3	5.7±0.2	1.28±0.1	5.2±0.1	1.4±0.1
Fe, mg.kg ⁻¹	725.7±0.6	226.4±0.5	991.5±0.9	116.0±0.6	907.7±0.8	108.1±0.5
Mn,mg.kg ⁻¹	37.9±0.2	11.1±0.1	86.4±0.4	22.6±0.1	45.4±0.1	31.3±0.1
P, mg.kg ⁻¹	472.5±3.0	558.2±3.5	622.5±3.5	1331.7±5.1	643.1±3.6	1150.9±5.0
Ca,mg.kg ⁻¹	2101.2±7.8	2855.7±8.6	1095.0±5.6	875.5±3.8	1311.8±5.9	720.3±2.8
Mg,mg.kg ⁻¹	449.2±2.0	504.1±2.4	527.4±2.3	366.4±2.5	732.0±3.8	497.7±2.6
K, mg.kg ⁻¹	3169.3±3.8	10601.7±5.6	4224.5±4.0	8788.8±5.3	2940.6±2.9	9735.1±5.4

heavy metals into plants, and their accumulation in plant organs depends on their content in the soil. Plants grown on contaminated soils accumulate heavy metals and get them in their vegetative organs. In most cases, most of them accumulate in the roots of plants as they come into contact with the contaminated soil. However, some plants accumulate a substantial fraction of heavy metals in the shoots (Kabata-Pendias & Pendias 2001).

Table 3 presents the results obtained for heavy metal, micro-, and macro-element contents of roots in the aerial mass of vetiver.

Heavy Metal Content in the Essential Oil

The ability of vetiver to absorb heavy metals in the roots can lead to heavy metal contamination of the oil, as the oil is extracted from the roots of the plant. The results show that most of the heavy metals contained in the roots of the vetiver do not pass into the oil during the distillation. Therefore, their content in the oil is much lower. Pb content in the essential oil of vetiver when grown on S1 and S2 ranged from 0.57 to 0.58 mg.kg⁻¹, Zn from 11.3 to 13.2 mg.kg⁻¹, Cd from 0.05 to 0.07 mg.kg⁻¹, while the range of Hg is below the limits of the quantitative measurement of the method used (Table 4). Significantly lower results in the essential oil of vetiver grown at a distance of 15 km from NFMW - 0.02 mg.kg⁻¹ Pb and 5.5 mg.kg⁻¹ Zn, while the content of Cd is below the limits of the quantitative measurement of the method used. The Pb, Cd, and Hg amounts in the vetiver oil are lower than the accepted maximum values and meet the requirements of an environmentally friendly product (5 mg.kg⁻¹ Pb, 1 mg.kg⁻¹ Cd, 0.1 mg.kg⁻¹ Hg)(European Pharmacopoeia 2021).

The results confirm the ones established by Angelova et al. (2015), which found that the heavy metal content

Table 4: Content of heavy metals in vetiver essential oil.

Element	S1 [0.5 km]	S2 [3.5 km]	S3 [15 km]
Pb, mg.kg ⁻¹	0.58±0.1	0.54±0.1	0.24±0.01
Cd, mg.kg ⁻¹	0.07±0.01	0.05±0.01	Nd
Hg, µg.kg ⁻¹	Nd	nd	Nd
Zn, mg.kg ⁻¹	13.2±0.2	11.3±0.2	5.5±0.1

nd- not detected

in essential oil is very low and is not affected by the level of soil contamination with heavy metals. Essential oils contain only traces of heavy metals in distilled oils because these metals have too heavy and large molecules to be volatilized enough and to be concentrated by the distillation process.

Essential Oil Content and Composition of Vetiver Essential Oil

The results of the chromatographic analysis of essential oils obtained by processing the roots of vetiver grown at a different distance from NFMW-Plovdiv are presented in Table 5. Fig. 1 shows the chromatograms from the GC MS analysis of vetiver oil.

Vetiver essential oil has a very complex chemical composition, with many constituents (mainly sesquiterpenoids) showing a substantial structural diversity (Prasad et al. 2014). More than 150 components have been identified in vetiver oil produced in different countries (Chowdhury et al. 2002, Champagnat et al. 2006).

Fifteen components were identified in vetiver oil, accounting for 97.55-98.9% of the total oil components (Table 5). The bicyclic sesquiterpenes from the Caryophyllenes, Cadinane, Guaianes, Eudesmane, Eremophilane Nootkatane, Spirane, and the tricyclic sesquiterpenes from the group of Copaanes and Zizaanes were contained in the studied oils.

Table 5: Chemical composition of vetiver essential oil (%).

	Compound	RI	S1	S2	S3
	Oil content, %		1.26	0.98	0.78
1	α -Copaene	1378	1.05 $\pm$ 0.1	1.13 $\pm$ 0.1	1.08 $\pm$ 0.1
2	(E)-Caryophyllene	1419	1.34 $\pm$ 0.1	1.44 $\pm$ 0.1	1.39 $\pm$ 0.1
3	α -Amorphene	1485	2.30 $\pm$ 0.1	2.44 $\pm$ 0.1	2.38 $\pm$ 0.1
4	β -Vetispiorene	1495	3.14 $\pm$ 0.1	3.39 $\pm$ 0.1	3.26 $\pm$ 0.1
5	β -Vetivenene	1557	3.38 $\pm$ 0.1	3.55 $\pm$ 0.1	3.50 $\pm$ 0.1
6	10-epi- γ -Eudesmol	1619	3.85 $\pm$ 0.1	4.15 $\pm$ 0.1	3.99 $\pm$ 0.1
7	Junenol	1621	6.36 $\pm$ 0.1	5.87 $\pm$ 0.1	6.59 $\pm$ 0.1
8	7-epi- α -Eudesmol	1658	3.12 $\pm$ 0.1	3.37 $\pm$ 0.1	3.23 $\pm$ 0.1
9	Valerianol	1662	10.33 $\pm$ 0.1	9.07 $\pm$ 0.1	9.67 $\pm$ 0.1
10	Guaiol acetate	1724	7.83 $\pm$ 0.1	8.15	8.12 $\pm$ 0.1
11	Vetiselinenol	1728	7.27 $\pm$ 0.1	7.84 $\pm$ 0.1	7.53 $\pm$ 0.1
12	Khusimol	1736	30.25 $\pm$ 0.1	29.40 $\pm$ 0.1	28.23 $\pm$ 0.1
13	(E)-Isovalencenol	1794	5.95 $\pm$ 0.1	6.42 $\pm$ 0.1	6.16 $\pm$ 0.1
14	β -Vetivone	1809	5.21 $\pm$ 0.1	5.62 $\pm$ 0.1	5.40 $\pm$ 0.1
15	α -Vetivone	1847	6.79 $\pm$ 0.1	7.12 $\pm$ 0.1	7.03 $\pm$ 0.1
	HC		11.20	11.95	11.61
	Alcohols		67.14	66.12	65.40
	Esters		7.83	8.15	8.12
	Ketones		12.00	12.75	12.43
	Bicyclic		66.88	68.44	68.24
	Tricyclic		31.30	30.53	29.31
	Total		98.18	98.97	97.55

RI - Kovacs relative indices

No monocyclic sesquiterpenes of the group of Bisobalane and Elemol were detected.

The terpene hydrocarbons (α -Copaene, (E) Caryophyllene, α -Amorphene, β -Vetispiorene, β -Vetivenene), the terpene alcohols (-epi- γ -Eudesmol, Junenol, 7-epi- α -Eudesmol, Valerianol, Vetiselinenol, Khusimol, (E)-Isovalencenol), terpene ketones (β -Vetivone, α -Vetivone) and ester (Guaiol acetate) are contained in vetiver oil.

DISCUSSION

Content of Heavy Metals and Nutrients in Vetiver Roots and Shoots

Significant differences were found in the contents of the elements in different parts of the vetiver. Most of the heavy metals accumulated primarily in the root system of vetiver, which is consistent with the results of other authors. The higher accumulation of heavy metals in the roots is because vetiver has a dense and deep root system, which can create an ideal environment for microbial processes and contribute to the uptake of contaminants from the soil (Truong 2000). As

the source of contamination becomes more distant, there is a clear tendency for the heavy metal content of vetiver roots and above-ground mass to decrease. The amount depends primarily on the distance to the pollution source and the heavy metal content of the soil. The obtained results are similar to Roongtanakiat & Chairrojy (2001), Antiochia et al. (2007), Roongtanakiat et al. (2008), and Hasan et al. (2017), who found that heavy metal uptake by plant roots depends on their soil content. Pb content in vetiver roots ranged from 19.4 mg.kg⁻¹ (S2) to 709.6 mg.kg⁻¹ (S1), Zn from 28.0 mg.kg⁻¹ (S2) to 442.0 mg.kg⁻¹ (S1), Cd from 2.3 mg.kg⁻¹ (S2) to 36.0 mg.kg⁻¹ (S1) and Hg from 915.7 to 2870 μ g.kg⁻¹ (Table 3). Significantly lower values of 5.6 mg.kg⁻¹ Pb, 15.1 mg.kg⁻¹ Zn, and 0.35 mg.kg⁻¹ Cd and 223.6 μ g.kg⁻¹ Hg was found in vetiver roots grown on uncontaminated soil (S3). The values obtained for Cd, Pb, and Zn in vetiver roots from the highly contaminated soil were significantly higher than the values considered toxic to plants by Kabata-Pendias & Pendias (2001) (5 mg.kg⁻¹ Cd, 30 mg.kg⁻¹ Pb, 100 mg.kg⁻¹ Zn).

The Pb content in vetiver shoots grown at a distance of 0.1 km (S1) reached up to 190.8 mg.kg⁻¹, Zn up to

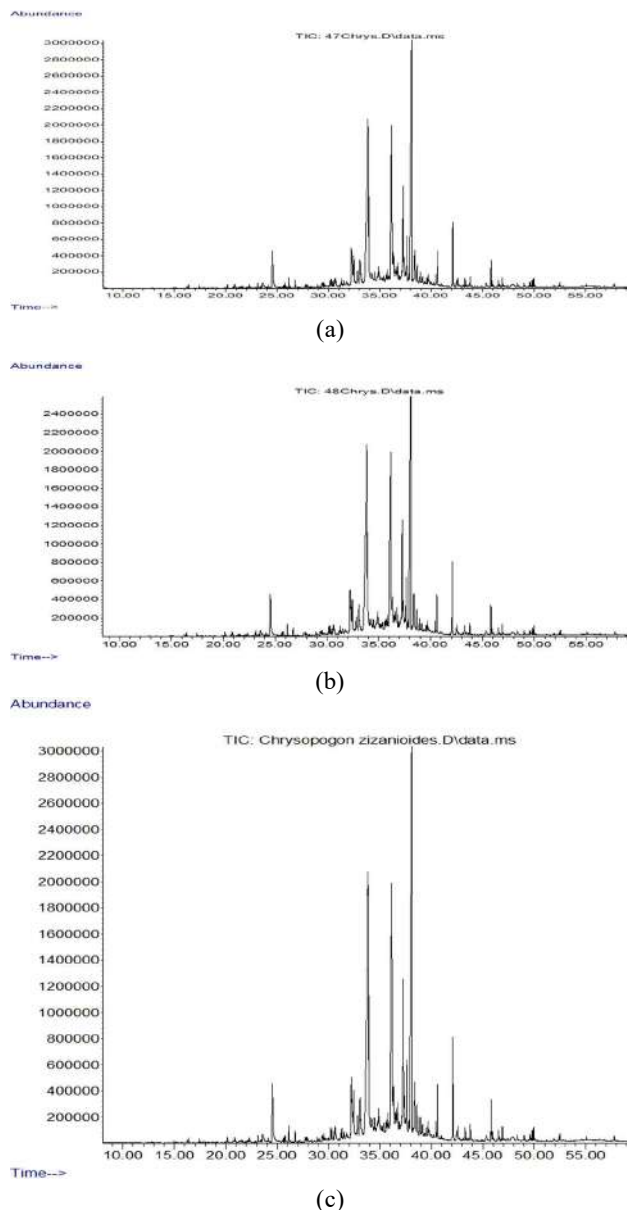


Fig. 1: GC-MS chromatogram of essential oil of vetiver: (a) S1; (b) S2; (c) S3.

113.9 mg.kg⁻¹, Cd up to 5.8 mg.kg⁻¹ and Hg up to 696.2 µg.kg⁻¹. Significantly lower concentrations were found in the shoots of vetiver than in the less contaminated and uncontaminated soils. Pb contents ranged from 2.6 to 3.8 mg.kg⁻¹, Zn from 5.7 to 7.6 mg.kg⁻¹, and Cd from 0.21 to 1.7 mg.kg⁻¹ (Table 3). The differences in heavy metal contents in vetiver shoots grown at different distances from the NFMW indicate significant aerosol contamination in the NFMW area, which decreased with increasing distance from the source of contamination.

The results obtained from this study agree with the

findings of Roongtanakiat et al. (2008), who reported that roots contained more metals than shoots. However, the results of Hasan et al. (2017) showed that the accumulation of metals was higher in the above-ground mass than in the roots due to the movement of heavy metals from roots to shoots.

The selective accumulation of heavy metals in vetiver roots indicates that the plants should be used primarily for phytostabilisation rather than phytoextraction (Banerjee et al. 2016).

The trace element content of the vetiver root system was higher than that of the above-ground mass, while the opposite

trend was observed for nutrients. The values recorded were as follows - in roots - 5.2 - 54.7 mg.kg⁻¹Cu, 725.7 - 991.5 mg.kg⁻¹ Fe, 37.9- 86.4 mg.kg⁻¹ Mn, 472.5- 643.1 mg.kg⁻¹ P, 1095.0-2101.2 mg.kg⁻¹Ca, 449.2- 527.4 mg.kg⁻¹ Mg, 2940. 6.- 4224.5 mg.kg⁻¹ P, in shoots 1.3 to 17.5 mg.kg⁻¹ Cu, 108.1 to 226.4 mg.kg⁻¹ Fe, 11.1 to 31.3 mg.kg⁻¹ Mn, 558.2 to 1331.7 mg.kg⁻¹P, 720.3 to 2855.7 mg.kg⁻¹ Ca, 366.3 to 504.1 mg.kg⁻¹ Mg, 8788.8 to 10601.7 mg.kg⁻¹ P.

High Pb content in the soil can lead to an imbalance in plant nutrient uptake. It is known that in most cases, Pb can block the uptake of K, Ca, Mg, Mn, Zn, Cu, and Fe into the root system (Kabata-Pendias & Pendias 2001). It has been found that P, Mg, Fe, and Mn contents are lower in vetiver roots than in contaminated soil.

The accumulation of elements by plants mainly depends on the content of mobile/immobile forms of the elements in the soil (Kabata-Pendias & Pendias 2001). The amount of mobile forms is related to the geochemistry of elements in the soil, and the accumulation of heavy metals in plants strongly correlates with soil contamination. The comparative analysis between the accumulation of metals in plants and the amount of mobile forms of metals in soil shows that heavy metal uptake and movement in vetiver depends on the amount of mobile forms in soil, and the mechanism of metal accumulation is the concentration gradient.

The distribution of heavy metals and micro and macro elements in the vetiver organs has a selective character and is specific to the individual elements, which follows the order: roots - K > Ca > Fe > Pb > Mg > Zn > P > Cu > Cd > Hg, above-ground mass - K > Ca > Mg > P > Fe > Pb > Zn > Cu > Cd > Hg.

Bioconcentration Factor (BCF) and Translocation Factor (TF)

To be able to give a definite answer to the question of what the ability of vetiver to extract heavy metals from soil and to evaluate the potential of vetiver for phytoremediation, bioconcentration factors (BCFroots and BCFshoots) and translocation factor (TF) were calculated. The bioconcentration and translocation factors (BCF and TF) are beneficial parameters for studying the movement pattern and accumulation of heavy metals from soil to plant (Baker 1981). Table 6 presents the results obtained for BCFroots, BCFshoots, and TF.

Bioconcentration Factor (BCFroots) represents the ratio of heavy metal content in plant roots to the content in soil (Yoon et al. 2006). BCF values concerning Pb were <1, indicating that the concentration of the element does not exceed its content in the soil. Similar results were obtained

for Zn. For cadmium, these values were around 1 (0.96) for vetiver from the highly contaminated soil (S1).

The translocation factor (TF) gives information about the ability of plants to assimilate heavy metals through the roots and move them to the shoots. TF showed the same trend as BCF (values less than 1). Most of the absorbed Pb in plants accumulates in the roots, and only a small fraction of Pb moves to the shoots. It is known that when Pb enters the plant roots, it immediately interacts with phosphates and carbonates present in high concentrations in the intercellular spaces and precipitates as phosphates or carbonates (Boonyapookana et al. 2005). The formation of insoluble Pb compounds leads to decreased Pb translocation in plants (Berti & Cunningham 2000). Data from Banerjee et al. (2016) for TF showed that except for Cu, other metals (Fe, Al, Zn, Cr, Mn, and Ni) assimilated by vetiver were retained mainly in the roots when vetiver was grown for three months on ore soils. Similar results were also obtained by Rotkittikhun et al. (2000). It was found that when vetiver was grown for 120 days, the heavy metal content was higher in the roots. In contrast, when vetiver was grown for 60 days, the heavy metal concentrations were higher in the shoots than in the roots (Roongtanakiat et al. 2001). Vetiver is a hyperaccumulator of Pb in some studies (Antiochia et al. 2007, Roongtanakiat et al. 2008). It was found that 4940 mg.kg⁻¹ Pb accumulated in roots and 359 mg.kg⁻¹ Pb in shoots, or over 0.22% Pb. Pb content reached in vetiver up to 1390-1450 mg.kg⁻¹ after nine months of cultivation on polygon soils (Wilde et al. 2006). It was established vetiver to be a hyperaccumulator of Pb and Zn, accumulating over 0.4% Pb in shoots and 1% Pb in roots, as well as 1% in Zn in roots and above-ground mass, respectively, when irrigated daily with PbCl₂ and ZnCl₂ solution for 30 days (Antiochia

Table 6: TF and BCF coefficients in vetiver grass.

Element	Coefficient	S1 [0.1 km]	S2 [3.5 km]	S3 [15 km]
Pb	BCFroots	0.57	0.09	0.17
	BCFshoots	0.15	0.02	0.08
	TF	0.27	0.20	0.46
Zn	BCFroots	0.26	0.08	0.22
	BCFshoots	0.07	0.02	0.10
	TF	0.26	0.27	0.44
Cd	BCFroots	0.96	0.74	0.70
	BCFshoots	0.15	0.54	0.42
	TF	0.16	0.74	0.6
Hg	BCFroots	1.36	1.88	3.60
	BCFshoots	0.33	0.48	1.07
	TF	0.24	0.26	0.3

et al. 2007). The higher uptake and translocation of Pb and Zn to the shoots are the available Pb and Zn content (Chiu et al. 2006, Antiochia et al. 2007).

The phytoextraction efficiency is also determined by the bioconcentration factor. The bioconcentration factor is defined as the ratio of the metal concentration in the above-ground mass of the plant to the soil and is a measure of the plant's ability to uptake and move metals to the above-ground mass (Baker et al. 1994). Metal concentration in plants can indicate the effectiveness of plants for remediation of heavy metal-contaminated soils (Chen et al. 2004). The results of Chen et al. (2004) showed that the ratio of Pb in shoots in vetiver to total Pb content in soil was very low (0.002-0.009).

The results obtained in this experiment showed that for Pb and Zn, the bioconcentration factor was lower than 1 when vetiver was grown on both contaminated and uncontaminated soils. For cadmium, the bioconcentration factor approaches 1 (0.96) when grown on highly contaminated soil and decreases to 0.7 on lightly contaminated soil (Table 6). For Hg, the bioconcentration factor is higher than 1, irrespective of the degree of soil contamination.

The phytoremediation capacity of plants is assessed by the bioconcentration factor (BCF_{shoots}) and translocation factor (TF). According to Baker et al. (1981), plants are classified as non-accumulators, accumulators, or hyperaccumulators if the values of BCF are as follows: <1, >1, and >10. The values obtained for BCF for plants grown on highly contaminated soils are less than 1 for Pb, Zn, Cd, and Hg. Similar results were obtained by Banerjee et al. (2016) for vetiver when grown on soils from mining activities (mining soils)

BCF and TF values greater than 1 indicate that the plant is a potential accumulator of heavy metals and can efficiently move metals from the roots to the above-ground mass (Baker et al. 1981). The results indicate that in vetiver, both BCF and TF were less than 1; hence, vetiver can be classified as a non-accumulating plant with low metal movement to shoots. Increased concentrations of heavy metals in the roots and low translocation to the aerial parts indicate the suitability of the plants for phytostabilisation (Baker et al. 1981). Since the content of heavy metals in the aboveground mass is low, an opinion can be made for farmers regarding the content of the aboveground mass for animal feed or mulch. An essential advantage of the collected aboveground mass of vetiver is that it is not considered hazardous waste, unlike the residues of hyperaccumulators. It can be safely used for bioenergy production or compost. The obtained results do not support the finding of Antiochia et al. (2007) and Roongtanakiat et al. (2008) that vetiver is a hyperaccumulator of Pb and Zn and can be used for phytoextraction.

Content of Heavy Metals in Vetiver Essential Oil

Metals in essential oils are associated with plant contamination, which varies depending on the plant species, climate, soil composition, plant age, harvest period, or geographical origin. Therefore, contaminants' sources are anthropogenic, and their establishment in plant organs is mainly from the soil via the root system. The transfer of metals from leaves, bark, roots, seeds, flowers, and fruits to essential oils depends on the extraction technology. According to European Pharmacopoeia (2021), "the risk of contaminants in essential oils is related to the type of contaminants, the origin of the plant material used for production, and the production processes." However, contaminants such as heavy metals, pesticides, and aflatoxins are not considered critical for essential oils. They should be considered on a case-by-case basis based on a risk assessment. In particular, the risk is higher for cold-pressed essential oils than for distilled ones.

For vetiver essential oil to be successfully used in perfumery, it must not contain any toxic substances that could adversely affect the health of the users. Studies have shown that vetiver can absorb heavy metals, such as Pb, Zn, and Cd, in its roots. The ability of vetiver to accumulate heavy metals in its roots can lead to contamination of the essential oil with heavy metals. For this reason, essential oils from vetiver grass extracted by steam distillation were further analyzed for heavy metal content using inductively coupled plasma emission spectroscopy (ICP-OES).

When properly distilled, essential oils should not contain the following heavy metals (e.g., mercury, lead, etc.), as these types of molecules are too heavy and large to evaporate under normal distillation conditions. The results show that the content of heavy metals in vetiver essential oil is low and below the maximum permissible limits (5 mg.kg⁻¹ Pb, 1 mg.kg⁻¹ Cd, 0.1 mg.kg⁻¹ Hg) (European Pharmacopoeia 2021) and does not pose a threat if used in perfumery and cosmetics.

Essential Oil Content and Composition of Vetiver Essential Oil

The yield and composition of vetiver essential oil can be influenced by environmental conditions, soil geochemistry, and geographical origin (Chowdhury et al. 2002, Champagnat et al. 2006, Prasad et al. 2014). According to Massardo et al. (2006), vetiver oil production is closely related to the metabolic activity of plant roots, which is influenced by changes in ambient temperature. Low temperature (e.g., in winter) leads to a decrease in the metabolic activities of the plants and, hence, to a reduction of oil production (Massardo et al. 2006).

It has been found that oil yields range from 0.29 to 9.61% on a dry-weight basis (Adams et al. 2003). Depending on the method of cultivation, the age of the roots, the method of storage and preparation of the roots before the distillation process, the method and duration of distillation, about 0.42 to 1.29% (Rotkittikhun et al. 2010) and 0.3 to 2 % (Lavanina 2003) essential oil can be obtained on a fresh weight basis. The yield progressively decreases with the storage period, while cutting fresh roots into 2.5-5 cm length increases the yield (Balasankar et al. 2013). Increasing the distillation time in a Clevenger apparatus for 16 h (extraction time) allows for break off of the cells of the vetiver roots and thus removes the heavier components (sesquiterpenes) from the vetiver oil, resulting in an increase in yield to 1.8% (Leite 2012).

Heavy metals were also found to affect oil yield and composition. Pb and Cd influence metabolic processes, including essential oil synthesis (Zheljazkov et al. 2006). Heavy metals in the soils increase the yield of essential oils from vetiver (Sulastri & Tampubolon 2019). The increase in the percentage yield of essential oil from plants growing in areas contaminated with heavy metals is due to the plant's response to heavy metal stress, leading to an increased release of secondary metabolites such as essential oils. Results obtained showed that vetiver grass grown on contaminated soil (S1) produced essential oil (1.26%) compared to unpolluted soil (S3) (0.78%) (Table 5). The lowest essential oil yield was obtained from vetiver grown on soils located 15 km from the NFMW (S3), which have the lowest heavy metal content since vetiver is not subjected to metal stress as a stimulating factor for releasing secondary metabolites. Similar results were obtained by Sulastri & Tampubolon (2019), who reported an increase in essential oil content by up to 101.5% in vetiver grown on soils enriched with 100 ppm Cd compared to untreated soils. Prasad et al. (2014) found that applying 50 ppm Cd to uncontaminated soils led to an increase of 33.33% in essential oils from plants grown on such soils.

Elevated levels of Pb in plants have a significant impact on oil content and sometimes lead to changes in the chemical composition of the oil, which can affect the quality and safety of the products produced from it. While for most aromatic plants such as mint, basil, and lavender, the oil yield decreases when they are grown on soils contaminated with heavy metals (Champagnat et al. 2006, Zheljazkov et al. 2006), for vetiver, the results are ambiguous. It was found that oil yield increased significantly from 0.25 to 0.45 % (v/w), and the number of components in the oil increased from 47 to 143 compounds when soils were treated with Pb (Rotkittikhun et al. 2010). Increased vetiver essential oil production was associated with an increase in root

biomass and an increase in root essential oil concentration (Rotkittikhun et al. 2010). Contrary results were obtained from Kotoky et al. (2013), which suggested that adding Cr, Pb, and Ni negatively affected root and essential oil yield.

Vetiver essential oil has a very complex chemical composition, with many constituents (mainly sesquiterpenoids) showing a substantial structural diversity (Massardo et al. 2006). More than 150 components have been identified in vetiver oil produced in different countries (Chowdhury et al. 2002, Champagnat et al. 2006).

The results show that alcohols are predominant in oil (65.40-67.14%) (Table 6), but significant differences in their content are observed in oils from slightly contaminated and uncontaminated areas. Khusimol and Valerianol were higher in oil from vetiver grown on highly contaminated soil, while the contents of Vetiselinol, E-Isovalencenol, 10-epi- γ -Eudesmol, and 7-epi- α -Eudesmol were lower.

Hydrocarbons ranged from 11.20 to 11.95% of the total oil composition. There was no significant difference in the hydrocarbon content of the oils from the contaminated and non-contaminated regions.

Ketones ranged from 12.0 to 12.75%, and their content was lower in the oil from the highly contaminated soil. Guaiol acetate ester was also detected in the oil, which ranged from 7.83 to 8.15%.

Khusimol, β -vetivone, and α -vetivone are among the major components contained in vetiver oil, and their presence is often considered to be the "fingerprint" of the oil and impart a characteristic odor to vetiver oil (Demole et al. 1995).

The values of β -Vetivenene, β -Vetivenene, khusimol, α -vetivenene, (E)-isovalencenol of the essential oil of vetiver from this study are compared with the standard for vetiver oil ISO 4716, in which the values from China, Haiti, Indonesia, Brazil and in Bourbon-type oils are specified).

The content of khusimol and β -Vetivone in vetiver oil grown in Bulgaria is significantly higher than the values specified in ISO 4716 (Table 7). The β -Vetivenene range of the Bulgarian-grown vetiver oils (3.38 -3.55%) is similar to the values given in the standard for essential oils from Haiti (0.7 to 3%), China (2 to 8%), Bourbon type (3 to 6%). Still, it is significantly lower in essential oils from Indonesia (4 to 9%) and oils from Brazil (6 to 19%). The β -Vetivenene content reaches 9.76% in oils from Turkey (Kirici et al. 2011). The content of isovalencenol in the essential oils of vetiver grown in Bulgaria (5.95 to 6.42%) is in line with the values indicated in the standard (for the oils from China (1 to 11%), from Indonesia (1 to 7%), and Brazil (5 to 11%) and lower than the oils from Haiti (10 to 16%) and Bourbon-type oils (6 to 14%).

Table 7: Comparison of the values of the main components of Bulgarian vetiver oil with ISO 4716:2013 and the literature.

Component	Bulgaria	Bourbon type	China	Haiti	Indonesia	Literature
β -Vetivenene	5.21-5.62	3-6	2-8	0.7-3	6-9	1.6-9.7
β -Vetivone	3.36-3.55	2-5	2-4	2-4	2-4	3.12-4.82
Khusimol	28.23-30.25	12-18	5-15	9-15	6-11	3.4-26.94
α -Vetivone	6.78-7.12	3-6	2-5	2-4	2-4	2.5-6.3
Isovalencenol	5.95-6.16	614	1-11	10-16	1-7	

The quality of vetiver oil is related to the metabolism of its root system and the agro-climatic conditions under which it is grown. It was found that the content of some of the components in the oil is affected by heavy metal contamination of the soil. e.g., the content of α -vetivone is higher in Pb-contaminated soil, while the amount of beta-vetivone is very low in Pb and Cd-contaminated soils (Kotoky et al. 2013). The content of khusimol and valerenol also decreased by 16% and 14% in Cd and 17% and 19% in Pb-contaminated soils, respectively. Cu, Zn, and Ni contamination of soil did not affect the individual components (Kotoky et al. 2013).

Opposite results were obtained from Prasad et al. (2014), who found that khusimol significantly increased due to Pb and Ni application and minor to moderate amounts of Cr and Cd. The significant change in khusimol content may be due to vetiver uptake of essential and phytotoxic metals. Heavy metals may increase the catalytic activity of some enzymes due to enzyme-substrate complex formation with metals and hence affect the synthesis of monoterpene compounds (Prasad et al. 2014).

The results obtained in this study confirm that soil contamination with heavy metals affects the chemical composition of essential oils. Khusimol, the main constituent of vetiver essential oil, varied from 28.23% in plants grown on unpolluted soils to 30.25% in plants from heavily polluted soils. The vetiver oil from contaminated soil was found to have a higher content of alcohols and lower content of ketones and esters and did not significantly affect the hydrocarbon fraction. Soil contamination with heavy metals decreased the total range of bicyclic sesquiterpenes from 68.24 to 66.88 and increased tricyclic sesquiterpenes from 29.31 to 31.30%.

The results confirm that vetiver is a crop tolerant to heavy metals and can be grown on soils contaminated with heavy metals. This crop can be referred to as a non-accumulating plant for Pb, Cd, and Zn and can be used for phytostabilisation of Pb, Cd, Zn, and Hg-contaminated soils in situ. The quality of vetiver oil usually depends on the amounts of alcohol (mainly khusimol) that contribute to the desirable woody odor of the oil (Kotoky et al. 2013).

Cultivating vetiver on soils contaminated with heavy metals has a beneficial effect on the yield and production of oil of high commercial value (high in khusimol). This can be an economic incentive for farmers to grow vetiver on heavy metal-contaminated soils.

CONCLUSIONS

The present study is the first attempt to establish vetiver cultivation possibilities in Bulgaria's industrially polluted areas. Vetiver grass was tolerant to heavy metals and could be grown on soils contaminated with heavy metals (37.7 mg.kg⁻¹ Cd, 1238.7 mg.kg⁻¹ Pb, and 1676.4 mg.kg⁻¹ Zn). Heavy metals (Pb, Cd, Hg) and trace elements (Zn, Cu, Fe, and Mn) accumulate mainly in the root system, while macroelements (P, K, Ca, Mg) get in the vetiver shoots. In vetiver, both BCF and TF are less than 1; therefore, vetiver can be classified as a non-accumulating plant and successfully used for the phytostabilisation of heavy metal-contaminated soils. The Pb, Cd, and Hg amounts in vetiver oil are lower than the accepted maximum values and meet the requirements for an environmentally friendly product. For the first time, data on the composition of vetiver essential oil are published. The results obtained for the production and composition of the essential oil are auspicious. Vetiver can be cultivated in areas contaminated with heavy metals without the risk of transferring metals from the soil to the essential oil. Cultivation of vetiver on soils contaminated with heavy metals has a beneficial effect on the yield and production of oil of high commercial value (high in khusimol). This study indicates the ability of vetiver grass to produce essential oil under heavy metal stress, making it useful for both essential oil production and an important candidate for phytoremediation. The results show that vetiver can be used for soil stabilization in industrial areas of Bulgaria and worldwide. Research is needed to expand the scope of the search with new vetiver varieties or to investigate genetic modifications to improve the phytoremediation capabilities of vetiver. This can be an economic incentive for farmers to grow vetiver on heavy metal-contaminated soils. The advantages of using vetiver for phytoremediation of contaminated soils are the simplicity of the technology,

low cost, and ease of maintenance. However, further research is needed to clarify the real potential of vetiver when grown in larger areas, as there is still no practical experience in developing this crop in the country and the appropriate treatment of the roots before essential oil distillation to receive high yields and good quality oil.

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Exploring the Role of Selective Earthworm Species in Microbial-Mediated Heavy Metal Conversion: Implications for Environmental Bioremediation

Purbasha Priyadarshini^{ID} and Sunita Satapathy^{†ID}

Department of Zoology, School of Applied Science, Centurion University of Technology and Management, Odisha, India

[†]Corresponding author: Sunita Satapathy: sunita.mishra@cutm.ac.in

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ABSTRACT

Heavy metal contamination poses a significant threat to soil ecosystems and the health of living organisms, necessitating sustainable remediation strategies. Selective earthworm species play a pivotal role in enhancing microbial activity, thereby influencing heavy metal transformation processes. This study investigates the synergistic interactions between earthworms and soil microbes in heavy metal bioconversion. Using a controlled experimental design, specific earthworm species that significantly enhance microbial-mediated reduction, immobilization, and detoxification of heavy metals, including lead, cadmium, and arsenic. Key findings indicate that *Eisenia fetida*, *Eudrilus eugeniae*, *Dendrobaena octaedra* and *Lumbricus terrestris* stimulate microbial populations capable of producing bioavailable heavy metal chelators and reducing agents. Enhanced enzymatic activities, such as phosphatases and dehydrogenases, were strongly correlated with the presence of these earthworms. Despite these promising results, critical gaps remain in understanding species-specific microbial dynamics and long-term impacts on heavy metal bioavailability. Furthermore, the effects of varying soil physicochemical conditions on bioremediation efficiency require a comprehensive investigation. This study underscores the potential of integrating selective earthworm species into bioremediation frameworks for sustainable soil management. Future research should prioritize field-scale trials, advanced metagenomic analyses, and the assessment of eco-toxicological implications to optimize earthworm-mediated bioremediation strategies.

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INTRODUCTION

The present study explores the role of specific earthworm species in aiding microbial activity to convert and stabilize heavy metals in contaminated soil environments. The research encompasses ecological, microbiological, and biochemical perspectives to understand the synergistic relationships between earthworms and soil microbes. By studying selective species of earthworms, their interactions with microbial communities, and their impact on heavy metal bioavailability, this topic aims to identify sustainable strategies for environmental bioremediation.

The industrialisation and urbanisation throughout the world are leading to a huge generation of waste from various sources of domestic, municipal, and industrial. The overproduction of waste causes severe environmental issues due to its inappropriate disposal and requires immediate attention to prevent further degradation. The direct, undifferentiated land dispersion of wastes has increased unbalanced soil nutrition in agricultural land, soil, and plant toxicity (Millati et al. 2019). The waste contains excess metals along with organic residues that interfere with soil microbial activities, plant growth, and transfer non-biodegradable concentration in the ecological system. Heavy metals are primary constituents of both municipal and industrial wastes, in relation to concentration, and have

adverse effects on soil activities and crop growth (Swati & Hait 2017). Therefore, it is highly challenging to ensure safe disposal and management of wastes with proper treatment that undergoes recycling to enhance stabilization of organic composition and immobilization /depletion of heavy metals with reducing toxicity for sustainable agricultural practices.

At present, several eco-friendly and cost-effective techniques are being applied for recycling, reducing, and reusing of wastes, which have gained much attention to prevent the waste from stabilizing for agronomic purposes. Nowadays, besides chemical, physical, and biological methods, new techniques like bioremediation, phytoremediation, and especially nano-bioremediation are used to convert heavy metal pollutants. In the technique of nanobioremediation, the nanoparticles enter the pores of soil to degrade the toxicity of heavy metal pollutants (Hemalatha et al. 2022).

Among various techniques, vermicomposting is a biological remediation process using earthworms to convert wastes into sustainable agricultural products. Vermicomposting is especially gaining attention for a low-cost cost-effective waste stabilization technique involved in metal remediation with sustainable land renovation practice as compared to other expensive and laborious methods (Ojha et al. 2023). The familiar term vermicomposting is a natural process that transforms organic waste into a valuable soil conditioner with the involvement of earthworms known as vermicompost. The vermicompost is an organic fertilizer renowned for its nutrient richness and soil conditioning benefits. The water-soluble nutrients in vermicompost enhance the availability of essential nutrients, improve soil structure, and contribute to better drainage elucidated by Thakur et al. (2021).

Through vermicomposting, organic matter is broken down by earthworms, resulting in nutrient-rich vermicast resembling peat. These vermicast enhance excellent water retention properties and are laden with essential nutrients, making them ideal for soil enrichment. Significantly, the efficacy of vermicomposting hinges on the earthworms' gizzard of digestive system. Nutrients are solubilized, beneficial microbial populations are enriched, and bioremediation processes are facilitated within the earthworms' guts, as elucidated by Kaur (2020). Earthworm processing emerges as a promising way for mitigating heavy metal content in waste streams without compromising the physiological integrity of these organisms. Specifically, *Eisenia fetida*, among other earthworm species, demonstrates a remarkable capacity for bioaccumulating various metals, including those classified as heavy metals, particularly when these metals are predominantly in non-bioavailable forms.

While there are thousands of earthworm species globally but only a select few are preferred for this composting method, as highlighted by Cui et al. (2023). With even minute quantities bearing detrimental consequences, heavy metal contamination stands as a significant environmental threat. Heavy metals, in contrast to organic pollutants, do not break down naturally and have a tendency to accumulate in living organisms. Many of these metal ions are toxic or even carcinogenic. Each of these groups of metals is characterized by an atomic number exceeding 20 and a density surpassing 5 g.cm^{-3} . Their extreme toxicity and resistance to biodegradation directly correlate with environmental contamination challenges reported by Balali-Mood et al. (2021). Its persistent presence across various industries, including electroplating, mining, leather tanning, and chemical production, underscores its hazardous environmental impact.

Heavy metal pollution arises from both natural phenomena and human activities. Natural sources include events such as volcanic eruptions and various geological processes. However, anthropogenic activities are the predominant contributors, involving practices like metal mining, landfill leaching, and vehicular emissions. This observation is supported by Briffa et al. (2020), who emphasise the significant impact of human-induced sources on heavy metal contamination. Agricultural practices further contribute to heavy metal contamination through the application of insecticides, fertilizers, and pesticides, thus compounding the environmental challenge. The heavy metals (HM) and metalloids, consisting of Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, Pb, and other metals, have enormous toxic impacts. Heavy metals, toxic to the environment, can directly affect an integral part of the plants and modify their biochemical, metabolic, and physiological processes. The cropping intensity was increased, and more area was brought under agriculture, reported by Jayakumar et al. (2021).

While trace amounts of certain heavy metals are essential for enzymatic and metabolic functions in organisms, their beneficial roles as cofactors and catalysts occur only in minute concentrations. However, elevated levels impede organismal growth, with metals like mercury, cadmium, chromium, and arsenic proving harmful even at low doses. Given their non-degradability, the accumulation of heavy metals in water bodies and soil poses grave risks to both flora and fauna. Unlike organic contaminants, heavy metals present formidable challenges in removal or degradation from the environment, aggravating environmental concerns (Bhattacharya et al. 2019).

According to environmental science, heavy metals (HMs) are defined as elements with densities higher than that of

water. This classification includes not only metals but also metalloids, such as arsenic, which are known to cause toxicity even at low exposure levels. This understanding is supported by the findings of Angulo-Bejarano et al. (2021), highlighting the potential environmental and health risks associated with these substances. Using vermicompost as soil amendments instead of industrial fertilizers can enhance soil health, boost biodiversity, and improve animal welfare while reducing dependence on non-renewable resources and promoting sustainable practices. Vermicompost is characterized by its finely divided, black, peat-like appearance and is produced through a non-thermophilic process that involves the biodegradation and stabilization of organic materials by earthworms and microorganisms reported by Kiyasudeen et al. (2016). Bioconcentration factors (BCFs) serve as crucial indicators of heavy metal availability to organisms within a substrate. As elucidated by Petroy et al. 2023 and further supported by Xie et al. (2020), higher BCF values correspond to increased chemical uptake, heightening the risk of adverse effects on organisms and subsequent trophic levels. Similarly, the accumulation of metals in earthworms' tissue is quantified using the Bioaccumulation Factor (BAF) reported by Suleiman et al. (2017).

The magnitude and hazardous nature of coal ash (CA), also known as fly ash, render it a formidable challenge as an industrial waste, as discussed by Bhattacharya & Kim (2016). The coal-based power industry contributes significantly to CA or fly ash generation, with approximately eighty percent of fly and bottom ash stemming from this sector. Notably, India, ranking as the world's third-largest energy consumer, heavily relies on coal, with 76% of its energy derived from this source, resulting in the annual production of around 200 million tons (MT) of CA, as highlighted by Spencer et al. (2018). Projections indicate a potential increase in CA production to 270 MT by the period of 2021-2022, according to Ojha et al. (2022). In addition to thermal power plants, numerous medium-sized enterprises in India, such as paper mills, tea processing factories, cement, brick, and steel production units, utilize coal for energy generation. This diverse industrial landscape significantly contributes to the overall volume of CA generated, as emphasized by Mondal et al. (2020a).

The primary objectives of this topic are to identify the selective earthworm species most effective in environments contaminated with heavy metals, focusing on their ability to thrive and influence soil properties. To explore how earthworm activity impacts microbial diversity, population dynamics, and enzymatic activities involved in heavy metal transformation. Examine the biochemical and microbial pathways facilitated by earthworm activities, such as bioaccumulation, biotransformation, and stabilization of

heavy metals. Evaluate the potential of earthworm-mediated microbial processes to reduce heavy metal toxicity, improve soil health, and enhance plant growth in contaminated areas. The identified earthworm species and their interactions with microbial communities approach heavy metal conversion effectively to explore the broader ecological implications for eco-friendly bioremediation, including their effects on soil ecosystem stability. These objectives align with advancing knowledge in eco-biotechnology and providing practical solutions for addressing environmental remediation challenges.

This review focuses on the basics of vermicomposting, explores new techniques in the field, and examines vermicompost production along with the factors that influence its quality and quantity. Previous studies have shown that vermicompost positively impacts plant growth by increasing soil organic carbon, nitrates, phosphates, exchangeable calcium, and other essential nutrients, even though it converts heavy metals. Additionally, vermicompost production presents a viable alternative to chemical fertilizers, reducing heavy metal contamination and providing scope for agricultural drive by offering farmers opportunities for cost savings, income generation, and access to new markets.

Exploring Different Sources of Heavy Metals

The heavy metals are generated from different bio-resources, varying depending on various factors such as the type of resource, geographical location, agricultural practices, and industrial activities in the surrounding area. Different organic wastes that were earlier reviewed, along with some inorganic waste, were used to formulate for sustainable existence.

Organic Matter

Organic materials such as compost, manure, and sewage sludge can contain heavy metals derived from various sources, including agricultural inputs, industrial waste, and urban runoff. The composition of heavy metals in organic matter depends on factors such as the feedstock used, the composting process, and the presence of contaminants in the original materials (Sohail et al. 2021)

Plant Materials

Heavy metals can accumulate in plant tissues through uptake from soil, water, and air. Crops, fruits, vegetables, leaves, and stems may contain varying levels of heavy metals depending on factors such as soil contamination, irrigation water quality, and atmospheric deposition. Certain plant species have a higher affinity for specific heavy metals, reported by Edelstein & Ben-Hur (2018), leading to differential accumulation patterns.

Agricultural Byproducts

By-products of agricultural processes, such as rice husks, sugarcane bagasse, and wheat straw, may also contain heavy metals. These residues can accumulate heavy metals through contact with contaminated soil, water, or air during growth or processing. The composition of heavy metals in agricultural by-products can influence their suitability for various applications, such as animal feed, biofuel production, or soil amendment (Zwolak et al. 2019).

The advancement within the agriculture practice with recent technologies, such as the application of fertilizers, has led the agricultural field into a polluted area with heavy metals. The rate of metals or metalloid concentrations within the agricultural soil is rising rapidly, and it affects plant growth, food safety, and soil microflora. Heavy metals depend on green plants for their biological and geological rehabilitation, reported by Jayakumar et al. (2021).

Animal-Derived Substances

Heavy metals can bioaccumulate in animal tissues through dietary intake of contaminated feed or water. Milk, eggs, meat, and poultry products may contain varying levels of heavy metals depending on factors such as the animal's diet, age, and exposure to environmental contaminants. Monitoring the heavy metal composition in animal-derived substances is essential for ensuring food safety and preventing human exposure to toxic substances Michalak et al. (2022).

Industrial Biowaste

Industrial bio-waste refers to organic waste materials generated by industries such as agriculture, food processing, pharmaceuticals, biotechnology Industries, and bio-based manufacturing. These wastes typically consist of biodegradable organic materials from food processing industries, such as plant residues, animal waste, food scraps, spoiled food, slaughterhouse waste, and other by-products from biological processes. In addition to food-related sources, the pharmaceutical and biotechnology industries also generate organic waste in the form of by-products from fermentation, tissue cultures, and microbial processes. The forestry and paper industry contributes wood residues, pulp, and other lignocellulosic materials, which are rich in organic content. Similarly, brewery and alcohol production units produce spent grains, yeast, and other fermentation residues, all of which are biodegradable and can be utilised in various waste-to-energy or composting processes (Gontard et al. 2018, Chavan et al. 2022). Proper management of industrial bio-waste is crucial to minimize environmental impact and improve sustainability reported by Karić et al. (2022).

Industrial Waste

Industrial waste is generated mainly from different sources of industries, like sludge, waste outlets, chimneys, burners, etc. These are abundant with Pb, Cd, Zn, Co, Ni, Cu, Mn, Cr, Sn, Hg, and other metals with a high concentration of toxic influences (Khalef et al. 2022), as industrial waste fly ash. Heavy metals raise toxicity in the environment and cause hazardous impacts. However, the increase and remodelling of technology can reduce the toxicity of heavy metals and make them available for recycling processes. Interestingly, recent reports have recorded converting heavy metals using a natural technique, vermicomposting, to make it suitable for agricultural purposes (Bhattacharya et al. 2019).

MATERIALS AND METHODS

Methodology for Heavy Metal Analysis

This literature review initially identified 355 papers with the concepts of vermicomposting from different reputed journals, conference databases; however, we have considered 150 recent papers focused on the conversion of heavy metals. The review was further narrowed down to 150 as including the microbial-mediated heavy metal conversion. Heavy metal determination encompasses various analytical techniques, each with its own set of advantages and limitations (Table 1).

However, achieving accurate results requires adherence to standard procedures, with sample preparation, handling, and sampling being among the crucial steps (Baltic Marine Environment Protection Commission, 2018). In the realm of heavy metal analysis, various analytical techniques are available, each offering distinct advantages. Among the most popular approaches the heavy metals (Mn, Zn, Cu, Ni, Cr, As, Pb, Cd, Co, Mo) (Table 1) are detected from various waste residues by X-ray fluorescence spectrometry (XRF) Fořt et al. 2021 inductively coupled plasma mass spectrometry (ICP-MS) (Raclavská et al. 2021) inductively coupled plasma optical emission spectrometry (ICP-OES) Smolka-(Danielowska & Jabłońska 2022), UV/VIS spectroscopy, and atomic absorption spectroscopy (FAAS) (Madjar et al. 2020). Each method presents unique strengths and can effectively measure heavy metal concentrations with accuracy (Guo et al. 2022)

Heavy Metals Analysis of the Ashes

Ash, a by-product of combustion, is primarily generated by power plants burning coal and wood. Despite its diverse potential uses in industries such as cement, concrete, and farming, its utilization in agriculture remains uncommon, as noted by Phan et al. (2019). In the analysis of ash samples, a digestion or solubilize process may be employed depending

on the chosen analytical method to enhance the accessibility of mineral elements. Aqua regia (a mixture of nitric acid and hydrochloric acid in a ratio of 1:3, v/v) is commonly utilized for this purpose, with occasional addition of hydrofluoric acid (HF) to facilitate digestion. Following digestion, the samples can be analysed using various techniques such as inductively coupled plasma mass spectrometry (ICP-MS) (Yao et al. 2020, Praspaliauskas et al. 2020), inductively coupled plasma optical emission spectrometry (ICP-OES) (Smółka-Danielowska & Jabłońska 2022, Praspaliauskas et al. 2020), or flame atomic absorption spectrometry (FAAS) (Madjar et al. 2020). Alternatively, the digestion step can be omitted when utilizing the high definition X-ray fluorescence (HDXRF) detected by Senila et al. (2020) and Kamperidou et al. 2021.

Various solid wastes result from combustion processes, including flue gas, boiler slag, fly ash(coal ash), and bottom ash. Both fly ash and bottom ash have been investigated as soil amendments. Studies by Okmanis et al. (2015) have observed changes in the concentrations of beneficial and harmful mineral elements in the upper layers of soil following the addition of fly ash and bottom ash. The concentration of heavy metals in ashes can be influenced by the temperature at which combustion occurs. High temperatures, particularly in large-capacity furnaces, have the potential to volatilize elements such as mercury, cadmium, and lead, as reported by Ibrahim et al. (2018). However, temperatures exceeding 700°C can lead to the fusion of small particles, resulting in the formation of a coarse ash fraction that may not be suitable for use as soil fertilizer, as noted by Okmanis et al. (2015).

Heavy Metals Analysis of the Vermicompost

Vermicompost, derived from crop residues, animal dung, and other organic materials commonly found on farms, stands as a

vital organic fertilizer in agricultural practices, as emphasized by the Food and Agriculture Organization (FAO) in 2010. Acknowledged as one of the primary inputs of organic matter in agriculture, composting serves various functions in soil management. According to the FAO, composting and vermicomposting contribute to soil improvement by enhancing physical characteristics such as water infiltration and erosion resistance, increasing soil fertility and biodiversity, and sequestering carbon in the soil reported by Sayara et al.(2020). The European Commission underscored these attributes in its communication titled “Towards a Thematic Strategy for Soil Protection” (COM (2002) 179), highlighting the pivotal role of composting in sustainable soil management practices. However, the efficacy of composting in managing heavy metal soil pollution is contingent upon both the type of compost utilized and the characteristics of the soil. Research has revealed that soil organic matter exhibits a negative correlation with the availability of heavy metals and a positive correlation with the total concentration of heavy metals. This finding underscores the complex interplay between composting organic residues (Xu et al. 2022) (Table 2), soil organic matter, and heavy metal dynamics in soil pollution management, as noted by the Federal Ministry of Agriculture, Forestry, Environment and Water Management, Austria, and European Communities in 2003.

In the context of environmental considerations, only the soluble forms of heavy metals present in composts are deemed relevant. The European Commission has underscored the risks associated with relying solely on total heavy metal analysis. However, there is currently no widely accepted methodology for assessing soluble heavy metal content in composts. Consequently, much of the research on heavy metal content in compost has focused on the overall concentration of these pollutants. Various techniques

Table 1: Methods and Heavy Metal Analysis in Different Sample Types.

Sl. No.	Sample types	Methods	Analyzed heavy metal elements	References
1.	Briquettes from paper and cardboard waste	ICP-MS	As, Cd, Hg, Pb, Mn	Raclavská et al. (2021)
2.	Agricultural residues ashes	ICP - MS	Mn, Zn, Cu, Ni, Cr, As, Pb, Cd, Co, Mo	Praspaliauskas et al. (2020)
3.	Agricultural residues, forestry, and agri-food processing plants, energy crops	HDXRF	Cr, Mn, Zn, Cu, As, Pb, Ni, Fe	Kamperidou et al. (2021)
4.	Forestry biomass, energy, and agricultural residues	XRF	Cd, Cr, Cu, Ni, Pb, Zn, Fe	Fořt et al. (2021)
5.	Bottom and fly ashes from municipal solid waste	ICP - MS	Pb, Cd, Zn, Cu, Mn	Yao et al. (2020)
6.	Wood burning generates ashes & wood combustion in home fireplaces	ICP - OES	Cu, Ni, Pb, Zn, Mn, Cd, Ni, Cr	Smółka-Danielowska and Jabłońska (2022)
7.	Wood-based ashes	FAAS (LVS ISO 11047)	Cd, Cr, Cu, Ni, Pb, Zn	Madjar et al. (2020)

have been employed (Table 2) for such analysis, including inductively coupled plasma mass spectrometry (ICP-MS) demonstrated by Samah et al. 2020, inductively coupled plasma optical emission spectrometry (ICP-OES) (Klein et al. 2023, Mawede et al. 2023, Wydro et al. 2021), cold vapour atomic absorption spectroscopy (CV-AAS) demonstrated by Fabricius et al. 2020 for mercury measurement, and flame atomic absorption spectrometry (FAAS) detected by Dias et al. (2023) and Madjar et al. (2020).

Physico-Chemical Analysis of Soil

Soil analysis involves the utilization of various instruments and methodologies to assess different soil parameters. In the study conducted by Gee and Bauder in 1986, soil texture was analysed using a Bouycos hydrometer, while soil temperature, moisture content, and pH were measured using a soil thermometer, portable digital moisture meter, and digital pH meter, respectively, as reported by Van Reeuwijk in 1992. Organic carbon content was determined using the method developed by Walkley and Black in 1934.

Furthermore, available potassium (K), phosphorus (P), and nitrogen (N) were assessed using specific methods. Olsen et al. in 1954 employed the acid digestion method for potassium and phosphorus analysis, while nitrogen content was determined using flame photometry, as per the methodology outlined by Jackson in 1973. These standardized procedures ensure accurate and reliable assessment of soil characteristics, essential for understanding soil fertility and management strategies.

Method of Microbial Inoculation on Heavy Metals Bed

Incorporating beneficial microbes into composting processes

can significantly enhance humification and heavy metal (HM) passivation. Bacteria, characterized by a negatively charged cell wall, play a crucial role in binding positively charged HM ions through electrostatic adsorption, as confirmed by Guo et al. (2022). Moreover, microbes influence the functional groups and composition of organic matter (OM) during humification, indirectly facilitating complexation with HMs, as reported by Song et al. (2021). Furthermore, microbes contribute to the breakdown of lignocellulosic bulking agents into fatty acids, which can bind HMs with varied stability, as outlined by Cui et al. (2020). These microbial activities demonstrate their adaptability to HMs in composting processes, thereby improving overall composting efficiency. This adaptation and enhancement of the composting process were exemplified in studies on chicken manure composting, as verified by Chen et al. (2022a, b, c, d). Such findings underscore the pivotal role of microbes in optimizing composting practices for efficient HM passivation and organic matter decomposition.

The introduction of microbial agents into composting processes has demonstrated significant efficacy in reducing the bioavailability of heavy metals (HMs). In a study by Cao et al. (2022), composting livestock manure with domestic sludge, furfural, straw, and a biochar-straw combination, alongside *Bacillus* strains, led to varying degrees of HM immobilization (including Zn, Cu, Cr, As, Pb, Cd, Ni, and Mn). This reduction in bioavailability, particularly for Cu and Cr, was attributed to enhanced organic matter (OM) degradation and the production of humic substances (HS) capable of binding metal ions. Similarly, Zhang et al. (2018) found that incorporating *Phanerochaete chrysosporium* into sewage sludge composting increased the stabilization of HMs (such as Ni, Cu, Zn, and Pb). This effect was linked

Table 2: Methods used for heavy metal analysis of composts and vermicompost.

Sl. No.	Sample types	Methods	Analyses heavy metal elements	References
1.	Mature compost from the organic fraction of municipal waste	ICP-OES	Ni, Pb, Cd, Cr	Klein et al. (2023)
2.	Composted sewage sludge with the addition of straw and wood chips	FAAS	Cd, Pb, Cu, Zn, Cr, Ni, Mo, Co, Fe, Mn	Radziemska et al. (2022)
3.	Compost from Solid Waste Recycling and Composting Plant	ICP-MS	Cu, Ni, Zn, Pb, Zn, Cd	Samah et al. (2020)
4.	Compost from sewage sludge from a municipal sewage treatment plant	ICP-OES	Cu, Cr, Cd, Ni, Pb	Wydro et al. (2021)
5.	Bottom and fly ashes from municipal solid waste	CV-AAS	Hg	Fabricius et al. (2020)
6.	Compost from organic residues	ICP-OES	Cd, Co, Cr, Cu, Ni, Pb, Zn	Xu et al. (2022)
7.	Compost from fish waste, seaweed, and pine bark, 1:1:3	ICP-OES	Pb, Cd, Cu, Ni, Cr	Mawede et al. (2023)
8.	Compost from fish waste is 80% and pine bark 20%	FAAS	Cr, Ni, Cu, Zn, Pb, Cd	Dias et al. (2023)
9.	Compost originating from organic waste	FAAS	Zn, Pb	Madjar et al. (2020)

to enhanced OM transformation and the promotion of humus formation. These findings underscore the valuable role of microbial interventions in enhancing the efficacy of composting processes for HM remediation.

Inoculation of microbial communities plays a crucial role in regulating the availability of heavy metals (HMs) in composting processes. Tang et al. (2019) demonstrated that *Firmicutes* and *Proteobacteria* were instrumental in controlling the phytoavailability of Pb and Cr, while *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* regulated Zn and Cu levels in municipal sludge composting. The microbial community structure has a significant impact on HM passivation during composting, as highlighted by Guo et al. (2022), who identified dominant microbial phyla such as *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Chytridiomycota* in composting environments. Similarly, Shehata et al. (2021) identified *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* as primary phyla responsible for immobilizing HMs in compost derived from cow and pig dung. Although HMs are not biodegradable, microbial activity can facilitate their transformation into a stable state, thereby reducing their bioavailability and potential toxicity.

Microbial communities exhibit a remarkable capacity to adapt to heavy metals (HMs) through collaborative efforts and synergistic cooperation, often facilitated by quorum sensing mechanisms. Despite high HM concentrations, these microbial consortia can effectively decompose organic substrates such as animal dung. In composting processes, the integration of the fungus *Phanerochaete chrysosporium* into Pb-contaminated agricultural waste (with a C:N ratio of 30:1 and 60% moisture content, over a duration of 42 days) resulted in the conversion of bioavailable Pb to stable forms, as documented by Huang et al. (2017). This study highlights the pivotal role of microbial interactions and fungal contributions in mitigating HM contamination during composting, underscoring the importance of microbial cooperation in environmental bioremediation efforts.

Pinto et al. (2020) studied the effects of composting cow dung and tree litter, with and without the addition of beneficial bacteria, on the solubility of Pb, Cu, and Zn. Their findings revealed that beneficial bacteria reduced the bioavailability of Zn and Cu by binding them to stable humic acid (HA). However, the total concentration of heavy metals (HMs) remained too high for safe agricultural use. Microbial inoculation, especially with effective microbes such as photosynthetic bacteria, *Bacillus* spp., *Lactobacillus* spp., *Actinomyces* spp., and yeast spp., proved to be more effective in reducing heavy metals (HMs) compared to non-inoculated composting. However, inoculation on its own was insufficient

to achieve acceptable levels of HM passivation. To further enhance passivation, vermicomposting or the use of additional composting additives may be required. Incorporating effective microbes at a 0.5% concentration reduced the ecotoxicity of pig manure and significantly lowered the bioavailability of Pb and Cu. This reduction was associated with changes in the microbial community structure.

Zhou et al. (2020a, b) explored the association between HM speciation and different fungal and bacterial species, finding notable inactivation rates for Cu and Pb, with corresponding increases in residual fractions for Cu, Zn, Pb, and Cd. Chen et al. (2019a, b) observed that microbial activity on specific HMs varied depending on the composting feedstock, with certain microbial species contributing to reduced bioavailability. Guo et al. (2022) highlighted the influence of microbial species' relative abundance on HM bioavailability, noting that low relative abundance may not significantly affect HM bioavailability. Microorganisms demonstrate an ability to adapt to HMs during composting, with varying degrees of resistance observed. X. M. Chen et al. (2020a, b, c) reported that Cu>Zn>Cd in terms of hazard to microorganisms, indicating both the potential for remediation and the challenge posed by the proliferation of heavy metal-resistant bacteria.

Wei et al. (2020) investigated the biosorption of heavy metals (Cu, Zn, Pb, Ni, Cr, and Cd) in composting using humic microbial resistant biomass (HMRB) from compost and humin from the maturity phase. While both substances can bind heavy metals, HMRB from composting exhibited a higher metal binding potential than humin. The combination of both HMRB and humin significantly enhanced heavy metal elimination, suggesting that microbial biomass and humin's humification process work synergistically to biosorb heavy metals. This underscores the importance of using a combination of HMRB and humin for effective heavy metal removal in composting processes. The study's findings align with the beneficial effects of microbial tolerance to heavy metal contamination. Bacteria's resistance and adaptation to heavy metals may contribute to reducing their bioavailability for HMRB. Various processes such as biosorption, metal efflux, quorum sensing, biofilm formation, and extracellular polymeric substance (EPS) production could impact the immobilization, sequestration, and precipitation of heavy metals in compost. These mechanisms highlight the complex interplay between microorganisms and heavy metals in composting systems, underscoring the potential for microbial-mediated strategies in heavy metal remediation.

Li et al. (2021a, b) demonstrated that introducing a bacterial consortium, including *Acinetobacter pittii*, *Bacillus subtilis* subsp. *stercoris*, and *Bacillus altitudinis* at 1%

v/w into swine dung composting, enhanced humification and increased the residual fractions of heavy metals (Cr, Cd, and Pb) compared to the control. The humic acid (HA) exhibited a favourable connection with the residual fractions of heavy metals, indicating improved stabilization. Microbial inoculation resulted in the passivation of chromium (Cr) and reduced exchangeable cadmium (Cd), although it did not affect exchangeable lead (Pb). Moreover, bacterial inoculation enhanced the growth of Firmicutes, Proteobacteria, and Actinobacteria, contributing to heavy metal immobilization and stabilization within the composting system.

Qin et al. (2022) investigated hyperthermophilic composting with 5% *Ureibacillus terrenus* in a mixture of dewatered sludge and corn stalk. They found that this approach reduced the bioavailability of heavy metals more effectively compared to the control method. The hyperthermophilic composting raised the residual proportions of copper (Cu) and lead (Pb) by 6.3% and 15.3%, respectively, compared to the control, indicating enhanced stabilization of these metals. This process involved selective biosorption and biomineralization, demonstrating the potential for microbial-mediated strategies in mitigating heavy metal contamination in composting systems. Chen et al. (2019a, b) investigated the influence of physicochemical characteristics and bacterial community on the bioavailability of heavy metals (HMs) in composting using beef, cattle, and chicken dung (with 60% moisture, 20:1 C: N ratio, and 40 days duration). In chicken manure composting, factors such as temperature, organic matter (OM), pH, and moisture were identified as crucial in reducing HM bioavailability. Similarly, in beef manure composting, OM and temperature were found to be significant physicochemical elements affecting HM bioavailability. Throughout the thermophilic phase of chicken manure composting, the bioavailable concentration of cadmium (Cd) decreased, possibly due to temperature sensitivity or enhanced microbial activity at higher temperatures, leading to the release of metabolites. Quorum sensing mechanisms may induce the production of extracellular polymeric substances (EPS), which sequester HMs and reduce their bioavailability. Binding to OM can increase the residual proportion of HMs, while the degradation of OM promotes the production of humic substances (HS) that bind HMs. Additionally, high pH levels can limit the mobility of HMs, leading to precipitation and passivation.

Attributed reduced bioavailable Cd levels to high pH conditions. However, aging a 40-day animal manure compost for 50–365 days did not yield significant benefits in reducing HM bioavailability, as reported by Chen et al. (2019a, b). Shen et al. (2016) suggested that 20 days was the optimal

composting duration for achieving passivation of HMs (such as copper (Cu) and lead (Pb)) during a 50-day composting period. These findings underscore the complex interplay between physicochemical factors, microbial communities, and composting duration in influencing HM bioavailability in composting systems.

Toxicity Conversion in Heavy Metals

Various techniques are enforced for the efficient reduction of toxic levels of heavy metals generated from various sources for sustainable practices. Several processing techniques are reflected in formulating the toxicity of heavy metals. Vermicomposting (Fig. 1 and (Fig. 2)) is one of the bioprocessing methods reviewed and focused on the reduction of toxic content in heavy metals.

Role of Earthworms in Heavy Metal Conversion

In the domain of terrestrial ecology, the term “earthworms” encompasses a diverse array of over 1,800 species of worms classified under the class Oligochaeta within the phylum Annelida. Notably, members of the genus *Lumbricus*, among others, hold particular significance within this classification. These creatures, commonly known as angleworms, play pivotal roles in soil ecosystems and have garnered substantial attention in ecological research due to their ecological significance and widespread distribution. (Ghosal et al. 2024). In ecological literature, earthworms are categorized into three primary eco-physiological groups based on their habitat preferences and behaviour, such as epigeic earthworms, endogenic earthworms, Anecic earthworms, subsurface burrowing earthworms, etc. (Wang et al. 2022). Earthworms serve as integral contributors to terrestrial ecosystem functioning and play a pivotal role in maintaining soil fertility.

In the 1990s, Nepal witnessed the introduction and subsequent widespread adoption of vermicomposting technology. *Eisenia fetida*, *Lumbricus rubellus*, *Perionyx excavatus*, *Lampiti mauritii*, and *Eudrilus eugeniae* emerged as the most commonly utilized earthworm species for this purpose. This transition towards vermicomposting reflected a departure from the conventional support for chemical pesticides and fertilizers for enhancing food production. Not only are these petrochemical-based inputs often unavailable, but they also pose significant environmental and health risks. In contrast, vermicompost emerged as a sustainable alternative, offering an exceptionally nutrient-rich organic fertilizer (Fig. 1). When incorporated into soil, vermicompost enriches it with a high NPK (Nitrogen, Phosphorus, and Potassium) ratio, as demonstrated by research such as Sande et al. (2024). This organic fertilizer not only sustains

agricultural productivity but also promotes soil fertility, thereby contributing to long-term agricultural sustainability and environmental health.

Utilizing earthworms in the vermicomposting process represents an effective method for composting organic waste. Earthworms play a crucial role in breaking down and homogenizing organic matter, thereby indirectly increasing the surface area available for microbial activity. The symbiotic relationship between earthworms and aerobic microorganisms accelerates the biodegradation of organic waste, facilitating the conversion process. Furthermore, the vermicast produced by earthworms contains a rich array of beneficial bacteria that have traversed their digestive tracts, as documented by Kaur (2020).

The accumulation of metals in earthworms is quantified using the Bioaccumulation Factor (BAF), with heavy metal accumulation being influenced by various factors such as earthworm species (Fig.1), developmental stage (adults), overall metal concentration, pH, and organic carbon content, as discussed by Suleiman et al. (2017).

In their seminal work, they provide a comprehensive examination of BCFs for inorganic compounds in earthworms, shedding light on the mechanisms underlying heavy metal uptake in these organisms. Among the key composting earthworm species, *Eisenia fetida*, *Lumbricus mauritii*, and *Perionyx excavatus*, investigations into the concentration of heavy metals in both their tissues and the resulting vermicompost have been reported by Singh et al. (2018). These studies aim to ascertain whether vermicomposting can effectively reduce heavy metal concentrations in the final product. The focal point of such investigations lies in evaluating the concentrations of heavy metals, including lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn), in both earthworms and vermicompost after experiments

reported by Pegu et al. (2024). Utilizing BCFs, researchers, as exemplified by Xie et al. (2020), calculate the amount of metal accumulated by composting earthworms, providing insights into the efficacy of vermicomposting as a means of heavy metal remediation.

Allison et al. (2020) (Table 3) reported that *Pheretima alexandri* earthworms, in association with *Pseudomonas oxalaticus*, aid in depositing oxalate for soil fertility, comprising various components essential for their physiological functions. Notably, they contain 76% protein, with specific amino acids such as glutamic acid, tyrosine, lysine, and hydroxyproline present in significant proportions, which tend to reduce the effects of heavy metals. Raval et al. (2023) reported the conversion of heavy metals from various concentrations of fly ash using *Eisenia fetida*. Ozturk et al. (2020) (Table 3) noted that in a vermicomposting bed, this process influences the morphological and cytological characteristics of *Ricinus communis* L. In another report, Satapathy et al. (2021) achieved conversion of heavy metal content from fly ash of varying concentrations using *Eudrilus eugeniae*, which affects the growth of *Oryza sativa*.

Suleiman et al. (2017) further elucidated that, compared to *Eisenia fetida* and *Eisenia Andrei*, *Dendrobaena veneta* exhibits lower tolerance to the harmful effects of heavy metals in composted sewage sludge. This finding underscores the differential responses of earthworm species to metal contamination. Additionally, despite the inability of earthworms to regulate metal intake, they are known to display dietary selection behaviours, suggesting potential avenues for mitigating metal uptake.

Effects of Vermicomposting on Heavy Metal Dynamics

Vermicompost, an organic amendment abundant in nutrients, is derived from the collaborative efforts of earthworms



Fig. 1: Schematic Representation of Vermicomposting Process and Its Environmental and Agricultural Benefits (Vuković 2021).

and microorganisms in decomposing organic waste. While microorganisms predominantly drive the decomposition process, earthworms play a vital role in vermicomposting. Through the aeration and fragmentation of organic matter, earthworms indirectly enhance microbial biomass and activity. This augmentation in microbial activity results in an expanded surface area accessible to microorganisms, thereby influencing the composition and structure of microbial communities involved in the vermicomposting process, as discussed by Devi & Khwairakpam (2022).

Directly, Wong et al. (2020) and Selvi (2023) (Table 3) explained that vermicompost influences plant growth by producing hormones and enzymes that regulate various physiological processes essential for plant development. Indirectly, it contributes to plant health by mitigating the impacts of pests, nematodes, and plant diseases, thereby reducing yield losses. These dual mechanisms underscore the multifaceted role of vermicompost in promoting soil fertility and supporting sustainable agriculture practices, as elucidated by Oyege & Balaji Bhaskar (2023).

Vermicompost and its by-products offer sustainable and eco-friendly solutions for improving production and managing pests in grain crops such as maize, wheat, barley, rice, and pearl millet. These organic amendments significantly enhance soil quality, increase nutrient availability, and boost crop productivity reported by Edwards & Arancon (2022) (Table 3). Vermicompost, characterized by its nutrient-rich, peat-like composition boasting a low C:N ratio and high porosity, serves as a potent soil amendment with diverse benefits for plant growth and development (Satapathy et al. 2021).

Microbial Influence on Heavy Metals During Vermicomposting

Heavy metals exert detrimental effects on microorganisms by

binding to proteins and disrupting enzymatic function, thereby impairing microbial viability. Notably, the oligodynamic nature of heavy metals confers potent antibacterial activity against *Staphylococcus aureus* even at low concentrations, as reported on *Eisenia fetida* by Eghomwanre et al. (2016) and Chowdhury et al. (2023) (Table 3). This characteristic underscores the significant ecological implications of heavy metal contamination in soil environments. The rod-shaped bacterium *C. metallidurans* thrives in heavy metal-enriched soils, highlighting its adaptation to environments rich in these toxic substances. Furthermore, certain minerals present in soil undergo decomposition over time, *Microscolex dubius* associated with bacteria *R. meliloti* L5-30R, leading to the fixation of nitrogen with increased root nodulation (Suresh & Lakshmi 2022) (Table 3) and the release of hydrogen and hazardous heavy metals into the atmosphere, as documented by Fashola et al. (2016).

The proliferation of bacteria experiences a decline with increasing concentrations of heavy metals, reflecting their toxic impact on microbial growth. Furthermore, research has elucidated the diverse defense mechanisms adopted by different microbial species to counter the toxicity of specific heavy metals and suppress the activity of plant pathogens (Pathma et al. 2019) (Table 4). Consequently, the effects of each heavy metal vary significantly depending on the microbial species under investigation, as documented by Ameen et al. (2021). This discrepancy in lead's impact on microbial growth may be attributed to bacteria's ability to mitigate lead toxicity by depositing it as a phosphate salt using membrane transport pumps and intra- and extracellular binding mechanisms. These findings corroborate the conclusions drawn by Longhi et al. (2022). And other researchers, indicating that while lead may not pose a significant hazard to microbes compared to other heavy metals, its toxic effects on microbial growth should not be overlooked.

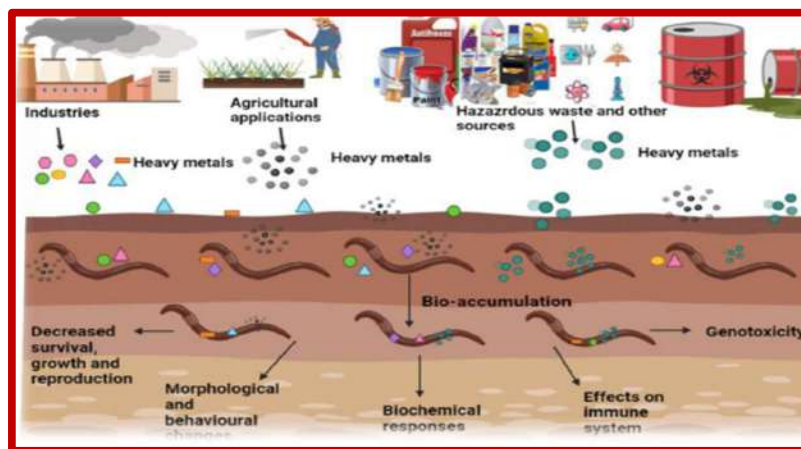


Fig. 2: Emission of heavy metals from various sources and role of earthworms on it (Renu et al. 2020).

Microbial activity plays a pivotal role in the breakdown of organic matter (OM) during composting processes, essential for humification and the overall composting efficacy. Moreover, microbes exhibit the capacity to sorb, transform, or attenuate heavy metals (HMs), thereby reducing their bioavailability and toxicity within composting environments. (Guo et al. (2022) elucidated that the negatively charged bacterial cell wall facilitates electrostatic binding with positively charged metal ions, thereby enhancing metal absorption. This phenomenon, known as biosorption, involves various mechanisms including adsorption, chelation, ion exchange, complexation, precipitation, or bioaccumulation. The interaction between HMs and bacterial cell walls is influenced by both the characteristics of the cell wall and the surrounding environment. Understanding these microbial traits and their interaction with heavy metals is crucial for optimizing composting processes and mitigating heavy metal contamination in composted materials (Aryal 2021).

The phenomenon of biosorption extends to dead microbial cells, allowing for heavy metal (HM) binding irrespective of cellular metabolic activity. In contrast, bioaccumulation involves the intrusion of HMs through the cell wall into the cytoplasm, where they may undergo

localization, precipitation, complexation, or transformation processes. Extracellular polymeric substances (EPS) play a crucial role in enhancing HM biosorption by bacteria, owing to their absorbent properties. EPS, composed mainly of proteins and polysaccharides, features functional groups such as phenolic, phosphoryl, carboxyl, sulfhydryl, and hydroxyl, capable of chelating HMs, as highlighted by Cui et al. (2021).

Additionally, EPS comprises phospholipids, lipoproteins, lipopolysaccharides (LPS), lipids, humic acid (HA), nucleic acids, and uranic acid. LPS, particularly in gram-negative bacteria, serves as a potent chelator, binding HMs and interacting with membrane proteins and functional groups on peptidoglycan. In gram-positive bacteria, LPS, phospholipids, and lipoproteins on the cell wall may exert a similar effect, potentially leading to stronger HM interactions. Microbial enzymes play a vital role in detoxifying and converting HMs, subsequently facilitating their elimination via efflux pumps. Through contact with microbial metabolites and extracellular enzymes, HMs can undergo conversion into less harmful chemicals, as reported by Cui et al. (2021). This comprehensive understanding of microbial-mediated processes in HM interaction underscores their potential for environmental remediation and waste management strategies.

Table 3: Earthworm Species, Associated Bacteria, and Their Beneficial Traits.

Sl. No.	Earthworm Species	Associated Bacteria	Beneficial Traits	References
1.	Pheretimaspp., Pheretima alexandri	Pseudomonas oxalaticus	Oxalate degradation	Allison et al. (2020)
2.	Unspecified	Rhizobium trifolii	Nitrogen fixation and growth of leguminous plants	Edwards and Arancon (2022)
3.	Lumbricus rubellus	R. japonicum, P. putida	Plant growth promotion	Wong et al. (2020)
4.	L. terrestris	Bradyrhizobium japonicum	Improved distribution of nodules on soybean roots	Selvi (2023)
5.	Aporrectodea trapezoids, A. rosea	P. corrugata 214QR	Suppresses Gaeumannomyces graminis in wheat	Pathma et al. (2019)
6.	A.trapezoids, Microscolex dubius	R. meliloti L5-30R	Increased root nodulation and nitrogen fixation in legumes	Suresh et al. (2022)
7.	Eisenia foetida	Bacillus spp., B. megaterium, B. faecalis DSM2570, Staphylococcus, B. pumilus, B. subtilis	Antimicrobial activity against Staphylococcus aureus	Chowdhury et al. (2023)
8.	L. terrestris	Fluorescent pseudomonads	Suppresses Fusarium oxysporum f. sp. asparagi and other pathogens in various crops	Elmer (2016)
9.	Eudrilus sp.	Filamentous actinomycetes, free-living nitrogen-fixers (Azospirillum, Azotobacter, etc.), phosphate solubilizers	Plant growth promotion through nitrification, phosphate solubilization, and disease suppression	Singh et al. (2020)
10.	E. foetida	Proteobacteria, Bacteroidetes, Verrucomicrobia, Actinobacteria, Firmicutes	Anti-fungal activity against Colletotrichum coccodes, R. solani, P. ultimum, P. capsici, F. moliniforme	Ozturk et al. (2020)
11.	Unspecified	Eiseniicola composti YC06271T	Antagonistic activity against F. moniliforme	Selvi et al. (2023)

Table 4: Effects of Heavy Metals on Different Earthworm Species: A Summary of Toxicological Studies.

Sl. No.	Earthworm Species	Heavy metals	Effects	References
1.	<i>Eudrilus eugeniae</i>	Pb and Ni	Ni and Pb have dose-dependent detrimental effects on earthworm survival, growth, and reproduction	Renu et al. (2020)
2.	<i>Eisenia fetida</i>	Pb	No effect on survival, but growth was inhibited	Zaltauskaite et al. (2020)
3.	<i>Eisenia fetida</i>	Cd	No mortality	Liu et al. (2020)
4.	<i>Dendrobaena octaedra</i>	Ni, Pb, and Hg	Significant decline in survival and reproduction	Bindesbol et al. (2009)

Bacterial enzymes, including reductases, play a crucial role in the transformation of heavy metals (HMs) into less harmful forms, either directly or indirectly. Additionally, Singh et al. (2020) examined microbes that possess the capability to bio-mineralize HMs, converting them into stable mineral forms such as Fe-Mn oxides, phosphates, and carbonates. Research conducted by Leśnianańska et al. (2022) revealed that composting processes can elevate zinc (Zn) levels in Fe-Mn oxides. Furthermore, earthworms contribute significantly to HM mitigation by absorbing and converting heavy metals within their gut and intestines, leading to passivation and reduced pollution, as documented by Swati & Hait et al. (2017). Earthworms also play a vital role in enhancing compost humification and microbial activity, thereby facilitating HM passivation. Moreover, earthworms can effectively reduce HM levels in compost through bioaccumulation, thereby minimizing HM contamination.

This review paper highlights the profound impact of heavy metal toxicity on microbial physiology and underscores the need for further research to elucidate the underlying mechanisms governing microbial responses to heavy metal stress.

Eudrilus eugeniae is highly sensitive to heavy metals such as lead (Pb) and nickel (Ni). Renu et al. (2020) reported dose-dependent toxic effects of these metals, resulting in significant reductions in survival, growth, and reproduction. *Eisenia fetida* exhibits a degree of tolerance to Pb contamination, with no observed effects on survival. However, growth is significantly inhibited, indicating sub-lethal toxicity. Zaltauskaite et al. (2020) demonstrated that while this species can withstand Pb exposure, its reduced growth highlights potential ecological risks. For cadmium (Cd) exposure, *Eisenia fetida* shows resilience, with no recorded mortality under tested conditions. Liu et al. (2020) emphasized the species' potential as a bioindicator for Cd contamination, as it tolerates Cd while still reflecting sub-lethal impacts on soil health. *Dendrobaena octaedra* is particularly sensitive to heavy metals such as Ni, Pb, and mercury (Hg). Bindesbol et al. (2009) observed significant declines in survival and reproduction, highlighting the species' vulnerability and its potential use as a bioindicator in assessing soil contamination and ecosystem health.

Transformation of Heavy Metal Contents During Vermicomposting

During the vermicomposting process, two key processes, namely bioaccumulation by earthworms and volume reduction due to organic decomposition, influence heavy metal concentrations. As organic matter undergoes mineralization and breakdown, heavy metal levels may concentrate and escalate; as a result, the weight and volume reduce during vermicomposting. Research conducted by Mekersi et al. (2024) supports this phenomenon, highlighting the potential for heavy metal accumulation during vermicomposting processes (Fig.3). This review paper emphasizes the importance of understanding the dynamics of heavy metal concentrations in vermicompost and the need for effective management strategies to mitigate heavy metal pollution while harnessing the benefits of vermicomposting for organic waste management and soil improvement.

In contrast to conventional composting, vermicomposting introduces a dynamic interaction between microorganisms and earthworms, significantly complicating the dynamics of heavy metal evolution within the system (Fig.3). The inclusion of earthworms inherently elevates the complexity of heavy metal dynamics during vermicomposting. Firstly, the observed increase in heavy metal concentrations within earthworm bodies and reduction of toxicity in the content of heavy metal by increasing nitrogen, phosphorus, and potassium (Satapathy et al. 2021).

Moreover, the interaction between earthworms and microorganisms during mineralization and humification processes also influences heavy metal transformation during vermicomposting. Changes in pH, electro-conductivity, organic matter content, and humic acid levels affect the mobility and speciation of heavy metals. Overall, the accumulation of heavy metals by earthworms and alterations in Physico-chemical characteristics of substrates play pivotal roles in heavy metal transformation during vermicomposting, ultimately reducing their mobility and availability.

Role of Converted Heavy Metal in Vermicompost

Vermicompost, a product of earthworm-mediated decomposition of organic matter, holds immense potential as a soil conditioner. This paper explores the diverse ways

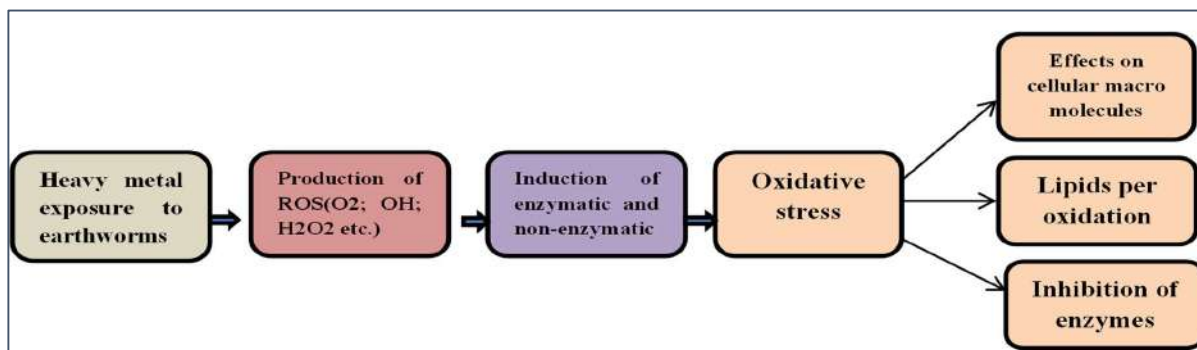


Fig. 3: Biochemical responses of earthworms on heavy metal conversion (Renu 2023).

in which Vermicompost enhances soil texture, aeration, water retention capacity, and overall biological, physical, and chemical qualities. Oyege & Balaji Bhaskar (2023).

Soil texture enhancement: Vermicompost contributes to the improvement of soil texture by fostering soil aggregation and reducing compaction. The organic compounds secreted by earthworms promote the formation of stable soil aggregates and improve soil porosity, facilitating better root penetration and nutrient distribution (Fig. 4).

Aeration improvement: The presence of earthworms in vermicompost enhances soil aeration through their burrowing activity, which creates channels that support gas exchange and microbial activity. Improved soil aeration, in turn, promotes root respiration, nutrient cycling, and overall soil health (Vuković et al. 2021).

Enhanced water retention capacity: Vermicompost increases soil water retention capacity by enhancing soil structure and organic matter content. Humic substances and polysaccharides present in Vermicompost act as natural hydrogels, improving soil moisture retention and

mitigating drought stress (Fig. 4). Vermicompost enriches soil with diverse microbial communities, including beneficial bacteria, fungi, and actinomycetes. These microorganisms play crucial roles in nutrient cycling, disease suppression, and plant growth promotion, thereby enhancing soil fertility and ecosystem resilience. In addition to soil structure improvement, Vermicompost reduces soil erosion, compaction, and surface crusting. These physical enhancements contribute to better soil tilth, facilitating sustainable land management practices and promoting soil health. Khan, et al. (2024).

Chemical properties: Vermicompost enriches soil with essential nutrients such as nitrogen, phosphorous, and potassium, organic matter as organic carbon, and bioactive compounds, enhancing soil fertility and plant nutrition. The presence of humic substances improves nutrient retention and availability, supporting optimal nutrient uptake by plants (Aransiola et al. 2024).

Vermicompost serves as a versatile soil conditioner, offering a multitude of benefits for soil health and agricultural

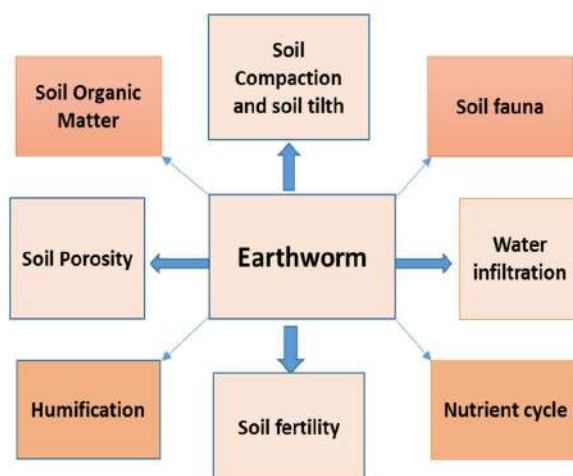


Fig. 4: Impact of earthworms on soil property enhancement.

productivity. By improving soil texture, aeration, water retention capacity, and fostering biological, physical, and chemical qualities, Vermicompost plays a crucial role in sustainable soil management practices. Its widespread adoption can contribute significantly to mitigating environmental degradation and promoting resilient agricultural ecosystems (Oyege & Balaji Bhaskar 2023).

Impact of Earthworms on Soil Properties Containing Heavy Metals

Earthworms are integral to soil ecosystems, playing a crucial role in improving soil structure through their burrowing activities and influencing various soil properties essential for enhancing soil richness and primary production (Hallam & Hodson 2020, Kumar et al. 2020). Their profound impact on soil's chemical and physical qualities, including nutrient cycling, soil aeration, moisture regulation, and overall structure, underscores their significance in ecosystem functioning (Figs. 4 and 5)

The burrowing activities of earthworms are instrumental in forming macro pores in soil, which enhance soil aeration, water infiltration, and root penetration, ultimately fostering nutrient uptake by plants and supporting primary production. Moreover, earthworms play a crucial role in nutrient cycling by consuming organic matter and releasing nutrient-rich casts, thereby facilitating decomposition and nutrient redistribution in soil ecosystems. Their activities also influence soil properties such as organic carbon content, pH levels, and the mobility of heavy metals, which have implications for plant growth and environmental quality. In addition to their role in nutrient cycling and soil structuring, earthworms contribute to trash decomposition, accelerating the breakdown of organic matter and facilitating nutrient release (Vásquez et al. 2023).

Heavy metals pose a significant threat to earthworm populations, with implications for soil fertility and ecosystem functioning. Liu et al. (2020) investigated the effects of cadmium (Cd) on leaf litter breakdown and soil fertility, highlighting the indirect impact of heavy metals on soil qualities through their effects on earthworm activity. The inhibition of leaf litter breakdown and reduction in soil fertility attributed to Cd exposure underscore the importance of understanding the interactions between heavy metals, earthworms, and soil properties. Cd not only affects leaf litter breakdown but also influences the physical and chemical qualities of soil through earthworm activities, as demonstrated by Liu et al. (2020).

Mechanisms of Heavy Metals Passivation in Compost

Improved humification: During composting, heavy metals

interact with humic substances, leading to the formation of organo-metal complexes. These complexes are crucial in immobilizing heavy metals and reducing their bioavailability in composted materials. Studies by De Souza et al. (2019) and Song et al. (2021) have provided insights into the mechanisms of organo-metal complex formation during composting. Microbial-derived fatty acids, produced during composting, play a significant role in binding heavy metals. These fatty acids interact with heavy metals by breaking down lignocellulose molecules, thereby reducing their mobility and toxicity in composted materials. Research by Cui et al. (2020) has elucidated the mechanisms underlying heavy metal binding by microbial-derived fatty acids. Microbial processes are essential for heavy metal passivation in composting, influencing the fate and behaviour of heavy metals in composted materials. Understanding these processes is critical for optimizing composting practices and enhancing heavy metal passivation. Further research is needed to explore the intricate interactions between microbes, organic matter, and heavy metals in composting systems.

Humification influences the physicochemical properties of compost, which subsequently affects the redistribution and speciation of heavy metals. The high pH of compost promotes the passivation of heavy metals (HMs) and their transformation into residual fractions, as HMs become less mobile at higher or alkaline pH levels, according to Vandecasteele et al. (2023), Shen et al. (2016), and Guo et al. (2022). Humic acid (HA) has been shown to have a positive correlation with the passivation of heavy metals (Cu, Cd, Zn, and Pb), as reported by Li et al. (2021a, b) and Pinto et al. (2020). Additionally, various humic substances (HS) have demonstrated a relationship with heavy metal passivation, as noted by Zhang et al. (2018) and Cao et al. (2022). Total phosphorus (TP) is also positively correlated with the passivation of certain heavy metals, including Pb, Zn, and Cd, as reported by Guo et al. (2022) and Xu et al. (2022). Fulvic acid (FA), humus, and organic matter (OM) have significant effects on certain heavy metals, such as Cu, Zn, Cd, and Pb, as reported by Pinto et al. (2020 and Li et al. (2019a, b, c).

Through electrostatic interaction, CEC enhances HM charcoal sorption and influences HM speciation (Jain et al. 2019, Liu et al. 2017). Phosphate enhances the conversion of heavy metals (HMs) into stable compounds, primarily zinc, copper, and lead. Kulikowska & Gusiatin (2019) reported that Fulvic acid (FA), which is abundant in compost feedstock, is transformed into more stable humic acid (HA). The HA contains active functional groups, such as hydroxyl, hydroxyl quinone, carboxyl, and quinone, which enhance

as well as utilize mechanisms like efflux pumps and cell wall interactions to sequester, immobilize, inactivate, complex, chelate, absorb, or precipitate heavy metals.

CONCLUSIONS

In conclusion, the exploration of selective earthworm species in microbial-mediated heavy metal conversion highlights a promising avenue for advancing environmental bioremediation. This review emphasizes that certain earthworm species, due to their unique interactions with soil microbiota, play a pivotal role in enhancing the transformation and detoxification of heavy metals. These earthworms facilitate microbial activity through their feeding habits, burrowing, and the enhancement of soil structure, which in turn improves the efficiency of microbial processes responsible for heavy metal conversion. The implications of these results are significant for bioremediation strategies by harnessing specific earthworm species that positively influence microbial communities involved in heavy metal detoxification. In the future, we can develop more targeted and effective bioremediation approaches, such as for industrial waste, along with organic waste. This could lead to the restoration of contaminated soils and sediments more efficiently, reducing environmental and health risks associated with heavy metal pollution. Future research should focus on identifying and characterizing the specific microbial strains and metabolic pathways involved in these processes, as well as evaluating the long-term stability and efficacy of earthworm-mediated bioremediation, integrating and interacting with microbes in diverse environmental settings. However, this research predominantly focuses on the mechanism of conversion of heavy metals with the activation of earthworm through gut analysis, microbial culture, and later with antimicrobial property and molecular analysis to be carried out from vermicompost. Additionally, integrating these findings with practical applications, such as the design of engineered bioremediation systems, could further enhance the utility of earthworms in sustainable environmental management.

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A Concurrent Review on Plant-Derived Biopesticides and Synthetic Pesticides: Their Importance in Plant Protection and Impacts on Human Health

R. Venkat Ramchandara^{1#}, Abhirama Kumar^{1#}, E. Priyanka¹, Madhu Rani Bharati¹, Laxmi Kant Bhardwaj², Piyush Khandelia¹ and Kumar Pranav Narayan^{1†}

¹Department of Biological Sciences, Birla Institute of Technology and Sciences Pilani, Hyderabad Campus, Hyderabad-500078, India

²Amity Institute of Environmental Toxicology, Safety & Management, Amity University, Noida, Uttar Pradesh, India

†Corresponding author: Kumar Pranav Narayan; pranav@hyderabad.bits-pilani.ac.in

#Authors contributed equally

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ABSTRACT

This review explores the impacts of synthetic pesticides and biopesticides on human health, aiming to provide a comprehensive understanding of their benefits and risks. Currently, farmers are using synthetic pesticides to increase the yield of crop production, but they pose significant health risks, such as acute poisoning, cancer, endocrine disruption, anaphylactic shock, and other severe health issues. On the other hand, biopesticides, derived from natural organisms or plant-derived secondary metabolites, are considered safer alternatives, offering effective pest management with reduced risk to human health. This review draws attention to plant-derived materials in pest control management. Further, deciphering plant diseases with phytopathogenic bacteria and their control by organic bio-pesticides. Conclusively, this review suggests that future research should focus on integrated pest management approaches that combine the strengths of both synthetic and biopesticide applications while mitigating health risks. The findings underscore the imperative for ongoing evaluation of pesticide usage and provide a framework for informed decision-making regarding human exposure to these substances.

INTRODUCTION

The significant effects of synthetic pesticides and biopesticides on human health emphasize the critical need for a nuanced understanding of their respective benefits and risks. The use of synthetic pesticides remains prevalent in modern agriculture. This review aims to explore the potential impact of synthetic pesticides on human health and the surrounding environment. To improve the production yield, humans are increasing the utilization of synthetic pesticides day by day, which leads to generating new diseases in humans after long use. Recent statistics indicate that around 2.5 million tons of chemical pesticides are applied annually worldwide, reflecting their continued reliance on effective pest control and increased crop yields (Massawe et al. 2018). Agriculture serves as the backbone of the Indian economy, accounting for 18% of the total Gross Domestic Product (GDP) and employing approximately 45% of the population. Ensuring food security for over 1.27 billion residents in the face of diminishing arable land presents a significant challenge (Reddy et al. 2021). However, the associated health risks cannot be ignored. For example, exposure to synthetic pesticides has been linked to a range of acute and chronic health issues, including acute poisoning, certain types of cancer, endocrine disruption, and severe allergic reactions such as anaphylactic shock. Prenatal exposure of mother or father to synthetic pesticides exposure of children

and young adults to synthetic chemical pesticides could increase the pesticide risks. Pesticide exposures have been correlated to several human diseases such as Parkinson's disease (PD), amyotrophic lateral sclerosis, Alzheimer's disease, asthma, bronchitis, infertility, congenital anomaly, attention deficit hyperactivity disorder, minimal brain disorder, autism, diabetes, and obesity, respiratory diseases, organ diseases, and system failures, etc. Recent studies have revealed concerning data about the health impacts of synthetic pesticide exposure. Researchers have found that agricultural workers exposed to these chemicals experience a considerably higher incidence of various health issues, such as neurological disorders and different cancer types, particularly non-Hodgkin lymphoma and leukemia. Moreover, the disruption of endocrine function, which can influence reproductive health and developmental processes, has surfaced as a significant public health issue associated with specific pesticides. These findings highlight the necessity of understanding not only the effectiveness of these products but also their broader effects on human health and environmental sustainability. These concerns have spurred heightened scrutiny from both researchers and regulatory authorities, further underscoring the urgency for investigating safer, alternative pest management strategies that could mitigate these severe health risks while sustaining agricultural productivity (Pergner & Lippert 2023). On the other hand, Biopesticides offer a promising alternative to synthetic options derived from natural organisms or plant-based derivatives. Biopesticides are considered to be significantly safer for human health and the environment (Hao et al. 2024). They function through various mechanisms, such as plant defenses, plant growth stimulators, protection from bacterial and fungal infections, including the most destructive pathogens such as *Xanthomonas* species, *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya dadantii*, *Dickeya solani*, *Pectobacterium carotovorum*, etc. Biopesticides such as plant-derived materials, phyto extracts, microbial biopesticides, antagonistic microorganisms, biochemical pesticides, etc., could be useful for the control and management of plant diseases. Furthermore, biopesticides can enhance the sustainability of agricultural practices by promoting soil health and biodiversity, aligning with the principles of integrated pest management (IPM). Comparative analyses in recent studies have begun to elucidate the mechanisms by which synthetic pesticides and biopesticides function. Synthetic pesticides typically target specific physiological pathways in pests, resulting in rapid pest elimination but potentially causing toxicity in non-target organisms, including humans. Conversely, biopesticides generally utilize environmentally sustainable

modes of action, such as competitive exclusion and the induction of pathogen resistance, providing a dual advantage of effective pest control alongside diminished toxicity and selectively killing targeted pathogens and pests by multiple modes of action. This comparison underscores the critical need for systematic assessments of these agents in relation to their efficacy in pest management and their associated health risks. In conclusion, the findings of this review highlight the necessity for ongoing assessment and informed decision-making regarding pesticide application in agriculture. It is crucial to establish frameworks that prioritize the protection of human health while addressing the complexities of pest management. As agricultural methodologies continue to advance, the incorporation of biopesticides into pest management strategies represents a vital opportunity to reduce health risks linked to synthetic pesticide use, ultimately promoting a safer and more sustainable agricultural landscape.

PLANT DISEASES

Bacterial infections caused by various pathogens such as *Xanthomonas* spp., *Pseudomonas* spp., *Ralstonia solanacearum*, etc., pose severe threats to major crops, including vegetables such as tomatoes, potatoes, cabbage, cauliflower, fruits, cereals, and fiber crops such as cotton. These diseases often lead to wilting, rotting, and stunted growth, resulting in substantial economic losses for farmers and the agricultural sector as a whole. The increasing prevalence of bacterial resistance to conventional pesticides further complicates management strategies, prompting researchers and practitioners to seek alternative solutions. Extensive crop losses were caused by phytopathogenic bacteria belonging to more than 25 genera and over 200 species (Sharma et al. 2022). The most important plant pathogenic bacteria genera are *Pseudomonas*, *Ralstonia*, *Agrobacterium*, *Xanthomonas*, *Erwinia* (*Pantoea*), *Xylella*, *Pectobacter*, and *Dickeya* (Buttimer et al. 2017, Mansfield et al. 2012). For example, a few common infections in the cotton plant, such as bacterial blight of cotton, a black arm of cotton, and angular leaf spot of cotton caused by *Xanthomonas citri* pv *malvacearum*, besides the fusarium wilt of cotton, caused by *Fusarium oxysporum* f. sp. *vasinfectum*, which leads to lesser production of cotton worldwide (Cox Jr et al. 2019). A few decades back, in 1996, in Eastern Georgia (USA), the causative agent of internal lint rot of cotton, *Pantoea agglomerans*, a bacterial pathogen, was identified. Bacterial pathogens causing the boll rot of cotton in India were *Pantoea agglomerans*, *Pantoea anthophila*, *Pseudomonas aeruginosa*, and *Xanthomonas citri* pv *malvacearum*. Bacterial wilt or Southern bacterial

blight is a major destructive disease caused by *Ralstonia solanacearum* in tomatoes, potatoes, and Solanaceae plants. *R. solanacearum* is a devastating pathogen with a dramatic

economic impact worldwide (Kumar et al. 2022a). *Ralstonia solanacearum* is the causative agent of bacterial wilt and infects over 200 plant species in 50 families (Xue et al.

Table 1: Various bacterial phytopathogens and diseases caused by them in crop plants.

Bacterial phytopathogen	Disease	Crop plant	Ref.
<i>Xanthomonas campestris</i> pv. <i>campestris</i>	Black rot	Cabbage, cauliflower, brussels sprouts, broccoli, Rape seed (canola), mustard, radish, and turnip.	(Ferby et al. 2024)
<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>	Bacterial leaf spot	Pepper, Tomato	(Ottens & Büttner 2021)
<i>Xanthomonas citri</i> pv. <i>malvacearum</i>	Bacterial blight of cotton, angular leaf spot of cotton, black arm of cotton, boll rot of cotton	Cotton	(Naqvi et al. 2022)
<i>Xanthomonas citri</i> pv. <i>viticola</i>	Grapevine bacterial canker	Grapes	(Ferreira et al. 2019b)
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Bacterial blight of rice	Rice	(Zhong et al. 2024b)
<i>Xanthomonas oryzae</i> pv. <i>Oryzicola</i>	Bacterial leaf streak of rice	Rice	(Yang et al. 2023)
<i>Xanthomonas euvesicatoria</i>	Bacterial spot disease	Pepper, Tomato	(Hernández-Huerta et al. 2021, Potnis et al. 2015)
<i>Xanthomonas hortorum</i> <i>gardneri</i>	Bacterial spot disease	Pepper, Tomato	(Bernal et al. 2021b)
<i>Xanthomonas fragariae</i>	Bacterial angular leaf spot	Strawberry	(Turechek et al. 2023a)
<i>Xanthomonas citri</i> pv. <i>punicae</i>	Bacterial blight of pomegranate	Pomegranate	(Radhika et al. 2021a)
<i>Xanthomonas citri</i> pv. <i>mangiferae indicae</i>	Bacterial black spot	Mango	(Liu et al. 2023a)
<i>Xanthomonas citri</i> pv. <i>fuscans</i>	Bacterial blight of bean	Bean	(de Paiva et al. 2020b)
<i>Xanthomonas campestris</i> pv. <i>raphani</i> (<i>Xanthomonas campestris</i> pv. <i>armoraciae</i>)	Bacterial leaf spot	Radish, turnip	(Fujikawa and Inoue, 2020a)
<i>Xanthomonas arboricola</i> pv. <i>juglandis</i>	Walnut blight	Walnut	(Kim et al. 2021a)
<i>Xanthomonas arboricola</i> pv. <i>pruni</i> ,	Bacterial spot of stone fruits and almonds	apricot, peach, nectarine, plum, and almond	(Garita-Cambronero et al. 2018)
<i>Xanthomonas albilineans</i>	Leaf scald of sugar cane	Sugar cane	(Bini et al. 2023a)
<i>Xanthomonas axonopodis</i> pv. <i>Glycines</i>	Bacterial pustule disease in soybeans	Soybeans	(Li et al. 2022b)
<i>Xanthomonas campestris</i> pv. <i>musacearum</i>	Enset wilt, Banana bacterial wilt	Banana	(Nakato et al. 2019)
<i>Xanthomonas translucens</i> pv. <i>Translucens</i>	Leaf streak of Barley	Barley	(Sapkota et al. 2020a)
<i>Xanthomonas translucens</i> pv. <i>Cerealis</i>	Leaf streak of wheat, barley	Wheat, Barley	(Shah et al. 2019b)
<i>Xanthomonas vasicola</i> pv. <i>Vasculorum</i>	Bacterial leaf streak of corn	Corn	(Ortiz-Castro et al. 2020)
<i>Xanthomonas cassavae</i>	Cassava bacterial necrosis	Cassava	(Zárate-Chaves et al. 2021)
<i>Xanthomonas oryzae</i> pv. <i>leersiae</i>	Streak disease	Rice, gross	(Lang et al. 2019)
<i>Xanthomonas oryzae</i> pv. <i>oryzicola</i>	Streak disease	Rice	(Tall et al. 2022b)
<i>Pseudomonas syringae</i> pv. <i>Syringae</i>	Speck, fleck, spot, blight, and canker diseases	Peach, stone fruit, lemon, kiwi, bean, sweet cherry, apricot, and rose	(Pinheiro et al. 2019)
<i>Pseudomonas syringae</i> pv. <i>actinidiae</i>	Bacterial canker of kiwi fruit	Kiwi fruit	(Vandelle et al. 2021)
<i>Pseudomonas syringae</i> pv. <i>aesculin</i>	Horse chestnut bleeding canker	horse chestnut	(James et al. 2020)
<i>Pseudomonas syringae</i> pv. <i>aptata</i>	Leaf spot disease of beet	Beet	(Nikolić et al. 2023)
<i>Pseudomonas syringae</i> pv. <i>Atrofaciens</i>	Glume rot of wheat	Wheat	(Butsenko et al. 2020)

2020). Bacterial spot of tomato and pepper is a destructive disease caused by four distinct devastating *Xanthomonas* pathovars, *Xanthomonas vesicatoria*, *X. euvesicatoria* pv. *euvesicatoria* (Xee), *X. euvesicatoria* pv. *perforans* (Xep) and *X. hortorum* pv. *gardneri* (Xhg) (Lamichhane et al. 2021). Bacterial canker of tomato is caused by a Gram-positive actinobacterium, *Clavibacter michiganensis*. It causes unilateral wilt, marginal leaf necrosis, stem cankers, bird's eye lesions on fruits, and ultimately, plant death. Causing an economically challenging problem for tomato growers worldwide (Peritore-Galve et al. 2021). Bacterial speck disease of tomato, caused by *Pseudomonas syringae* pv. *tomato*, is another widespread disease causing huge crop losses of tomato (El-Fatah et al. 2023). Black rot is caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Xcc) is the most common and destructive seed-borne bacterial disease of the cabbage family worldwide (Sumi et al. 2022). In cabbage and cauliflower, losses due to black rot disease range from 50-70%. *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) (synonym *Erwinia carotovorum*) is a causative agent of soil-borne soft rot in a broad range of vegetables such as cabbage, tomato, potato, cucumber, and in flowering plants such as *Amorphophallus konjac* and *Zantedeschia hybrida* (Cui et al. 2019). Bacterial leaf spot caused by *Pseudomonas syringae* pv. *maculicola* (Psm) occurs globally in at least 25 plants of crucifers, including cabbage and cauliflower (Peters et al. 2004). The details of bacterial plant diseases and associated bacterial phytopathogens are summarized in Table 1.

PESTICIDES

According to Robert Finger, pesticides are chemical substances or biological agents designed to prevent, destroy, or control pests that harm crops, livestock, and human health. Pests can include insects, weeds, fungi, bacteria, and other organisms that compete with crops or pose health risks. Pesticides work by targeting specific biological processes in these pests, leading to their elimination or suppression (Finger 2024).

Types of pesticides

The pesticides are classified into major categories such as insecticides, herbicides, fungicides, bactericides, and rodenticides. This review article explores the use of bactericides in pest control. Bactericides, which manage bacterial pathogens, are divided into two major categories: synthetic pesticides and Biopesticides, i.e., explained below.

Synthetic Pesticides

Synthetic pesticides are chemically manufactured substances designed to prevent, destroy, or control pests that can harm

crops or human health. Unlike natural pesticides derived from plants or minerals, synthetic pesticides are typically created in laboratories and often exhibit higher potency and specificity in their action. An example of a synthetic pesticide is glyphosate, widely used in agriculture for weed control. It is an herbicide that targets specific enzymes in plants, thus inhibiting their growth without affecting most crops. In agriculture, synthetic pesticides play a critical role in enhancing crop yields and protecting food production systems (Cheng 1990). They have significantly increased agricultural productivity by efficiently controlling a wide range of pests, including insects, weeds, and diseases, which can otherwise devastate crops. The use of these chemicals allows farmers to maintain healthy crops and secure food supply chains, thus contributing to global food security.

Biopesticides

Biopesticides are natural products derived from living organisms, including plants, bacteria, fungi, and other microorganisms, designed to control pests. Biopesticides could mean living organisms (bacteria, Fungi, protozoa, viruses, and algae), their products (biochemicals produced by them), and also plant byproducts. EPA recognizes three categories of Biopesticides. These are microbial pesticides, plant-incorporated protectants, and biochemical pesticides. They are recognized for their effectiveness in pest management and are considered environmentally friendly alternatives to synthetic pesticides. A well-known example of a biopesticide is the bacteria *Bacillus thuringiensis* (Bt), which may infect insects of Lepidoptera, Coleoptera, and Diptera. *Bacillus thuringiensis* (Bt) is a soil-dwelling bacterium that produces toxins lethal to specific insects. Bt is widely used to protect crops such as cotton and corn from pest infestations (Barssoum et al. 2023). These are naturally produced biochemical materials that are non-toxic to the environment and can be employed in pest control. The biopesticides would be more effective when formulated in the nanosize range for all the plant cells (Kumar et al. 2024b, Maurya et al. 2022a, Maurya et al. 2022b, Sharma et al. 2020a, Srivastava et al. 2021b).

Nano-synthetic Pesticides

Nano-pesticides refer to pesticides that contain chemicals in nanoscale materials or nanoparticles. Those materials have specific characteristics, such as size and shape, and are designed with ideal and unique physical, chemical, and biological properties. Nano-pesticides are pesticides formulated in nanomaterials fixed on a hybrid substrate, encapsulated in a matrix, or functionalized nanocarriers. Nano-agrochemicals (NACs) are nanomaterials and formulations specifically designed and controlled at the nanoscale (Chaud et al. 2021). The U.S. Environmental

Protection Agency (EPA) proposed that the size of nano-pesticides should be in the size range of 1 to 100 nm. Some authors suggested that the particle size of nano-pesticides should be less than 500 or 1000 nm (Shangguan et al. 2024). The European Commission defined nano-pesticides as a natural, incidental, or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate, and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm-100 nm. Nano-pesticides, as nanoparticles, are now produced and used as novel carriers for the delivery of pesticides. Different types of formulation suggested, including nano-emulsions, nano-encapsulations, nano-vesicles, nano-fibers, etc., are used to improve the efficacy of existing pesticides. Nano pesticides may be either nano-based synthetic pesticides or Nano- nano-nanobiopesticides (Abdollahdokht et al. 2022).

Nano-biopesticides

Nano-biopesticides derived from plant materials represent a promising advancement in pest control, offering an environmentally friendly alternative to traditional chemical

pesticides. Additionally, it could be analyzed by HPLC, LC-MS, and different types of analytical methods. (Kumar et al. 2024a, Maurya et al. 2021, Rana et al. 2024). These biopesticides utilize nanoparticles to improve the efficacy and delivery of active compounds while reducing the environmental impact associated with chemical pesticides (Fig. 1). They could be used as a plant growth stimulator for plants and therapeutics management for cancer or metabolic diseases (Kumar et al. 2022b). The incorporation of nanotechnology allows for enhanced targeting and controlled release of biocontrol agents, contributing to the sustainability of agricultural practices. Research indicates that various plant species can yield nanoparticles, further supporting the development of effective pest management solutions. Overall, the potential of plant-derived nano-biopesticides is significant for sustainable agriculture (Krishnamurthy et al. 2020). Nano-based biopesticide formulations such as liposomes, micelles, nano-emulsion, nanoparticle, mesoporous, etc., could deliver active lead compounds inside the plant cells easily compared to other conventional formulations (Kumar et al. 2025, Sharma et al. 2020b, Srivastava et al. 2021a).

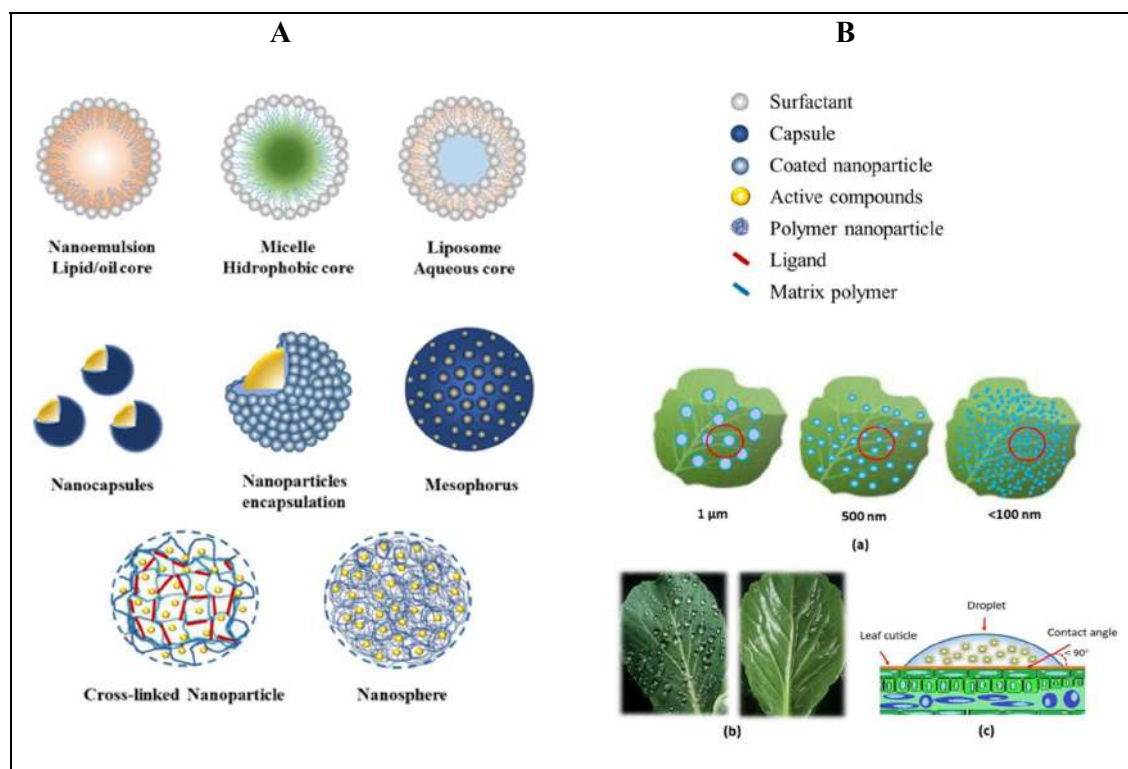


Fig. 1: (A) Various types of nano-delivery biopesticide formulations could be prepared for the protection of plant diseases (B) An illustration depicting the distribution of droplets containing a nano-delivery system based on droplet size and the wettability of the leaf surface (a), a comparative image showing nanomicelle droplets with low wettability (left) and high wettability (right) on the leaf surface (b), and a representation of the reduction in surface tension in droplets containing a nano-sized suspension on the leaf surface (< 90° C). CC by 4.0 (Melanie et al. 2022, Sharma et al. 2020a).

PROTECTION OF PLANT DISEASES

Protection of plants from bacterial infections is an essential aspect of modern agriculture, as these diseases can significantly affect crop yield and food security. Efforts to manage bacterial plant diseases have evolved, focusing on integrated approaches that combine chemical, biological, and cultural practices to enhance plant health and minimize disease incidence (Maciag et al. 2023).

Biological Control Strategies

Biological control is a promising alternative to synthetic pesticides for managing bacterial diseases. Utilizing naturally occurring microorganisms and plant-derived biomaterials as biocontrol agents has gained traction in recent years. Beneficial bacteria, such as *Bacillus subtilis* and *Pseudomonas fluorescens*, are known for their ability to inhibit plant pathogens through competition, secretion of antimicrobial compounds, and induction of plant defenses (Morikawa 2006). These biocontrol agents enhance crop resilience and reduce disease incidence, providing an eco-friendly approach to pest management. Plant growth-promoting rhizobacteria (PGPR) are particularly effective in promoting plant health. PGPR can enhance nutrient uptake, stimulate root development, and induce systemic resistance in plants, thereby improving their capacity to combat bacterial infections. Research shows that the application of PGPR not only suppresses pathogen growth but also promotes overall plant vitality and yield (Bonaterra et al. 2022b).

Integrated Disease Management

An integrated approach to managing bacterial diseases involves combining biological control with other practices such as crop rotation, the use of resistant crop varieties, and cultural practices that optimize plant health. Crop rotation helps to disrupt the life cycles of pathogens and reduces their population in the soil. Additionally, the selection of resistant plant varieties is a crucial component of a comprehensive disease management strategy, offering a sustainable solution to bacterial infections (Sundin et al. 2016).

Synthetic Pesticides Approach

The protection of plants from bacterial infections using synthetic pesticides has been a common practice in agriculture. Synthetic pesticides are effective at controlling a range of bacterial pathogens, such as *Ralstonia solanacearum*, which causes wilt disease in crops like tomatoes. However, the reliance on these chemicals raises concerns regarding environmental impact and the development of resistance in pathogens, leading to decreased efficacy over time (Ayilara et al. 2023).

Biopesticides Pest Control Approach

The protection of plants from bacterial infections using biopesticides is an effective and environmentally friendly approach in agricultural practices (Higgins et al. 2024). Biopesticides, which include beneficial microorganisms and natural substances, enhance plant health and suppress pathogens without the harmful effects associated with synthetic chemicals. One notable example is the use of *Bacillus subtilis*, a beneficial bacterium that acts as a biocontrol agent against various bacterial diseases (Habazar et al. 2018, Higgins et al. 2024). A details Summary of plant diseases and causative bacterial pathogens, and pest control management are reported in Table 2.

Microbial Biopesticides

Microbial pesticides, Plant-incorporated protectants, and Biochemical pesticides. Biological control is an effective strategy and sustainable alternative method or supplement to conventional chemical pesticides for microbial plant disease management. Several research studies show the role of beneficial bacteria in promoting plant growth, disease resistance, and disease control in crops. Bacteria strains belonging to *Bacillus*, *Paenibacillus*, *Agrobacterium*, *Bradyrhizobium*, *Acinetobacter*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Rhizobium*, and *Streptomyces* have been reported as biocontrol agents for the control and management of various diseases in major crops (Ayaz et al. 2021, Massawe et al. 2018). Some of the most intensively studied are bacteria belonging to the genus *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* spp., which have already been registered as commercial products and marketed. Nowadays, in the EU, there are 13 bacterial-based biocontrol agents (BCA) registered as biopesticides for the control of bacterial and fungal diseases (*Bacillus amyloliquefaciens* strains: QST 713, AH2, MBI 600, FZB24, and IT 45, *Bacillus amyloliquefaciens* subsp. *plantarum* strain D747, *Bacillus firmus* I-1582, *Bacillus pumilus* strain QST 2808, *Bacillus subtilis* strain IAB/BS03, *Pseudomonas* sp. strain DSMZ 13134, *Pseudomonas chlororaphis* strain MA 342, *Streptomyces* K61, and *Streptomyces lydicus* strain WYEC 108) (Bonaterra et al. 2022a). *Bacillus* species such as *Bacillus amyloliquefaciens*, *Bacillus subtilis*, and *Bacillus pumilus* isolates could inhibit the growth of Xcc to varying degrees. *B. subtilis*, *B. pumilus*, *Bacillus megaterium*, *Bacillus cereus*, *Bacillus velezensis*, *Paenibacillus*, and *Bacillus thuringiensis* also showed significant antibacterial activity against the Xcc. Strains identified as *Bacillus velezensis* X5-2, *Bacillus megaterium* X6-3, and *Pseudomonas orientalis* X2-1P were effective in vitro and in vivo when applied as a whole-cell suspension form and also

as a cell-free supernatant form against Xcc. The greenhouse *in vivo* tests on winter oilseed rape plants with three selected biocontrol strains lead to a disease reduction of 82.37% and 72.47% in preventive and curative treatments, respectively. Twenty-four isolates of *Paenibacillus* spp., obtained from New Zealand-grown brassica hosts or soil, were evaluated for *in vitro* antagonism and biocontrol against six Xcc isolates. Seven *Paenibacillus* spp. Isolates with different levels of *in vitro* suppressive activity against Xcc were screened in pot experiments for their ability to reduce black rot disease on cabbage. Two *Paenibacillus* isolates (P10 and P16) exhibited effective biocontrol activity against Xcc. *B. subtilis* R14, *B. megaterium* pv. *cerealis* RAB7, *B. pumilus* C116, and *B. cereus* C210 showed antibiotic activity *in vitro* against the bacterium *X. campestris* pv. *campestris*, the causative agent of black rot in crucifers (Luna et al. 2002). In their study, metabolic products released by *B. subtilis* R14 produced the highest inhibition zone against Xcc in agar diffusion assay. Fifty-four isolates from the rhizosphere soil of *Brassica campestris* were screened against Xcc. Two isolates, *Pseudomonas aeruginosa*, and *Bacillus thuringiensis*, showed higher inhibition against Xcc in the *in vitro* assay. The combined use of them produced the highest inhibition zones against Xcc. In the greenhouse study, both isolates were effective in reducing black rot lesions compared to the untreated control involving either a foliar spray or the combined seed soak and soil drench. However, the combined strains were significantly more effective when the mode of application was combined seed and soil drench (Mishra & Arora 2012). Seventeen strains of pathogenic Xcc were tested for sensitivity to 31 bacteriophages isolated from several black rot-infected farmers' fields in India. Bhoyar. It was found that some lytic phages, especially phage Xcc9SH3, possess superior ability as biocontrol agents. Xcc9SH3 isolated from a soil sample from Lucknow was found to lyse all 17 tested strains of Xcc *in vitro*. (Bhoyar et al. 2017).

Management of Bacterial Plant Diseases Through Phyto Extracts

Copper compounds such as copper sulfate, Copper Oxychloride, Bordeaux mixture (Copper sulfate plus Calcium hydroxide), lime sulfur, and antibiotics, formulations with a combination of antibiotics such as streptomycin (Streptomycin sulfate plus tetracycline chloride) are the only antibacterial choices to control phytopathogenic bacteria that are readily available in a large part of the world. Several researchers tried to identify crude phyto extracts and extract fractions as Biocontrol agents by *in vitro* laboratory experiments and field pot experiments. But so far, no effective crude phyto extracts or phyto components are

available in the market to control phytopathogenic bacteria on par with antibiotics and copper compounds. Fontana et al. (2023) showed that methanol and hydroalcoholic extracts of leaves of *Moringa olifera* and hydroalcoholic extract with maltodextrin are very effective against *Xanthomonas campestris* pv. *campestris* (Xcc) and reduced biofilm formation (Fontana et al. 2021). They showed that the MIC of methanolic, hydroalcoholic, and hydroalcoholic maltodextrin extracts of leaves of *Moringa olifera* are 0.5mg.mL⁻¹ and 0.1 mg.mL⁻¹, respectively. Methanol extract, hydro-alcoholic extract, and hydro-alcoholic extract with malto dextrin reduced biofilm formation by 62%, 77%, and 73%, respectively. They also found that treatment with *Moringa oleifera* extracts on Xcc-infected cabbage leaves *in vivo* cleared and unblocked of xylem by scanning electron microscopy. On the other hand, Fontana et al. (2021) found that Xcc was more susceptible to petal extracts of *Tagetes erecta* and *Chrysanthemum Coronarium* by the disc diffusion method (Fontana et al. 2023). They produced 24.0 mm and 23.50 mm growth in the inhibition zone, respectively, against Xcc. They also reported that *Acacia farnesiana* (11.5mm), *Anthocephalus cadamba* (10.5), *Bombax malabaricum* (11.0mm), *Lathyrus odoratus* (10.5mm), *Rosa damascena* (13.5mm), *Thevetia nerifolia* (11.5 mm) were also effective against *Xanthomonas campestris* pv. *Campestris*. Their study showed that Xcc is most sensitive to the petal extracts of *Tagetes erecta* and *Chrysanthemum coronarium*.

IMPACT OF CHEMICAL PESTICIDES ON THE INDIAN AGRICULTURAL SYSTEM

In response to growing concerns, the Government of India has completely banned the use of Streptomycin and Tetracycline in agriculture, effective from January 1, 2024. This shift highlights the urgent need for alternative microbial biopesticides and antibacterial botanicals to control and manage bacterial diseases in crop plants. Each pesticide possesses unique properties and toxic effects. The indiscriminate use of synthetic pesticides over time has led to various hazards and toxicity. Pesticide residue refers to any specific substance present in food, agricultural commodities, or other products and animal feed resulting from the use of pesticides. This term also includes any derivatives of pesticides, such as conversion products, metabolites, and reaction products that are toxic. Pesticide residues can significantly contaminate the environment, including soil, water, air, and food, posing a threat to both plant and animal life. The intergovernmental standards-setting organization for food standards - the Codex Alimentarius Commission - establishes maximum residue limits (MRLs) for pesticides in food based on acceptable daily intakes (ADIs). Pesticide

Table 2: Summary of plant diseases and causative bacterial pathogens, and pest control management.

Plant species	Disease	Symptoms	Bacterial pathogen	Pest control management	Ref.
Rice	Bacterial blight of rice	Yellowish stripes on leaf blades.	<i>Xanthomonas oryzae</i> pv. <i>Oryzae</i>	Bordeaux (copper sulfate: lime: water), Streptomycin copper oxychloride	(Zhong et al. 2024a) Sabri et al. 2023
Cotton	Bacterial blight of cotton	Angular leaf spots, Black arm, boll rot.	<i>Xanthomonas citri</i> pv. <i>malvacearum</i>	Copper oxychloride 0.25 % + Streptomycin 100 ppm Copper oxychloride 0.25 % + Agrimycin 100 ppm	(Wheeler et al. 2021)
Cabbage, Cauliflower	Block rot of crucifers	Yellow V-shaped lesions appear along the leaf margins, and then the entire leaf may yellow, wilt, and fall off. Leaf veins in affected areas turn from green to dark brown to black.	<i>Xanthomonas campestris</i> pv. <i>campestris</i>	0.1% Streptocycline Copper Sulphate Lime Sulfur Copper sulphate + Streptocycline	(Greer et al. 2023)
Sugar cane	Leaf scald of sugarcane	White to yellow spots on leaves. A diffuse yellow border of varying widths runs parallel to the pencil line streak.	<i>Xanthomonas albilineans</i>	Hot water treatment of planting material streptomycin + tetracycline (60 g/ha/500 l water)	(Bini et al. 2023b) Govindaraju et al. 2020
Apricot, Peach, Nectarine, Plum Almond	Bacterial spot of stone fruits and almonds	Small, water-soaked, grayish areas on leaves. Later, the spots become angular and purple, black, or brown in color.	<i>Xanthomonas arboricola</i> pv. <i>pruni</i> ,	Copper hydroxide The mixture of copper hydroxide and mancozeb	(Garita-Cambronero et al. 2018)
Walnut	walnut blight	Reddish-brown spots with a yellow halo, irregular lesions on the leaf blade, Small black cankers on twigs, black lesions on shoots, and cankers on the stem.	<i>Xanthomonas arboricola</i> pv. <i>juglandis</i>	Copper-based sprays	(Kim et al. 2021b) Jenkins 2010
soybeans	Bacterial pustule disease in soybeans	Necrotic lesions surrounded by chlorotic haloes on the leaf surfaces of fruits.	<i>Xanthomonas axonopodis</i> pv. <i>glycines</i>	, copper talc, and copper + sulfur dust. Streptocycline plus copper salts	(Li et al. 2022a) Patidar 2023
Barley	Leaf streak of Barley	Light brown lesions, several centimeters long.	<i>Xanthomonas translucens</i> pv. <i>translucens</i>	Cupric acetate (0.5%)	(Sapkota et al. 2020b)
Wheat, Barley	Leaf streak of wheat, barley	Light brown lesions, several centimeters long.	<i>Xanthomonas translucens</i> pv. <i>cerealis</i>	Cupric acetate (0.5%)	(Shah et al. 2019a)
Corn	Bacterial leaf streak of corn	Lesions can be brown, orange, and/or yellow and are often yellow when backlit. Lesions usually have slightly wavy edges.	<i>Xanthomonas vasicola</i> pv. <i>vasculorum</i>	Copper combinations	(Ortiz-Castro et al. 2020) Pietrobon et al. 2021
Cassava	Cassava bacterial necrosis	Angular, water-soaked spots that turn brown and may have a chlorotic blackening of the tips of the stem ring.	<i>Xanthomonas cassavae</i>	Sanitary control, cultural practices Crop rotation	(Zarate-Chaves et al. 2021)
Cassava	Bacterial Blight of cassava	brown to dark-brown water-soaked translucent angular spots on the leaf tissue, browning at later stages, occasionally surrounded by a chlorotic halo. Diseased shoots die, leaving bare leafless stems pointing upwards.	<i>Xanthomonas phaseoli</i> pv. <i>manihotis</i>	Sanitary control, cultural practices Crop rotation	(Zarate-Chaves et al. 2021)
Gross, rice	Streak disease	The streaks are initially dark green but turn translucent, yellowish-orange, and finally brown.	<i>Xanthomonas oryzae</i> pv. <i>leersiae</i>	Copper-based combinations	(Tall et al. 2022a)

Table Cont....

Plant species	Disease	Symptoms	Bacterial pathogen	Pest control management	Ref.
Banana	Enset wilt, Banana bacterial wilt	A yellowish ooze appears on the leaves. Leaves wilt and turn yellow.	<i>Xanthomonas campestris pv. musacearum</i>	Copper-based combinations, Streptomycin	(Nakato et al. 2018)
Radish Turnip	Bacterial leaf spot	Small greasy spots are observed that gradually become necrotic, then dry.	<i>Xanthomonas campestris pv. raphani</i> (<i>Xanthomonas campestris pv. armoraciae</i>)	Copper-based combinations	(Fujikawa and Inoue, 2020b)
Bean	Bacterial blight of bean	Water-soaked spots that may expand and turn brown, surrounded by a yellow halo. Sunken, tan lesions with reddish-brown margins on the stem and pods.	<i>Xanthomonas citri</i> pv. <i>fuscans</i>	Copper sulfate, copper hydroxide, and potassium methyl di thio carbamate can be used to control foliage infection effectively.	(de Paiva et al. 2020a)
Mango	Bacterial black spot	Angular black spots, chlorotic spots on leaves, necrotic patches on leaves, and black irregular or oblong lesions on fruits.	<i>Xanthomonas citri</i> pv. <i>mangiferae indicae</i>	Copper combinations, Streptomycin Tetracycline combination.	(Liu et al. 2023b) Sossah et al. 2024
Pomegranate	Bacterial blight of pomegranate	Dark brown and oily lesions leading to premature defoliation. Water-soaked necrotic spots on stems, branches, and fruits.	<i>Xanthomonas citri</i> pv. <i>punicae</i>	Bordeaux mixture, Copper oxy chloride, Copper combinations Streptomycin, Streptomycin + Tetracycline,	(Radhika et al. 2021b) Ambadkar et al. 2015
Pepper, Tomato	Bacterial leaf spot	Circular water-soaked lesions 3mm surrounded by a yellow halo, pale green spots on fruits later become dark brown.	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>	Spraying copper-based compounds, streptomycin sulfate, and Kasugamycin	(Otten and Buttner, 2021) Pontis et al., 2015
Strawberry	Bacterial angular leaf spot	Small, translucent spots turn reddish brown.	<i>Xanthomonas fragariae</i>	copper-containing bactericides, streptomycin, and Kasugamycin	(Turechek et al. 2023b) Kim et al. 2016
Pepper and Tomato	Bacterial spot	Irregular dark brown greasy lesions turn necrotic on leaves, pale green spots on tomatoes, and scab-like necrotizing lesions on pepper fruits.	<i>Xanthomonas hortorum gardneri</i>	Copper oxychloride, Copper hydroxide, Bordeaux mixture	(Bernal et al. 2021a)
Pepper, Tomato	Bacterial spot disease	dark brown to black with a wet to greasy appearance, fruit lesions.	<i>Xanthomonas Euvesicatoria</i> (<i>X. perforans</i>)	Spraying copper-based compounds, streptomycin sulfate, and Kasugamycin	(Hernandez-Huerta et al. 2021) Pomis et al. 2015
Grapes	Grapevine bacterial canker	Angular reddish-brown spots surrounded by a yellow halo. Leaves become necrotic and die. Stunted shoots. Flowers become black and die. Wilting of fruits.	<i>Xanthomonas citri</i> pv. <i>viticola</i>	copper sprays after pruning and budding, disinfection of pruning tools using a 2% active sodium hypochlorite or a 0.1 % quaternary ammonia solution	(Ferreira et al. 2019a)
Citrus	Citrus canker	Raised, tan to brown lesions on leaves, stems, and fruit.	<i>Xanthomonas citri</i> pv. <i>citri</i>	Agrimicina containing 15% streptomycin and 1.5% Tetracycline, Streptomycin plus copper sulfate	(Shahbaz et al. 2022)
Sorghum	Bacterial Leaf Blight of Sorghum	Yellow or rust color streaks on leaves.	<i>Enterobacter asburiae</i>	Recently, I detected a disease. Control measures have not yet been established.	(Chen et al. 2023)

residues have been found in groundwater-surface water, and potable water samples in India (Behera et al. 2024). Centre for Science and the Environment (CSE), New Delhi, carried analysis of pesticide residues in soft drinks in 2006 and published their findings.

They examined 57 soft drink samples of 11 brands (7 brands of PepsiCo and 4 brands of Coca-Cola) for 15 organochlorine pesticides and 13 organophosphorus pesticides. The range of concentration of total pesticides (organochlorines and organophosphorus) was 2.65 ppb to 31.55 ppb in all 57 samples. The average concentration of total pesticides in all 57 samples was 11.85, which is 24 times the BIS limit for total pesticides in soft drinks. Prolonged reliance on chemical-based pesticides has caused numerous adverse effects, such as soil and water contamination, pesticide residues on crop products, the development of insecticide resistance, and the biomagnification of insecticides in living organisms (Mandakini and Manamgoda, 2021). The extensive application of pesticides over the years has released substantial amounts of chemicals into the atmosphere, contributing to global climate change. Numerous undesirable environmental impacts have been reported in various countries, including contamination of soil, surface water, groundwater, and air, as well as pollution-related diseases, excessive mortality, reproductive toxicity, changes in species abundance, and a loss of ecosystem diversity. Additionally, pesticides have led to the destruction of beneficial insects, the depletion of natural resources' productive potential, and the development of pesticide resistance in both target and non-target species (Kumar et al. 2021).

LIMITATIONS AND CHALLENGES FOR BIOPESTICIDES

Regardless of their great capability, biopesticides are yet to become fundamental tools in agriculture for managing pests and pathogens. It isn't easy to establish biopesticides that are more potent than chemical pesticides and have broad-spectrum activity, long shelf life, and high environmental tolerance. Fundamental issues in biocontrol research have impeded India's efforts to produce high-quality biopesticide products. The short shelf life of biopesticides is the key concern, influencing both the efficiency of biopesticides against target pests and their competitiveness with chemical alternatives (Samada & Tambunan 2020). The extensive adoption of biopesticides in India faces several complications, including challenges in efficacy, shelf life, production techniques, and performance in the field. Often, biopesticides have a narrow range of target pests or pathogens, and issues with delivery systems, high production costs, and regulatory setbacks further hinder their use. Intense regulatory approvals, which

biopesticides must endure just like synthetic pesticides, are tedious and costly. Moreover, varying regulations across countries can hamper the development and distribution of biopesticides and restrict their market presence (Assadpour et al. 2024). Higher production costs than synthetic pesticides also deter farmers, who may find chemicals more economical. Presently, biopesticides make up only 1-5% of the global pesticide market, highlighting the need for greater awareness and support for their use. Despite these hurdles, biopesticides hold significant potential to support sustainable agriculture. Continued research and innovation, such as nanoencapsulation technology to improve efficacy and stability, as well as streamlined regulatory frameworks, could significantly boost their viability and acceptance within the agricultural sector (Moosavi & Minassian 2021).

LIMITATIONS AND CHALLENGES OF CHEMICAL PESTICIDES

Chemical pesticides have broad-spectrum activity, long shelf life, high environmental tolerance, higher effectivity, and performance, etc., against target pests and pathogens but are responsible for several hazards like pesticide residues in food and water, biomagnification or bioaccumulation, phytotoxicity towards crop plants, development of pesticide or antibiotic resistance in microorganisms and subsequent transfer of antibiotic or pesticide resistance to clinical pathogens, etc. Phytopathogenic bacterial strains have developed copper resistance (Cu) based pesticides (Lamichhane et al. 2018) and antibiotics such as streptomycin (Batuman et al. 2024). However, antibiotics' use in agriculture is less compared to human and animal healthcare, but their increased use in agriculture has contributed to the creation of new resistant strains of bacterial pathogens (Islam et al. 2024) and the spread of antibiotic resistance from phytopathogenic bacteria to human pathogenic bacteria. Spontaneous development of streptomycin resistance was identified in *Clavibacter michiganensis*. Streptomycin-resistant strain- *Clavibacter michiganensis* BT-0505R was produced in the laboratory from the streptomycin-sensitive strain *Clavibacter michiganensis* BT-0505 on exposure to streptomycin (Lyu et al. 2019). Detailed advantages and disadvantages of synthetic and biopesticides for the management of plant diseases and protection are summarized in Table 3.

SYNTHETIC PESTICIDES AND THEIR IMPACT ON HUMAN HEALTH

Synthetic pesticides are chemical factors used to prevent or control pests, including insects, rodents, fungi, weeds, and other unwanted organisms. Instead of their advantages

Table 3: Summary of the advantages and disadvantages of synthetic and Biopesticides for the management of plant diseases and protection.

Commercial Aspect and Human Health	Synthetic pesticides		Biopesticides		Ref.
	Potential Advantage	Limitations	Potential Advantage	Limitations	
Effective Control	Often provide rapid and extensive control of a wide range of pests and diseases, leading to increased crop yields.	Develop resistance to synthetic pesticides, rendering these chemicals less effective.	Generally, more environmentally friendly and biodegradable, reducing soil and water contamination risks.	Biopesticides may exhibit variable effectiveness depending on environmental conditions such as temperature, humidity, and soil type.	(Mawcha et al. 2024, Siddiqui et al. 2023)
Cost-Effective	Economies of scale make synthetic options generally cheaper for farmers, especially for large-scale use.	The price of synthetic pesticides can be significant, especially for high-quality products required for effective pest management.	Typically presents a lower risk to human health and non-target organisms compared to synthetic options.	The price of biopesticides can be higher than synthetic pest control.	(Felsot & Racke 2006)
Availability	Synthetic pesticides are easily available	It is only sold by authorized vendors.	Biopesticides are quite expensive compared to synthetic pesticides.	It isn't easy to achieve viability in all places.	–
Resistance Management	Synthetic pesticides act quickly and are highly effective against a broad range of pests.	Prolonged usage has led to resistance to several pests, directing the development of new chemical classes and application strategies.	Pests develop less resistance to biopesticides than to synthetic pesticides.	Only a few may be applicable to resistance management.	(Daraban et al. 2023, Fenibo et al. 2022)
Health Risks	Diverse doses and long-term exposure cause health risks. Management of doses can decrease the health risk.	High doses can lead to immediate health effects such as respiratory distress, carcinogenicity, and neurological disorders.	Biopesticides have fewer side effects and do not cause health hazards.	Biopesticides can disrupt hormones, which can lead to several health issues, but they are comparatively less harmful than synthetic pesticides.	(Haroon et al. 2024).
Variable Efficacy	Synthetic pesticides are highly effective against a broad spectrum of pests and diseases, ensuring immediate results.	As pests develop resistance, they cause a decrease in efficacy.	Due to fewer side effects and precision, the efficacy is high.	They may have a narrower action spectrum and can be less effective under certain environmental conditions.	(Ayilara et al. 2023)
Environmental Impact	When used as directed, synthetic pesticides do not leave harmful residues on food.	Pesticides can be toxic to many organisms, including birds, fish, beneficial insects, and non-target plants.	These pesticides are derived from natural sources, such as plants, bacteria, fungi, animals, and minerals. They are generally safer for use.	Biopesticides can target only a particular range of pests.	(Basaid & Furze, 2024).
Commercial potential	Synthetic pesticides have a longer shelf life than most organic pesticides.	Synthetic pesticides can take decades to degrade.	Biopesticides are less toxic to non-target organisms than synthetic pesticides.	Biopesticides can negatively affect soil microorganisms by limiting their ability to produce plant growth-promoting traits.	(Kaur et al. 2024)
Agricultural benefit	High Efficacy: Synthetic pesticides are typically more potent and can quickly reduce pest populations, offering immediate results.	Health Risks: There are potential health risks associated with synthetic pesticide use, including acute poisoning and long-term health effects on humans and animals	Biopesticides tend to be less toxic to non-target organisms, including humans, wildlife, and beneficial insects. This reduces the risk associated with pesticide application compared to synthetic options.	Efficacy: Biopesticides may not always be as effective or long-lasting as synthetic pesticides, potentially requiring more frequent applications to achieve the desired pest control	(Rani et al. 2021) & (Alewu & Nosiri, 2011)

Table Cont....

Commercial Aspect and Human Health	Synthetic pesticides		Biopesticides		Ref.
	Potential Advantage	Limitations	Potential Advantage	Limitations	
Impact on crop yield	Synthetic pesticides improve crop yield by decreasing pests.	Synthetic pesticides can harm non-target plants, reducing their ability to photosynthesize and produce seeds.	Biopesticides can be used in integrated pest management (IPM) programs to reduce the need for synthetic pesticides while maintaining high crop yields.	Biopesticides can be slower to control pests than conventional pesticides.	(Ayilara et al. 2023)
Impact on soil	Synthetic pesticides can increase agricultural productivity by reducing losses from weeds, diseases, and insect pests.	Synthetic pesticides can make soil brittle, reduce soil respiration, and harm soil microorganisms.	Biopesticides are generally less toxic to non-target organisms and degrade more quickly in the environment, reducing pollution risks	Biopesticides can negatively impact soil microorganisms by limiting their ability to produce plant growth-promoting traits	(Wei et al. 2024)
Long-term use		Long-term exposure is linked to serious conditions, including cancer, reproductive disorders, and neurodegenerative diseases.	Biopesticides are generally considered to be environmentally friendly and sustainable. They can reduce the use of chemical pesticides, which can pollute soil, water, and air.	Biopesticides can be less effective than chemical pesticides, with efficacy dropping as low as 50% compared to 80% for chemical pesticides.	(Aktar et al. 2009) (Wend et al. 2024)

in crop and disease management, synthetic pesticides pose high pitfalls to the environment and health. Pesticides have high health risks, which can be divided into chronic and acute toxicities (Gupta et al. 2023). Acute toxicity can arise from severe doses through inhalation, skin contact, or ingestion, and chronic toxicity will occur from repeated exposure. The short-term acute adverse effects of pesticide exposure on humans are irritation of the skin, rashes, blisters, burning sensation of the eyes, blindness, nausea, dizziness, diarrhea, and death (Shah 2020). The chronic effects of pesticide exposure on humans are cancer, teratogenicity, reproductive harm, immunotoxicity, neurotoxicity, and endocrine disruption. The usage of pesticides has led to diverse contamination of the surroundings, which is severely affecting natural resources. This will give rise to long-term effects on the ecosystem (Ahmad et al. 2024). WHO and the United Nations Environment Programme (UNEP) report stated that three million people are being affected, and 2,00,000 deaths occurred due to severe exposure to pesticides in developing countries. Pesticides will increase the production of ROS (reactive oxygen species), which causes a reduction in antioxidant levels and their properties. Due to disparities, proteins and lipids affect cellular signaling pathways. Chronic health effects are caused by ROS and oxidative stress. Pesticides cause major effects on the ecosystem, which causes adverse effects on human health, from acute intoxication to chronic infection that causes various types of cancer (brain cancer, breast cancer, prostate cancer, and colon cancer), Alzheimer’s disease, Parkinson’s disease, infertility, leukemia, and diabetes (Mishra et al. 2020). Nonetheless, pesticide use must be harmonized with environmental and health considerations, such as integrated pest management should be developed to ensure sustainable and effective pest control strategies. Synthetic pesticides have a direct or indirect impact on human health (Fig. 2).

FUTURE DIRECTION AND CONCLUSION

Extensive research on plant-derived biopesticides has to be focused on minimizing the health impacts, reducing the toxic effects on the ecosystem, and maintaining sustainability. More research must be conducted on improving the stability of biopesticides and their high potency to make them better options. Various techniques must be developed for biopesticide absorption and persistence. Furthermore, investigating the effect of biopesticides could reduce their toxic effects and environmental impacts. Precision agriculture is also a hot target for pesticide application, potentially decreasing the toxic effects on humans and the ecosystem.

Additionally, research on the acute and chronic toxic effects, such as carcinogenicity and neurotoxicity, is

to be performed on safer usage guidelines, particularly for synthetic pesticides (Cappa et al. 2022). Expanding the screening of diverse plants, especially tropical or medicinal species, may give new bioactive compounds for sustainable pest control. Extensive research should be focused on several limitations of biopesticides, which can be overcome. To ensure the long-term effectiveness of both kinds of pesticides, research into resistance mechanisms will also aid in the creation of strategies to combat resistance. Instead of using frameworks that are focused on synthetic compounds, it would be safer and more efficient to develop safety and efficacy testing standards specifically designed for biopesticides. By bridging the gap between synthetic and natural components, consistent worldwide regulatory rules that support biopesticide research and application can enable sustainable pest management on a global scale. These future directions aim to create more effective and safe pest control solutions that balance plant protection needs with human health and environmental safety.

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ABBREVIATIONS

GDP	: Gross domestic product
PD	: Parkinson's disease
Xee	: <i>Xanthomonas euvesicatoria</i> pv. <i>euvesicatoria</i>
Xep	: <i>Xanthomonas euvesicatoria</i> pv. <i>perforans</i>
Xhg	: <i>Xanthomonas hortorum</i> pv. <i>gardneri</i>
Xcc	: <i>Xanthomonas campestris</i> pv. <i>campestris</i>
Pcc	: <i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>
Psm	: <i>Pseudomonas syringae</i> pv. <i>Maculicola</i>
Bt	: <i>Bacillus thuringiensis</i>

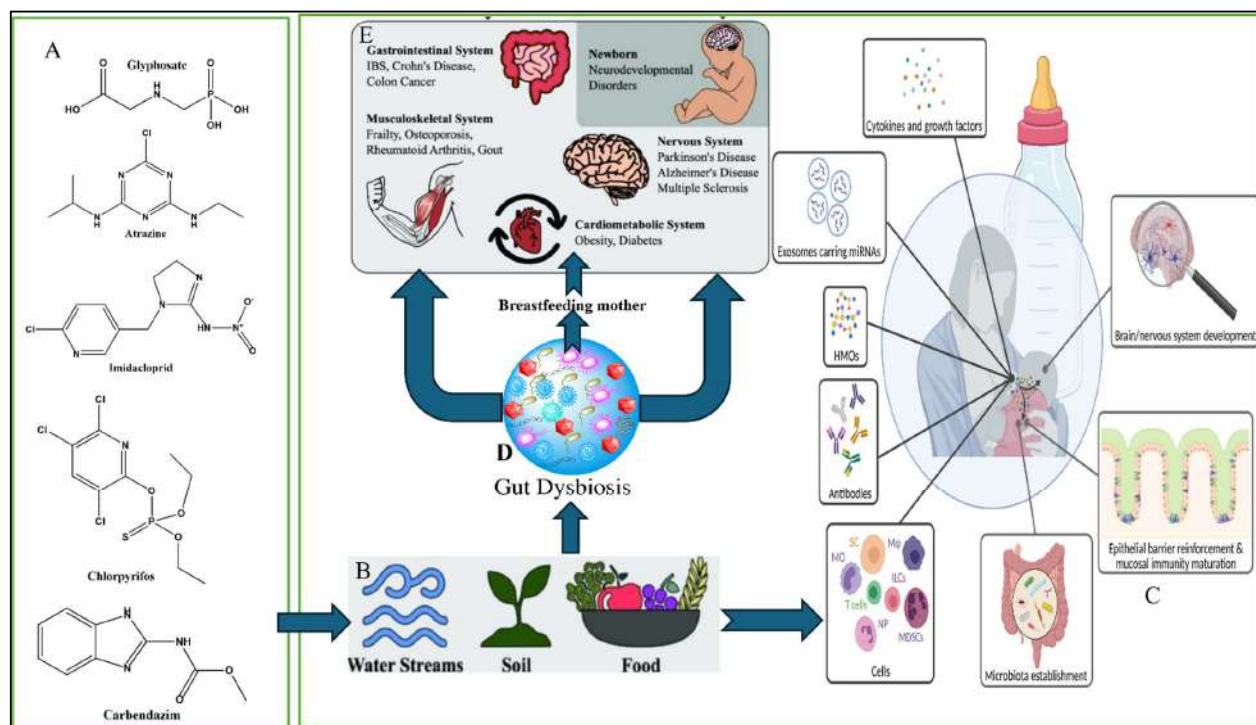


Fig. 2: (A) Structural representations of commonly used pesticides. Glyphosate is a widely utilized herbicide that inhibits the shikimate pathway in bacteria, which can alter the composition of gut microbiota. Atrazine is another herbicide known for its impact on gut microbiota and endocrine functions. Imidacloprid, a neonicotinoid insecticide, disrupts gut bacteria and immune responses. Chlorpyrifos, an organophosphate insecticide, is associated with gut microbiome disruptions and neurotoxicity. Carbendazim is a fungicide that negatively affects intestinal microbial diversity. (B) These synthetic pesticides come into direct contact with water streams, soil, and food, leading to environmental pollution that can adversely impact human health. (C) Special attention is given to breastfeeding mothers and their infants, as these pesticides can influence health during this critical development stage. (D) Pesticides are implicated in gut dysbiosis, which is linked to several health issues. (E) The illustration depicts the potential effects of pesticide exposure, including disruptions in the cardiometabolic system, neurodevelopmental disorders, gastrointestinal disorders, and an increased risk of colon cancer.

EPA : Environmental Protection Agency
 U. S : United states
 NACs : Nano-agrochemicals
 HPLC : High-performance liquid chromatography
 LCMS : Liquid chromatography- mass spectrometry
 PGPR : Plant growth-promoting rhizobacteria (PGPR)
 EU : European Union
 ADIs : acceptable daily intakes
 WHO : World Health Organization
 CSE : Centre for Science and Environment
 ROS : Reactive Oxygen Species
 UNEP : United Nations Environment Programme

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Eco-Friendly Bioremediation of Batik Dye Waste Using Indigenous Bacterial Consortia

Suhartini^{1†} , A. K. Prodjosantoso² , Bernadetta Octavia¹  and Isana Supiah Yosephine Louise² 

¹Biology Study Program, Department of Biology Education, Faculty of Mathematics and Natural Sciences, Yogyakarta State University, Yogyakarta, Indonesia

²Chemistry Study Program, Department of Chemistry Education, Faculty of Mathematics and Natural Sciences, Yogyakarta State University, Yogyakarta, Indonesia

†Corresponding author: Suhartini; suhartini@uny.ac.id

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ABSTRACT

This study explores the potential of indigenous bacteria in bioremediating batik dye wastewater, a major environmental pollutant that threatens aquatic ecosystems and human health. The research aimed to identify and characterize bacterial isolates capable of degrading natural dyes (*Indigofera tinctoria* L., *Caesalpinia sappan* L.) and synthetic dyes (methyl, naphthol, remazol, indigosol). The variable investigated in this study is the degradation rate of batik dye wastewater by bacteria, both individually and in bacterial consortia. Using isolation, purification, and 16S rRNA sequencing, 15 bacterial isolates were identified, with *Stenotrophomonas maltophilia* (D), *Stutzerimonas stutzeri* (H, I), *Micrococcus* sp. (J), and *Pseudomonas* sp. (N) exhibiting high effectiveness. *Micrococcus* sp. (J) achieved degradation rates of 99.62% for *Indigofera*, 85.74% for red remazol, and 83.05% for blue naphthol, while *Pseudomonas* sp. (N) degraded remazol red at 94.72%. A bacterial consortium (INJ: *Stutzerimonas stutzeri*, *Pseudomonas* sp., and *Micrococcus* sp.) efficiently degraded indigosol blue at 90.29%. Statistical analyses revealed no significant differences in decolorization between natural and synthetic dyes or dye colors. These bacteria demonstrated strong enzymatic activity under diverse environmental conditions, such as differences in pH, temperature, dye concentration, and chemical composition, providing an eco-friendly and cost-effective solution for batik dye waste bioremediation. The findings support SDG 6 (Clean Water and Sanitation) and SDG 12 (Responsible Consumption and Production) by improving wastewater quality and promoting sustainable waste management in the batik industry.

INTRODUCTION

Batik, acknowledged by the United Nations Educational, Scientific, and Cultural Organization (UNESCO) as an Indonesian cultural heritage in 2009, is commemorated annually on National Batik Day every October 2nd, as decreed by Indonesian Presidential Decree No. 33 of 2009. The intricacy of Batik patterns lies in their vivid coloration, ornamental display, and scale, rendering each piece uniquely distinguished (Kasim et al. 2016). Nevertheless, the production process of Batik involves the utilization of hazardous and enduring chemicals, resulting in elevated levels of pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), and heavy metals. This effluent contributes to the contamination of aquatic ecosystems (Syamwil et al. 2021).

The wastewater from Batik dye comprises deleterious elements such as chromium (Cr), sulfide (S²⁻), ammonia (NH₃), phenol, and oil-fat (Dewi et al. 2019). These substances encompass wax, dye, soda ash, metal, and fabric (Noviar et al. 2019). The utilization of synthetic dyes in Batik production has been associated with environmental pollution and health hazards, including the risk of skin cancer (Utami

2024). Dyeing one meter of Batik fabric can necessitate 25 to 50 cubic meters of water, often discharged untreated into rivers and soil, resulting in significant pollution (Indrayani & Triwiswara 2020). Batik waste significantly contributes to water, soil, and air pollution through the release of toxic chemicals, heavy metals, and emissions. Effective waste management and treatment strategies are essential to mitigate these environmental impacts and protect public health (Budiyanto et al. 2019, Linggawati et al. 2022).

Given the adverse environmental repercussions, it is imperative to conduct research and enhance public awareness regarding the management of Batik dye effluent before its release into the environment. Various methodologies have been explored, such as electrochemical degradation utilizing carbon-polyvinyl chloride (PVC) electrodes to disintegrate Batik waste through electrolysis, achieving up to 97.68% waste degradation and substantial reductions in lead (Pb) and COD (Riyanto & Nisaa 2017). Another approach involves the utilization of natural dyes and cleaner production methods to minimize pollution from synthetic dyes (Elsahida et al. 2020). However, these techniques are costly, necessitating more economical and environmentally sustainable solutions.

Bioremediation, employing microorganisms such as bacteria and fungi to break down, eliminate, immobilize, or detoxify pollutants, presents a promising alternative (Peer et al. 2022). This process involves biosorption, biodegradation, and bioaccumulation to eradicate contaminants from the environment (Kumar et al. 2019), aiming to transform harmful elements into less toxic alternatives (Paladhi et al. 2022). Bioremediation with indigenous microorganisms effectively decomposes waste, offering a viable solution for managing Batik dye effluent, particularly for small-scale Batik enterprises lacking appropriate waste treatment facilities.

This study aims to address Batik dye effluent, particularly methyl, naphthol, remazol, indigosol, and natural dyes through bioremediation. These variables were chosen because the dyes used, remazol, naphthol, indigosol, and natural dyes such as *Indigofera* and *Caesalpinia*, are the most dominant and commonly employed in the batik-making process in Indonesia. Remazol dyes are known for their water solubility and vibrant colors, naphthol dyes produce bright and long-lasting hues, indigosol dyes are widely used for their strong adherence to fabric and rich coloration, while natural dyes like *Indigofera* and *Caesalpinia* provide environmentally friendly options with *Indigofera* offering a rich blue hue and *Caesalpinia* producing vibrant red tones essential for traditional and sustainable batik production (Muchtasjar et al. 2019, Elshahida et al. 2020, Dewi et al. 2019, Rakhmania et al. 2022).

The research entails the identification and characterization of native bacteria in Batik dye effluent, measuring bacterial proliferation, testing their degradation capabilities in the laboratory, and molecularly identifying the most efficient bacteria via 16S rRNA sequencing. Additionally, degradation tests using combinations of bacterial isolates or bacterial consortia will be conducted to ascertain their potential to degrade Batik dye effluent. The hypothesis posits that indigenous bacteria can effectively decompose these dyes, mitigating the adverse effects of batik dye effluent on aquatic ecosystems. Furthermore, it is anticipated that this laboratory-based research can be translated into practical applications, leading to an effective, cost-efficient, and environmentally friendly Batik dye effluent management system.

This study addresses key research gaps in previous works by adopting a holistic approach that integrates microbial proliferation rate measurements, molecular identification via 16S rRNA sequencing, and degradation tests using bacterial consortia. Earlier studies, such as Mutiara et al. (2023), demonstrated the effectiveness of microbial consortia in degrading synthetic dyes but did not consider microbial growth dynamics, which are critical for understanding biodegradation efficiency. Similarly, Hussain et al. (2024) and Prabhakar et al. (2022) focused on specific bacterial strains but lacked assessments of consortia-based degradation and molecular-level characterization. By highlighting the high efficiency of indigenous bacteria in degrading both natural (*Indigofera*, *Caesalpinia*) and synthetic dyes (methyl, naphthol, remazol, indigosol), this research not only fills these gaps but also provides practical, cost-effective, and environmentally sustainable solutions. These findings advance prior studies by emphasizing the synergistic potential of bacterial consortia and their application in managing batik wastewater, particularly for small-scale enterprises lacking proper treatment facilities.

By harnessing the innate capabilities of local bacteria, this study seeks to develop sustainable, efficient, and cost-effective bioremediation strategies to alleviate pollution stemming from the Batik industry, ultimately safeguarding ecosystems and public health. The objectives are to identify and characterize predominant indigenous bacteria in Batik dye wastewater and determine the most effective bacteria for decolorizing these dyes through 16S rRNA gene sequencing. This research was conducted in the Yogyakarta Special Region, Indonesia, in 2023. Although this study was conducted in Yogyakarta, the use of synthetic dyes, such as remazol, naphthol, and indigosol, is widespread in Indonesia, particularly in commercial batik industries across major production areas like Pekalongan, Solo, and Yogyakarta, due

to their practicality and vibrant, long-lasting colors (Dewi et al. 2019, Indrayani & Triwiswara, 2020). In contrast, natural dyes like indigofera and caesalpinia are more commonly used in specific regions, such as rural areas in Yogyakarta and Central Java, where there is better access to raw materials and a focus on sustainability.

MATERIALS AND METHODS

Study Area and Sample Collection

This experimental research was conducted on batik dye residue acquired from the batik industry in Bantul, Special Region of Yogyakarta, Indonesia. Residual samples were directly collected from the site and preserved in sterile containers for subsequent analysis.

Isolation of Indigenous Bacteria

Isolation of autochthonous bacteria from batik wastewater was conducted utilizing the spread plate technique. The wastewater specimens were progressively diluted up to 10^{-8} and 10^{-15} with sterile isotonic sodium chloride solution (NaCl) for liquid wastewater and soil samples, respectively. The use of the spread plate technique for the isolation of indigenous bacteria in this study is associated with the selection of aerobic bacteria. Meanwhile, the sample dilution levels were determined based on the specific conditions of each sample. For instance, samples predicted to contain high concentrations of organic matter, such as batik dye effluent, are often densely populated with microbes. Therefore, dilution is required to a level that allows the separation of bacterial colonies within the sample. This approach ensures accurate isolation and characterization of bacteria for further analysis, particularly in complex environments like dye effluents, where microbial diversity and abundance are typically high (Madigan, 2018, Cappuccino, 2021). The diluted specimens were subsequently spread onto Nutrient Agar (NA) plates and incubated at 37°C for 24 hours to foster bacterial proliferation (Muchtasjar et al. 2019). The experimental samples in this study were replicated twice.

Purification of Bacterial Isolates

Individual bacterial colonies were purified utilizing the continuous streak inoculation method on nutrient agar slant media. Isolated colonies were aseptically transferred to fresh slant media to ensure the presence of single strains. The inoculated media were then placed in an incubator at a temperature of 37°C for 24 hours to facilitate optimal bacterial growth (Mambe et al. 2016). During the preliminary phase, each bacterial isolate is assigned an isolate code represented by an alphabetical letter for easy documentation.

Subsequently, following the screening process based on its capacity to degrade diverse batik dyes, the chosen bacterial isolate will undergo molecular characterization, after which the isolate code, represented by an alphabetical designation, will be replaced with the species name.

Phenetic Characterization of Bacteria

The phenotypic characterization of isolated bacteria comprised: a. Macroscopic observations: Examination of colony morphology (size, shape, color, texture) was conducted on agar plates (Rattray et al. 2022). b. Microscopic observations: Gram staining discriminated between Gram-positive and Gram-negative bacteria (Rohde, 2019). and c. Biochemical analyses: Various biochemical assays evaluated metabolic capacities, encompassing carbohydrate fermentation, starch hydrolysis, gelatin hydrolysis, catalase testing, citrate utilization, hydrogen sulfide (H_2S) production, indole production, motility, temperature resilience, pH tolerance, and Methyl red-Voges Proskauer (MR-VP) tests on media (Carr 2017).

Creation of Color Standard Curves

To quantify the removal of dyes, standard color curves were established for each dye under investigation: crimson naphthol, azure naphthol, scarlet remazol, cobalt remazol, crimson indigosol, azure indigosol, methyl crimson, methyl azure, *Caesalpinia*, and *Indigofera*. Initial solutions of 100 parts per million (ppm) were meticulously prepared for every dye in the nutrient broth media and subsequently diluted serially to concentrations varying from 10 ppm to 80 ppm. The optical density of each diluted solution was measured using ultraviolet-visible (UV-Vis) spectroscopy by a Thermo Scientific Genesys spectrophotometer to construct the standard curves.

Decolorization Assay and Bacterial Growth Measurement

The capacity of individual bacteria to decolorize and break down specific dyes was evaluated. Starter cultures were established by introducing single bacterial isolates into sterile nutrient broth media (20 milliliters/mL) and incubating for 24 hours at ambient temperature with agitation (Subashkumar, 2014). Subsequently, 1 mL (20%) of this initial culture was introduced into fresh media containing distinct dye concentrations, such as 50 ppm for red naphthol, red remazol, blue remazol, red indigosol, methyl red, methyl blue, and secang (*Caesalpinia*), and 100 ppm for blue naphthol, indigosol blue, and *Indigofera*. The cultures were once again incubated at room temperature with agitation for a duration of one week. Following the incubation period, bacterial cells

were isolated through centrifugation, and the remaining liquid was assessed utilizing a UV-Vis spectrophotometer to determine absorbance and compute the percentage of dye decolorized. Bacterial proliferation was tracked by measuring the absorbance of the culture at 600 nanometers (nm). The decolorization outcomes were derived using the following formula in Equation 1.

$$\% \text{ Decolorisation} = \frac{\text{Value of start Absorbance} - \text{Value of Absorbance Finish}}{\text{Value of start Absorbance}} \times 100\% \quad \dots(1)$$

McFarland Standard Curve Preparation

To quantify bacterial cell density, a McFarland standard curve was established. This standard was created by mixing specific proportions of 1% sulfuric acid (H₂SO₄) and 1% barium chloride (BaCl₂) to produce solutions with varying levels of turbidity. The optical density of each McFarland standard was assessed at 600 nm using UV-Vis (Dalynn Biologicals, 2014).

Measuring the Growth Rate of Bacterial Isolates (μ)

A bacterial inoculum was meticulously prepared by introducing a single dose of bacterial isolate into a sterile nutrient broth medium (20 mL). Subsequently, the medium underwent an incubation process within a shaker incubator at ambient temperature for a duration of 24 hours. The bacterial inoculum was then extracted using a micropipette under aseptic conditions and transferred into a sterile nutrient broth medium (50 mL). The initial Optical Density (OD) of the bacterial culture was determined against the 0.5 McFarland standard, yielding approximately 0.110-0.120 A at a wavelength of 600 nm using a UV-Vis spectrophotometer (Duedu & French, 2017). The blank utilized for this procedure consisted of sterile nutrient broth medium. Following this, the medium was once again subjected to incubation in a shaker incubator at room temperature. OD values were recorded at specific time intervals of 3, 6, 8, 16, 24, 32, 40, 48, 72, and 96 hours. The absorbance values obtained were then transformed into Klett Units by multiplying the absorbance value by 500 (OD $\times$ 500). Subsequently, a growth curve was constructed based on the results of the absorbance values converted into Klett Units (Cuevas and Edwards, 2017) and (Kochan et al. 2020). The rate of bacterial growth (μ) was determined using the following formula in Equation 2.

$$\mu = \frac{(\ln(X_t) - \ln(X_0))}{t} \quad \dots(2)$$

Where:

μ = Bacterial Growth Speed

X_t = Klett Value Bacterial Incubation Unit at t-th hour

X_0 = Klett Value Bacterial Incubation Unit at Hour 0

t = Bacterial Incubation Time (Hours)

ln = Natural Logarithm

16S rRNA Sequencing

For bacterial identification, 16 Svedberg ribosomal ribonucleic acid (16S rRNA) sequencing was conducted. Genomic deoxyribonucleic acid (DNA) was extracted from the bacterial isolates using a standardized extraction protocol. The 16S rRNA gene was amplified through polymerase chain reaction (PCR) with specific primers. Following amplification, the DNA fragments were sequenced bidirectionally. Subsequently, the acquired sequences were cross-referenced with known sequences in databases to ascertain the bacterial species present in the samples (Zemb et al. 2020).

Antagonist Test

An antagonist assay was undertaken to ascertain the bacterium's capacity to produce secondary metabolites possessing antibiotic properties that can impede the proliferation of other bacteria. This examination also assesses the efficacy of bacterial combinations or consortia in decomposing batik dye waste (Bosmans et al. 2016).

Bacterial Consortium Test (Combination of Bacteria) Degrading Batik Dye Waste

In nature, microbial communities are more commonly found in the form of consortia rather than single bacterial species, particularly when engaging in complex activities such as bioremediation. In a consortium, the metabolic by-products of one bacterial population can serve as substrates or resources for another population within the same community, creating a synergistic system. This interaction facilitates comprehensive bioremediation processes, such as those required for batik wastewater treatment, ensuring that the final products are environmentally safe (Ramaswamy et al. 2022, Barathi et al. 2022).

Naturally formed bacterial consortia are inherently stable when they are well-adapted to their environmental conditions. Recognizing this, the present study constructed a bacterial consortium tailored for potential application as a bioremediation agent in batik wastewater treatment. Such engineered consortia can mimic natural microbial dynamics while being optimized for specific environmental applications (Ihsanullah et al. 2020).

Bacterial consortium test for degrading batik dye waste involves the following steps:

- a. Establishment of a bacterial consortium: The amalgamation of bacterial consortia is formulated using three highly effective bacteria. To determine the bacterial consortium combinations from five effective bacteria, the combination theory is applied as shown in Equation 3.

$${}^5_3C = \frac{5!}{(5-3)!3!} \quad \dots(3)$$

By applying the combination theory, the resulting combinations consist of DHI, DHN, DHJ, DIN, DIJ, DNJ, HIN, HIJ, HNJ, and INJ bacteria.

- b. Discoloration of batik dye by the indigenous bacterial consortium: A bacterial starter culture is meticulously prepared by inoculating a single dose of bacterial isolate into a sterile nutrient broth medium. This medium is subsequently incubated in a shaker incubator at ambient temperature for a duration of 24 hours. Subsequently, 3 mL of the starter culture, each composed of 1 mL + 1 mL + 1 mL of pure starter culture based on the aforementioned combination of bacteria, is inoculated onto a medium consisting of Nutrient Broth and various dyes (naphthol red, naphthol blue, remazol red, remazol blue, indigosol red, indigosol blue, methyl red, methyl blue, *Caesalpinia*, and *Indigofera*). The medium is then subjected to incubation in a shaker incubator at room temperature with a speed of 121 revolutions per minute (rpm) for a span of 168 hours (equivalent to 1 week). Following this incubation period, the medium is transferred to a 15 mL capacity Eppendorf tube and centrifuged to precipitate the bacterial biomass using a centrifuge operating at 3000 rpm for 30 minutes. The discoloration of the dye is quantitatively assessed utilizing a UV-Vis spectrophotometer.

$$\% \text{ Decolorisation} =$$

$$\frac{\text{Value of start Absorbance} - \text{Value of Absorbance Finish}}{\text{Value of start Absorbance}} \times 100\% \quad \dots(4)$$

Data Analysis

The ability of bacterial isolates to degrade batik dye wastewater was assessed using Equation 1. The degradation capacity of these isolates for batik dye residues, including methyl, naphthol, remazol, indigosol, and natural dyes, was categorized into two color groups: blue and red. If the data met the assumptions of normality and homogeneity, an Analysis of Variance (ANOVA) was conducted to evaluate differences in degradation rates among bacterial isolates for blue and red dyes separately. Additionally, a t-test was conducted to compare the degradation rates of each bacterial isolate in breaking down red and blue dyes, both

synthetic (methyl, naphthol, remazol, indigosol) and natural (*Indigofera tinctoria* L., *Caesalpinia sappan* L.). However, if the data did not meet the normality or homogeneity assumptions for either ANOVA or the t-test, the Kruskal-Wallis test was used as a non-parametric alternative, as suggested by Kim (2019) and Morgan et al. (2019). These statistical approaches are widely applied in biological and environmental research to determine significant differences between independent groups.

RESULTS AND DISCUSSION

Characterization and Identification of Indigenous Bacteria

The isolation procedure resulted in a total of 15 bacterial isolates extracted from batik dye wastewater. Each isolate was assigned a unique alphabetical code, and the specific type of batik dye incorporated into the nutrient media is detailed in Table 1.

The principal aim of this research was to characterize and identify predominant autochthonous bacteria from batik dye wastewater containing remazol, naphthol, and indigosol dyes. This extensive isolation effort highlighted the diverse microbial community inherent in batik dye wastewater.

Characterization of Isolated Bacteria

The phenotypic characterization consisted of macroscopic observations of colony morphology and microscopic examinations (Gram staining) as delineated in Table 2,

Table 1: Bacterial isolation results from batik dye wastewater.

Bacterial Isolate Code	Isolate Name	Discription
A	RZ T 10	Remazol selective media
B	RZ T 10	Remazol selective media
C	NP AT 7	Naphthol selective media
D	NP AT 7	Naphthol selective media
E	RZ T 8	Remazol selective media
F	NP T 13	Naphthol selective media
G	NP T 13	Naphthol selective media
H	RZ ID 4	Remazol selective media
I	NP ID 4	Naphthol selective media
J	RZ NP 6	Remazol selective media
K	RZ ID 4	Remazol selective media
L	ID T 10	Indigosol selective media
M	ID T 13	Indigosol selective media
N	RZ AT 7	Remazol selective media
O	NP AT 7	Naphthol selective media

while the biochemical and physiological characterization of bacterial isolates is detailed in Table 3.

The bacterial isolates displayed a diverse array of colony shapes, colors, and configurations, showcasing the morphological richness within the microbial community. For example, isolate J, identified as *Micrococcus* sp., exhibited a circular yellow colony with a convex elevation and an entire colony edge. Besides isolating J, the details for all other bacterial isolates, identified by their respective codes, can be found in Table 2. Gram staining further distinguished these isolates, unveiling a combination of gram-positive and gram-negative bacteria, crucial for comprehending their structural and functional roles in dye degradation (Calgin & Kalayci 2024). The integration of advanced materials such as nanocomposites derived from natural waste can significantly enhance the degradation capabilities of indigenous bacteria. Haroon et al. (2023) demonstrated that nanomaterials synthesized through green processes, such as those derived from plant waste and fly ash, exhibit high biocompatibility and stability. These properties enable them to adsorb heavy metals and organic dyes effectively, thus complementing bacterial enzymatic activities in wastewater remediation. As indicated in Table 2, 11 are gram-positive, while 4 are gram-negative. The fundamental disparities between gram-positive and gram-negative bacteria reside in their cell wall composition, quorum-sensing signal molecules, and ecological functions. Gram-positive bacteria possess a thicker peptidoglycan layer and utilize peptides for quorum sensing, whereas gram-negative bacteria feature a thinner peptidoglycan layer, an outer membrane, and employ N-acyl homoserine

lactone molecules for quorum sensing (Bishop & McCue 2021).

The biochemical and physiological analysis (Table 3) offered profound insights into the metabolic capacities of these isolates. Assessments such as citrate utilization, starch hydrolysis, and fermentation of diverse sugars illuminated the enzymatic potential of these bacteria. Significantly, isolates J and N, which demonstrated the highest efficiency in dye degradation, also displayed favorable outcomes in multiple biochemical examinations, indicating their robust metabolic frameworks. This comprehensive assessment emphasizes the significance of comprehending bacterial metabolism for both environmental and industrial purposes, particularly in the realm of dye pollutant bioremediation.

The biochemical and physiological evaluation of bacteria is indispensable for grasping their roles and utility. This process aids in understanding bacterial physiology, pathogenesis, and metabolism (Malarney & Chang 2023) and is pivotal for the recognition and characterization of advantageous microbial strains through microbiological, biochemical, and molecular methodologies (Hossain & Sultana 2018). In industrial settings, fundamental enzyme assays and biochemical analysis are essential for various industrial enzymes and applications (Salwan et al. 2023). Bacteria may coordinate numerous unrelated genes concurrently in response to extreme environments like batik wastewater containing synthetic chemical dyes. This coordination entails global regulatory systems such as the lactose operon and the maltose regulon, which react to these

Table 2: Bacterial colony morphology.

Isolate code	Shape	Color	Elevation	Colony Edge	Configuration	Gram +/-
A	<i>Circular</i>	Yellowish white	<i>Convex</i>	<i>Entire</i>	<i>Round</i>	+
B	<i>Circular</i>	Yellowish white	<i>Convex</i>	<i>Entire</i>	<i>Round</i>	+
C	<i>Circular</i>	Brownish white	<i>Convex</i>	<i>Entire</i>	<i>Round</i>	+
D	<i>Circular</i>	Brownish white	<i>Convex</i>	<i>Undulate</i>	<i>Serrate</i>	+
E	<i>Circular</i>	White	<i>Convex</i>	<i>Entire</i>	<i>Round</i>	+
F	<i>Circular</i>	Yellow	<i>Convex</i>	<i>Entire</i>	<i>Round</i>	+
G	<i>Irregular</i>	Brownish white	<i>Convex</i>	<i>Lobate</i>	<i>Irregular and Spreading</i>	+
H	<i>Irregular</i>	White	<i>Raised</i>	<i>Undulate</i>	<i>Round and Scalloped</i>	-
I	<i>Irregular</i>	White	<i>Raised</i>	<i>Undulate</i>	<i>Round and Scalloped</i>	-
J	<i>Circular</i>	Yellow	<i>Convex</i>	<i>Entire</i>	<i>Round</i>	-
K	<i>Irregular</i>	White	<i>Raised</i>	<i>Undulate</i>	<i>Round and Scalloped</i>	-
L	<i>Circular</i>	Yellowish white	<i>Convex</i>	<i>Entire</i>	<i>Serrate</i>	+
M	<i>Irregular</i>	Brownish white	<i>Convex</i>	<i>Undulate</i>	<i>Round and Scalloped</i>	+
N	<i>Circular</i>	Brownish white	<i>Convex</i>	<i>Undulate</i>	<i>Serrate</i>	+
O	<i>Irregular</i>	White	<i>Convex</i>	<i>Undulate</i>	<i>Round and Scalloped</i>	+

Table 3: Results of biochemical and physiological characterization of bacterial isolates from batik dye waste.

Biochemical Test	Isolate Code															
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
Simmons Citrate	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Starch Hydrolysis	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	
Glucose Fermentation	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	
Sucrose Fermentation	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	
Galactose Fermentation	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	
Maltose Fermentation	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	
Lactose Fermentation	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	
Facultative Anaerobe	+	+	+	+	+	+	+	-	-	+	-	-	+	+		
Anaerobe	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+	
Gram Negative	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	
Gram Positive	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	
Growth pH 4	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	
Growth pH 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth pH 9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth NaCl 5%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth NaCl 10%	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	
H ₂ S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Growth Temperature -4°C	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	
Growth Temperature 25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth Temperature 55°C	-	-	-	+	+	+	+	+	+	+	-	+	-	-	+	
Gelatin	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	
Methyl Red	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	
+P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Growth on Echinulate Slant Agar	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	

Note: + = Positive Test - = Negative Test

conditions, enabling bacterial genes to acclimate to the synthetic dyes present in batik wastewater.

Bacterial Growth Rate

Quantifying bacterial growth rates not only provides insights into their physiological behavior but also holds practical implications for industrial, environmental, and medical applications. The table presented here (Table 4) summarizes the growth rates of various bacterial isolates under standardized conditions.

Understanding bacterial proliferation is crucial in environmental biotechnology, particularly for bioremediation applications. Certain bacterial isolates exhibit exceptional abilities to degrade environmental pollutants, such as dye wastes, even under growth-limiting conditions, emphasizing

the importance of metabolic capabilities over growth rates.

Growth rates ($\mu\mu$) of various bacterial isolates, along with key observations, reveal significant diversity in their proliferation potentials. Isolate J demonstrates the lowest growth rate due to inefficient substrate utilization, whereas isolate O exhibits the highest growth rate. Despite isolate J's sluggish proliferation, it shows remarkable dye-degrading efficacy, efficiently breaking down diverse dye wastes. This highlights the significance of functional capabilities beyond growth rates in bioremediation (Basan et al. 2020).

Bacterial proliferation rates are influenced by nutritional status, environmental factors, temperature, protein synthesis capability, and cellular dimensions. The interplay between these factors and waste degradation efficacy is determined by bacterial species, environmental conditions, and

Table 4: Bacterial growth rate.

No.	Isolate Code	Growth Rate [μ]
		Average
1	A	0.2733
2	B	0.2770
3	C	0.2892
4	D	0.2856
5	E	0.2766
6	F	0.2848
7	G	0.2895
8	H	0.2233
9	I	0.2704
10	J	0.1817
11	K	0.2246
12	L	0.3067
13	M	0.2986
14	N	0.3009
15	O	0.3100

waste composition, all of which impact the efficiency of bioremediation efforts (Zainol et al. 2020). Isolate J’s substantial dye waste degradation ability, despite its slow growth, underscores its value as a bioremediation agent, demonstrating that functional efficiency can outweigh growth constraints in such applications (Santal et al. 2024).

Decolorization Assay and Bacterial Growth Measurement

The efficacy of the isolates in degrading batik dye waste was assessed through decolorization assays. Figs. 1 and 2

depict the degradation percentages for azure and crimson batik dyes, respectively.

Fig. 1 illustrates the levels of degradation of blue batik dye waste by 15 bacterial strains. Isolate J (*Micrococcus* sp.) demonstrated the highest degradation efficacy, achieving 99.62% for *Indigofera* and 83.5% for naphthol blue. This remarkable efficiency can be attributed to its robust enzymatic prowess, encompassing a variety of oxidases and reductases, enabling the bacterium to metabolize a broad spectrum of dye molecules (Ikram et al. 2023). In addition to bacterial-based decolorization, the application of nanocomposites has been shown to provide significant advantages in treating dye-laden wastewater. According to Haroon et al. (2023), nanocomposites synthesized from agricultural waste achieve high dye adsorption efficiencies, effectively removing pollutants that are otherwise resistant to biological degradation. This synergy between adsorption and biodegradation presents a promising strategy for optimizing batik dye wastewater treatment.

Isolate I (*Stutzerimonas stutzeri*) also exhibited commendable performance, particularly in the degradation of blue remazol dye with an efficiency of 79.40%. The production of enzymes such as azoreductase, laccase, and peroxidase by this isolate plays a pivotal role in the decolorization and breakdown of dye molecules (Joshi et al. 2020).

Isolate N (*Pseudomonas* sp.) achieved an 85.26% degradation efficiency for *Indigofera*. *Pseudomonas* sp. is renowned for its metabolic versatility and capacity to produce an array of enzymes, including azoreductase and dioxygenase, crucial for the breakdown of complex organic pollutants like synthetic dyes (Patel et al. 2017).

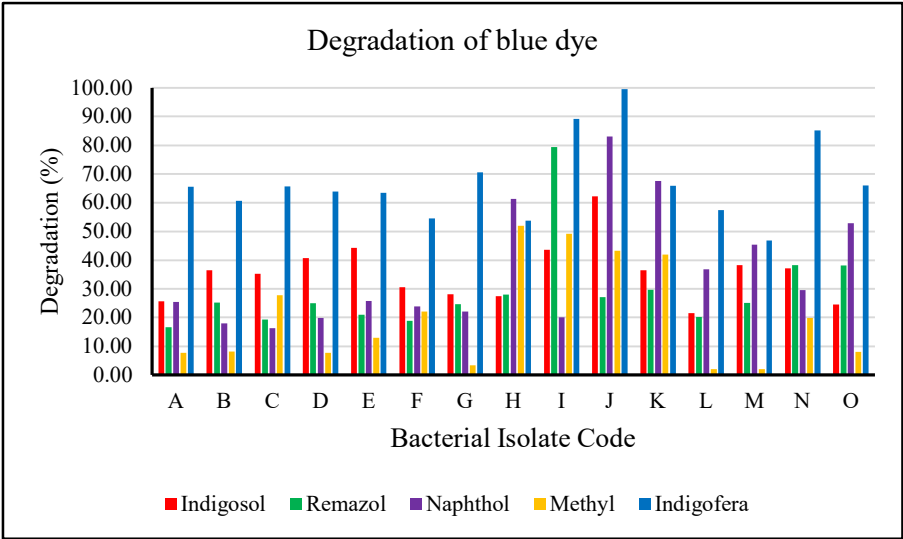


Fig. 1: Bar diagram of degradation of blue batik dye waste.

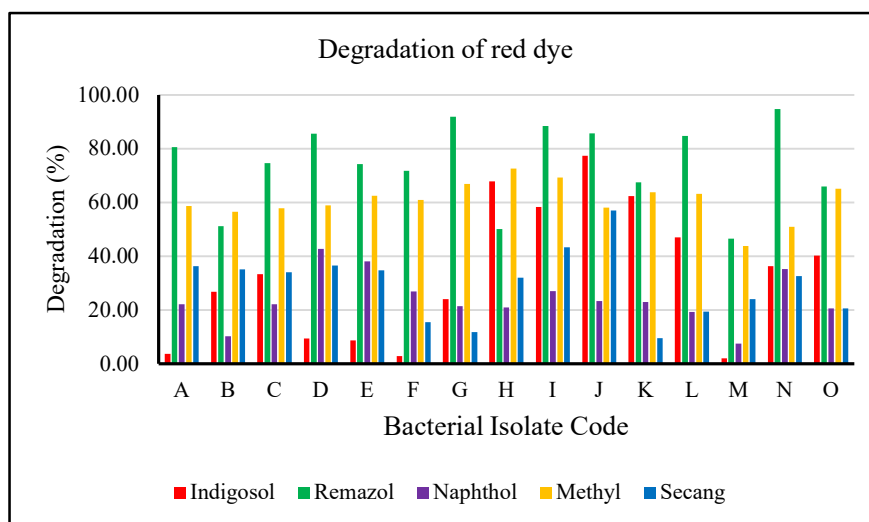


Fig. 2: Bar diagram of degradation of red batik dye waste.

Comparatively lower degradation efficiencies were observed in isolates H, with each achieving approximately 52.04-61.28% for methyl and naphthol blue dyes, respectively. These disparities can be attributed to variations in enzymatic capabilities and the environmental adaptability of the respective isolates (Misha 2022).

Fig. 2 illustrates the levels of degradation of red batik dye waste by the bacterial isolates. Isolate J (*Micrococcus* sp.) once again demonstrated exceptional performance, achieving an 85.74% degradation efficiency for remazol red dye. This achievement is consistent with prior research highlighting the extensive enzymatic capabilities of *Micrococcus* sp. (Ikram et al. 2023). Isolate H (*Stutzerimonas stutzeri*) attained a 72.68% degradation efficiency for methyl red dye. The presence of azoreductase, laccase, and peroxidase enzymes enables this isolate to efficiently break down various dye types (Joshi et al. 2020). In the case of remazol red dye, isolate D (*Stenotrophomonas maltophilia*) exhibited a noteworthy efficiency of 85.51%, comparable to isolate N with 94.72% and isolate J with 85.74%. This suggests that *Stenotrophomonas maltophilia* could be highly proficient in degrading specific dye varieties, particularly remazol red, possibly due to specialized enzymes targeting the molecular structure of this dye. *Pseudomonas* sp. (Isolate N) also excelled in degrading remazol red dye, achieving an efficiency of 94.72%. This indicates the significant potential of this isolate for practical applications in dye wastewater bioremediation (Ihsanullah et al. 2020). Lower degradation efficacy was observed in isolates A and B, particularly with secang red dye. This could be attributed to limitations in enzymatic activity or the incapacity of these isolates to adapt to the challenging conditions posed by synthetic batik dyes

(Salwan et al. 2023). Based on the decolorization capabilities of bacterial isolates towards batik dye waste as depicted in Figs. 1 and 2, isolates D, H, I, J, and N have been identified as effective. Moreover, these five efficacious bacterial isolates were discerned through 16S rRNA sequencing. To evaluate the degradation prowess of effective bacterial isolates (D, H, I, J, N) on blue batik dye waste containing natural dyes (*Indigofera*), methyl, naphthol, remazol, and indigosol, as well as red batik dye waste with natural red dyes (*Caesalpinia*), methyl, naphthol, remazol, and indigosol, an ANOVA analysis was performed. The outcomes are presented in Table 5 below.

The Anova results showed no significant differences in the decolorization abilities of bacterial isolates for blue ($F = 1.288$, $p = 0.308$) and red dyes ($F = 0.308$, $p = 0.869$), indicating comparable capacities among the isolates. Anova analysis was performed because the data met the assumptions of normality and homogeneity. Separate ANOVA analyses for blue and red dyes supported these findings, consistent

Table 5: The results of the ANOVA analysis on the ability of effective bacterial isolates to degrade various batik dye wastes.

Degradation rate of blue batik dye waste					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3085.841	4	771.460	1.288	.308
Within Groups	11975.115	20	598.756		
Total	15060.956	24			
Degradation rate of red batik dye waste					
Between Groups	806.592	4	201.648	.308	.869
Within Groups	13090.297	20	654.525		
Total	13896.889	24			

Table 6: The results of the independent t-test and Kruskal-Wallis test between the ability of effective bacterial isolates in degrading blue and red batik dye waste.

Types of batik waste dyes	Information	The t-test results				
		F	Sig.	t	df	Sig. (2-tailed)
Natural dyes	Equal variances assumed	3.616	0.94	3.941	8	0.004
	Equal variances not assumed.			3.941	6.208	0.007
Indigosol	Equal variances assumed	3.783	0.088	-0.403	8	0.697
	Equal variances not assumed.			-0.403	5.354	0.702
The Kruskal-Wallis test results						
	Chi-Square	df			Asymp. Sig.	
Methyl	5.771	1			0.016	
Naphthol	0.011	1			0.917	
Remasol	5.771	1			0.016	

with studies by Rakhmania et al. (2022), which demonstrated the effectiveness of dye-degrading bacteria in disintegrating azo dyes commonly used in the batik industry. The outcomes of this investigation include a comparison of the degradation abilities of bacterial isolates on red and blue batik dye waste for each dye type, both natural and synthetic. For natural dyes and indigosol, an independent t-test was conducted as the data met normality and homogeneity assumptions. Meanwhile, methyl and naphthol dyes did not meet the homogeneity requirement, and remazol dye failed the normality assumption; thus, these were analyzed using the Kruskal-Wallis test, as shown in Table 6.

The t-test results revealed a significant difference in the degradation abilities of bacterial isolates for natural blue and red dyes ($p = 0.004$), while no significant difference was observed for indigosol ($p = 0.697$). This suggests that the chemical structure of natural dyes like *Indigofera* and *Caesalpinia* affects degradation efficiency, unlike indigosol.

For synthetic dyes, the Kruskal-Wallis test indicated significant differences in degradation between blue and red dyes for methyl and remazol ($p = 0.016$), but no significant difference for naphthol ($p = 0.917$). These variations are likely linked to the interaction of enzymes such as azoreductase, laccase, and peroxidase with the dye molecules, as described by Mishra et al. (2022).

These findings align with Calderón-Tapia et al. (2024), emphasizing the importance of developing tailored bioremediation strategies for different dye types and colors, particularly in managing complex batik dye wastewater.

Prior research suggests that the efficacy of decolorization predominantly hinges on the specific bacterial strains utilized and environmental factors such as pH, dye concentration, and biomass dosage. For example, *Bacillus algicola* showcased the capacity to decolorize over 95% of azo dyes at pH 8.0 and

25°C (Chukowry et al. 2017). Additionally, *Pseudomonas* sp. and *Enterobacter cloacae* have demonstrated notable decolorization prowess across various dyes, encompassing red and blue variants (Ravi et al. 2024, Govindaraj et al. 2018).

Identification of Batik Dye-Degrading Bacteria Using 16S rRNA Sequencing

The outcomes of bacterial identification through molecular analysis using the 16S rRNA sequencing technique can be evaluated based on the following criteria:

1. **Percentage Identity:** This metric signifies the level of resemblance between the provided DNA sequence and the target DNA sequence. Samples of microorganisms utilizing the 16S rRNA marker are deemed identical at the species level when the “percentage identity” surpasses 97.5%, and at the genus level when it exceeds 95% (Teng et al. 2014).
2. **Query Coverage:** This parameter indicates the degree to which the provided DNA sequence aligns with the target DNA sequence. When the provided DNA sequence completely matches the target DNA sequence in the NCBI database, the query coverage reaches 100%.
3. **E Value:** The E value, or expectation value, gauges the probability of the alignment occurring randomly. A favorable E value approaches zero, signifying that the alignment is highly improbable to have happened by chance.

The molecular identification results obtained from 16S rRNA sequencing by comparing the DNA sequences acquired with the GenBank nucleotide database from NCBI for bacterial identification are delineated in Table 7.

To precisely ascertain the predominant bacterial strains accountable for dye degradation, 16S rRNA gene sequencing was conducted. This molecular methodology yielded intricate

Table 7: Molecular Identification.

Bacterial Isolate Code	Organism	Fragment	%Species identity	Query coverage [%]	E Value	Accession Number
D	<i>Stenotrophomonas maltophilia</i>	16S	99.93	100	0	ON197508.1
H	<i>Stutzerimonas stutzeri</i>	16S	99.93	100	0	MN889324.1
I	<i>Stutzerimonas stutzeri</i>	16S	99.93	100	0	MN889324.1
J	<i>Micrococcus</i> sp	16S	99.93	100	0	JQ658425.1
N	<i>Pseudomonas</i> sp	16S	100	100	0	MK696212.1

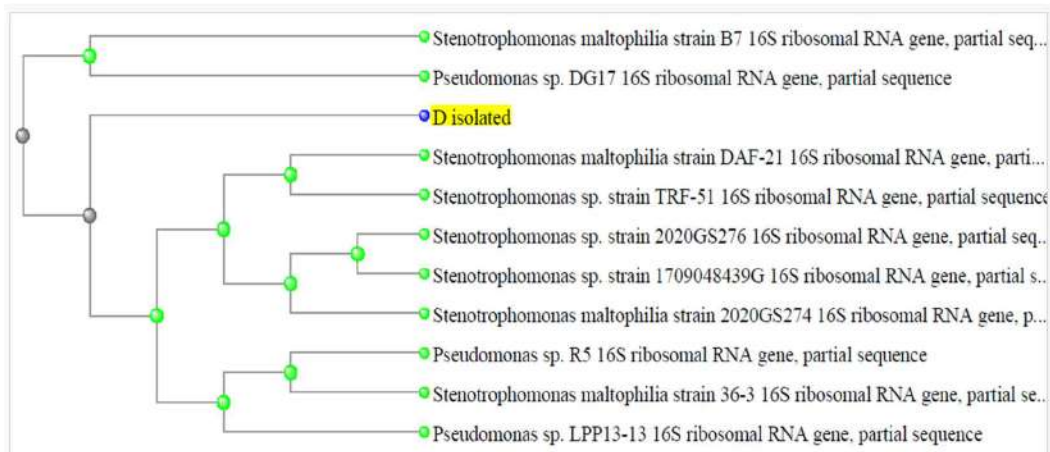


Fig. 3: Phylogenetic tree analysis for isolate D (NP-AT7).

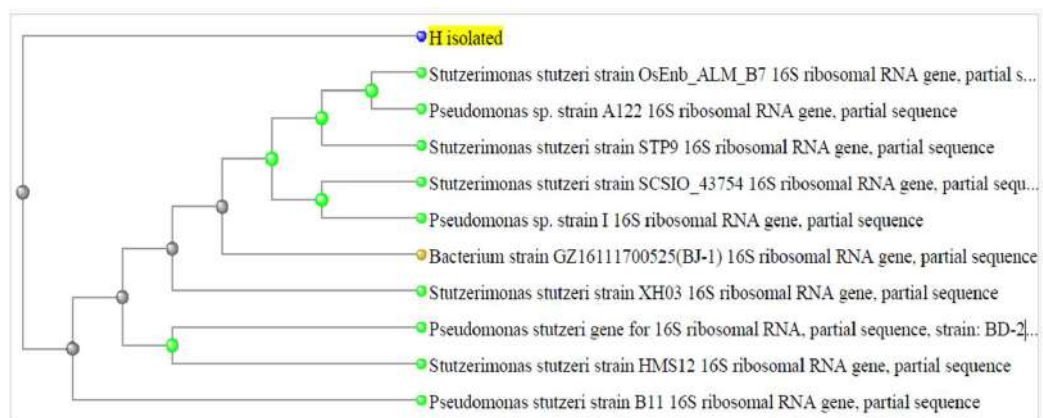


Fig. 4: Phylogenetic tree analysis for isolate H (RZ-ID4).

insights into the genetic affinities and potential functional capacities of the isolated bacteria. The outcomes of the phylogenetic analyses for isolates D, H, I, J, and N are depicted in Figs. 3, 4, 5, 6, and 7, respectively.

Fig. 3 depicts the phylogenetic tree of isolate D (NP-AT7), identified as *Stenotrophomonas maltophilia*, known for effectively degrading red naphthol and remazol dyes via laccase enzymes. These enzymes catalyze the oxidation of phenolic and non-phenolic compounds, including synthetic

dyes, by transferring electrons from dye molecules to oxygen, breaking down complex structures into simpler, less harmful forms (Vishani & Shrivastav 2022).

Fig. 4 shows the phylogenetic tree of isolate H (RZ-ID4), identified as *Stutzerimonas stutzeri*, which effectively degrades blue naphthol, red methyl, and blue remazol dyes. This is achieved through enzymes such as azoreductase, which initiates the breakdown of azo bonds in synthetic dyes, and laccase and peroxidase, which further

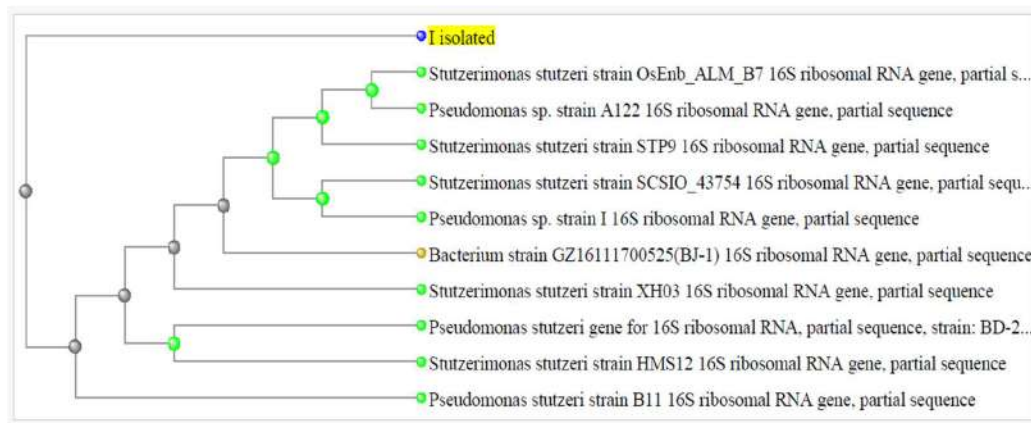


Fig. 5: Phylogenetic tree analysis for isolate I (NP-ID4).

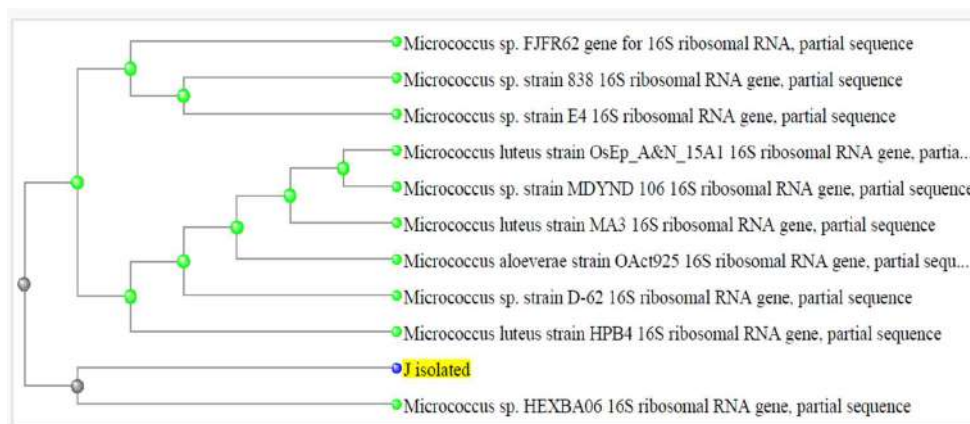


Fig. 6: Phylogenetic tree analysis for isolate J (RZ-NP6).

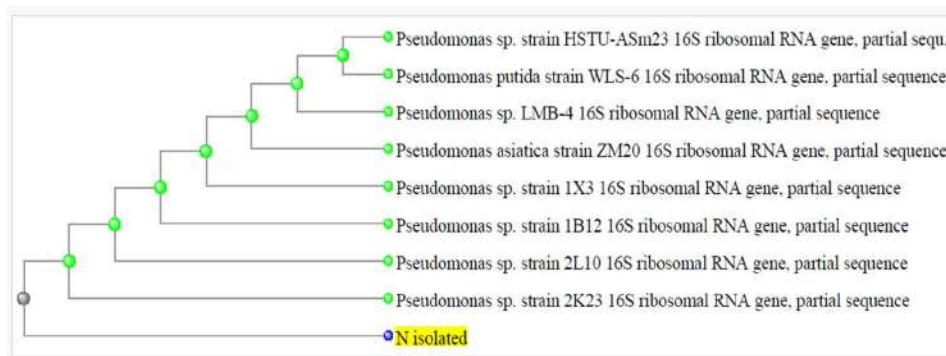


Fig. 7: Phylogenetic tree analysis for isolate N (RZ-AT7).

oxidize dye molecules into less harmful compounds (Joshi et al. 2020).

Fig. 5 depicts the phylogenetic tree of isolate I (NP-ID4), identified as *Stutzerimonas stutzeri*, which efficiently degrades natural *Indigofera*, red remazol, and blue remazol dyes. This ability is attributed to its enzymatic repertoire,

including azoreductase, laccase, and peroxidase, which collaboratively break down complex dye molecules into simpler compounds, accelerating decolorization and reducing environmental impacts (Mishra et al. 2022).

Fig. 6 illustrates the phylogenetic tree of isolate J (RZ-NP6), identified as *Micrococcus* sp., which exhibits versatility

in degrading dyes such as natural *Indigofera*, *Caesalpinia*, indigosol, remazol, and naphthol, attributed to its robust enzymatic systems. These capabilities make it a strong candidate for bioremediating diverse dye pollutants (Ikram et al. 2023). Similarly, studies by Calderón-Tapia et al. (2024) highlight the degradation potential of *Micrococcus aloeverae* for CN and Hg, achieving efficiencies up to 98.80%.

Fig. 7 illustrates the phylogenetic tree of isolate N (RZ-AT7), identified as *Pseudomonas* sp., which effectively degrades natural *Indigofera* and red remazol dyes through azoreductase and dioxygenase enzymes. These enzymes disintegrate dye molecules into simpler, harmless forms, enabling efficient decolorization and detoxification of dye-laden wastewater (Ihsanullah et al. 2020). Decolorization assessments revealed that Isolate N achieved a 94.72% degradation rate for red remazol, while Isolate J excelled with 99.62% for natural *Indigofera* blue dye, highlighting their potential for eco-friendly bioremediation.

Testing a Combination of Bacterial Isolates or Consortium Bacteria as Batik Dye Waste Degraders

The efficacy of a combination of bacterial isolates, or a consortium of bacteria, in the degradation of batik dye waste surpasses that of individual isolates. The superior performance of these indigenous bacterial consortia in the decomposition of batik dye waste is evident in the illustrations depicted in Figs. 8 and 9 below.

Fig. 8 illustrates that numerous bacterial consortia exhibited remarkable efficacy in degrading blue batik dyes. Particularly noteworthy is the INJ consortium, consisting of *Stutzerimonas stutzeri*, *Pseudomonas* sp., and *Micrococcus* sp., which demonstrated the highest degradation efficiency for indigosol blue at an impressive rate of 90.29%. In a related study, Sarwan et al. (2024) demonstrated that cellulolytic bacterial isolates not only achieved high efficiency in breaking down cellulosic wastes but also effectively deinked colored paper within 24 hours. These findings emphasize the potential of integrating cellulolytic bacteria into bioremediation consortia to target complex pollutants, such as batik dyes, for enhanced degradation and eco-friendly waste management. This exceptional performance can be attributed to the synergistic enzymatic activities of the three isolates, likely encompassing azoreductase, laccase, and peroxidase, well-known for their pivotal roles in dye degradation (Joshi et al. 2020).

The HIN consortia also displayed significant effectiveness, achieving degradation efficiencies of 79.94% for indigosol blue. This underscores the ability of these consortia to leverage the diverse metabolic pathways of their constituent isolates to effectively dismantle intricate dye molecules (Barathi et al. 2022).

When it comes to natural indigofera blue dye, the INJ consortium once again excelled with a degradation efficiency of 61.22%, demonstrating its versatility across various dye types. Additionally, consortia such as HNJ and DHJ

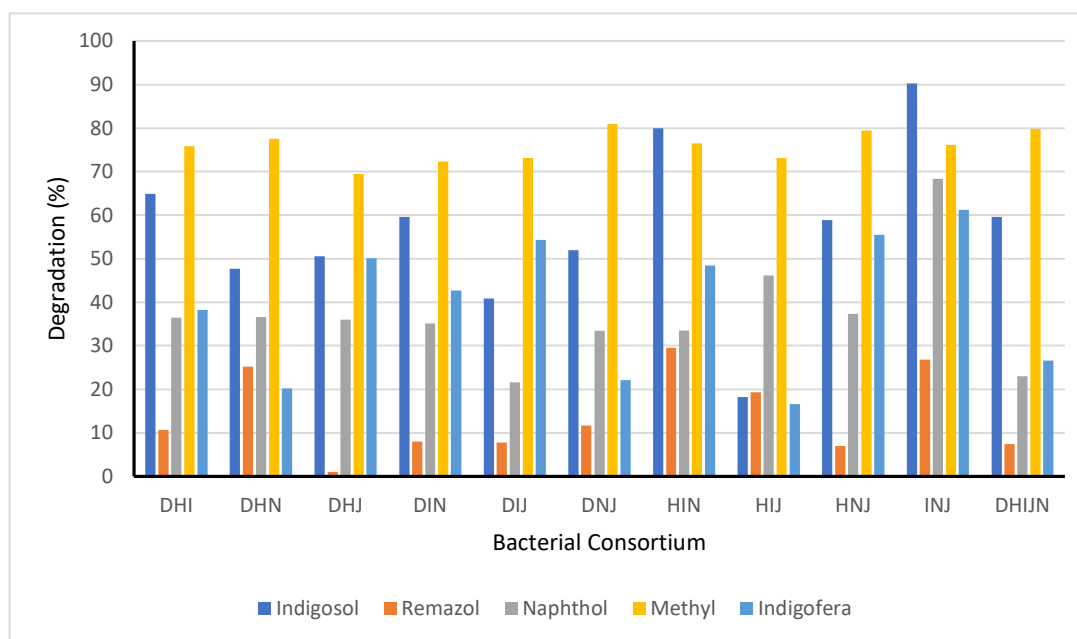


Fig. 8: Bar diagram of the ability of a combination of bacterial isolates/consortium bacteria to degrade blue batik dye waste.

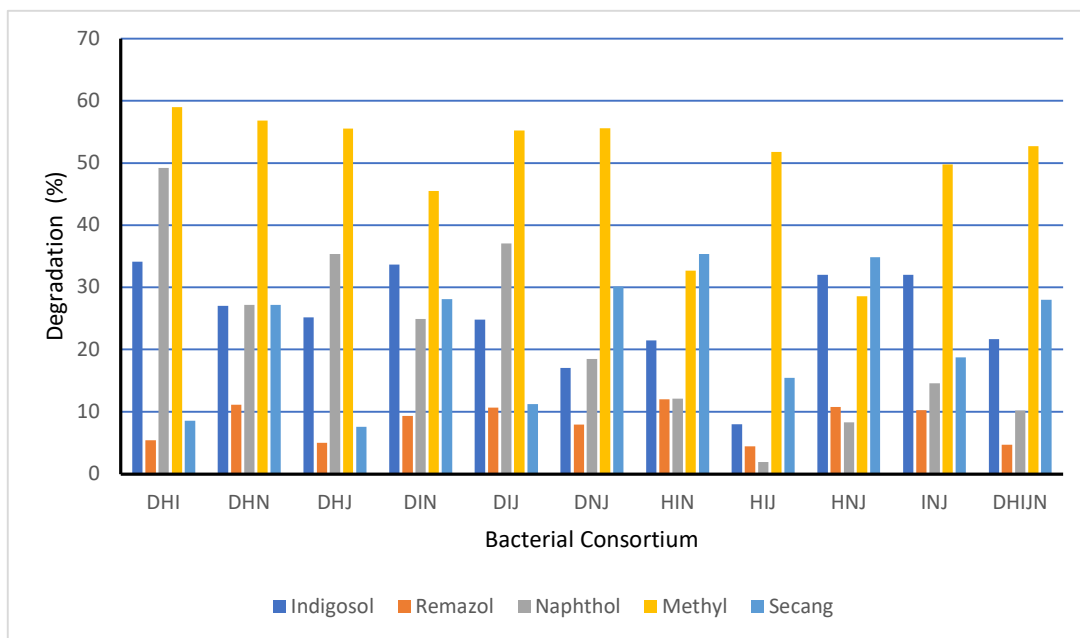


Fig. 9: Bar diagram of the ability of a combination of bacterial isolates/consortium bacteria to degrade red batik dye waste.

exhibited commendable performance, achieving degradation efficiencies of 55.52% and 50.16%, respectively.

In the case of methyl blue dye, the DNJ consortium attained the highest efficiency at 80.92%, closely trailed by DHN with 77.54% and HNJ with 79.45%. These findings underscore the significance of selecting consortia with complementary enzymatic capabilities to optimize degradation efficiency (Mutiara et al. 2023).

Fig. 9 illustrates the degradation efficiencies of various bacterial consortia for red batik dye waste. The findings indicate that the degradation capacity for red dyes was generally lower than that of blue dyes. Concerning secang red dye, the consortium HIN demonstrated the highest degradation efficiency at 35.34%, followed by DNJ at 30.14%. These outcomes suggest that specific combinations of bacteria are more adept at breaking down natural red dyes due to their distinct enzymatic profiles.

In the realm of synthetic naphthol red dye, the DHI consortium exhibited the highest degradation efficiency at 49.23%. This achievement is remarkable, especially considering the intricate chemical structure of naphthol dyes, which typically pose challenges for degradation (Mahmood et al. 2015). As for indigosol red dye, the DHI consortium showcased a relatively high degradation efficiency of 34.13%, closely followed by DIN at 33.63%. These results imply that certain bacterial combinations are proficient in targeting and disintegrating indigosol dye molecules (Abu Talha et al. 2018).

Isolate J (*Micrococcus* sp.) demonstrates superior performance both individually and within consortia, which can be attributed to its high enzymatic activity. This isolate is capable of producing key enzymes such as oxidase and reductase, which play a critical role in breaking down complex dye molecules. The study highlights that isolate J achieved the highest degradation rates for natural *Indigofera* dye of 99.62% (Fig. 1), remazol red (85.74%), and naphthol blue (83.05%).

At the consortium level, isolate J significantly contributes due to its complementary enzymatic activity when paired with other isolates, such as *Pseudomonas* sp. and *Stutzerimonas stutzeri*, in the INJ consortium. This consortium achieved a decolorization efficiency of 90.29% (Fig. 8) for indigosol blue dye, demonstrating synergy supported by the presence of enzymes such as azoreductase, laccase, and peroxidase. These results are consistent with analogous studies, such as (Elkema et al. 2023), where the singular isolate, *Enterobacter* sp. ASH decomposed 80% of crude oil. Conversely, another study by Calderón-Tapia et al. (2024) suggested that individual strains generally exhibit lower decolorization capabilities in comparison to consortia.

The superiority of isolate J over others may also be linked to its metabolic flexibility, enabling it to degrade a wide range of natural and synthetic dye compounds under various environmental conditions. Its high enzymatic activity and effectiveness within consortia make isolate J a promising candidate for large-scale bioremediation applications.

The capacity of bacteria to degrade batik dyes is significantly impacted by various factors, including: a) culture characteristics and ideal conditions, such as the presence of sodium chloride, initial dye concentration, inoculum size, and pH (Palanivelan et al. 2024), b) the utilization of specific carbon and nitrogen sources, such as mannitol and ammonium sulfate, which are pivotal for achieving optimal removal efficiency (Ramaswamy et al. 2022).

Drawing from these explanations, the benefits of employing bacterial consortia in the degradation of batik dye waste encompass: a) bacterial consortia offer accelerated degradation rates and heightened efficiency, rendering them efficacious for bioremediation (Kurade et al. 2023), b) consortia exhibit resilience to substantial environmental fluctuations, rendering them suitable for practical-scale operations and less reliant on sterile conditions (Ramaswamy et al. 2022), c) the synergistic effects of microbial consortia furnish significant advantages over singular cultures in mineralizing complex dye compounds, leading to enhanced decolorization (Pinheiro et al. 2022), d) the utilization of bacterial consortia in bioremediation is cost-effective and environmentally sustainable, presenting a promising technology for treating batik dye waste (Barathi et al. 2022).

The results of the ANOVA analysis for the consortium of bacterial isolates in decolorizing batik dye waste, in both blue and red colors, are delineated in the subsequent Table 8.

The ANOVA results in Table 8 show no significant differences in the ability of bacterial consortia to degrade blue ($F = 0.573$, $p = 0.811$) and red batik dye waste ($F = 0.294$, $p = 0.972$). ANOVA was performed because the data met the assumptions of normality and homogeneity, and separate analyses were conducted for blue and red dye degradation rates. These findings indicate that all tested consortia have comparable degradation capabilities for both dye types, consistent with previous studies highlighting the efficiency

of bacterial consortia in degrading complex dye structures due to their synergistic enzymatic activities (Barathi et al. 2022, Mishra et al. 2022).

The consistent performance of the consortia suggests they are well-suited for degrading batik dye wastewater, likely due to their ability to adapt to the chemical composition of the waste and produce diverse enzymes. These results support the feasibility of using any tested consortia for bioremediation purposes. Future research should focus on optimizing environmental conditions and scaling up these methods for industrial applications (Ramaswamy et al. 2022).

The results of this study can be applied on a field scale by developing bioreactors based on bacterial consortia to treat industrial wastewater. Pilot-scale testing is necessary to determine optimal conditions, such as retention time and nutrient requirements. These consortia can also be integrated into existing wastewater treatment systems to reduce COD and color intensity. Regular monitoring and safety evaluations of degradation products are essential to ensure that the process is safe and sustainable.

CONCLUSIONS

This study demonstrates that indigenous bacterial isolates, both individually and in consortia, possess significant abilities to decolorize batik dye wastewater, including both natural and synthetic dyes. The findings and implications of this research are as follows:

Identification and characterization of indigenous bacteria: The study isolated 15 bacterial strains from batik dye wastewater, five of which displayed substantial dye degradation abilities. These isolates were identified as *Stenotrophomonas maltophilia*, *Stutzerimonas stutzeri* (H and I), *Micrococcus* sp., and *Pseudomonas* sp. Each of these bacteria showcased the capacity to degrade specific dyes such as remazol, naphthol, and indigosol. The utilization of 16S rRNA gene sequencing was pivotal in precisely identifying these bacteria, emphasizing the genetic and functional diversity within the microbial community of the wastewater.

Effectiveness in dye decolorization: The biodegradation assays verified that the identified bacterial strains could efficiently decolorize batik dye wastewater. Isolate J (*Micrococcus* sp.) and isolate N (*Pseudomonas* sp.) demonstrated the highest overall degradation efficiency, underscoring their resilience and adaptability. Particularly, isolate J achieved a 99.62% degradation of natural dye *Indigofera*, 85.74% for red remazol, and 83.05% for blue naphthol, while isolate N excelled in degrading red remazol dye (94.72%). Moreover, bacterial consortia like INJ (comprising *Stutzerimonas stutzeri*, *Pseudomonas* sp., and

Table 8: ANOVA results on the ability of the bacterial consortium to degrade blue and red batik dye waste.

Degradation rate of blue batik dye waste					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3257.344	9	361.927	0.573	0.811
Within Groups	25280.041	40	632.001		
Total	28537.385	49			
Degradation rate of red batik dye waste					
Between Groups	820.905	9	91.212	0.294	0.972
Within Groups	12389.037	40	309.726		
Total	13209.943	49			

Micrococcus sp.) exhibited remarkable efficiency, achieving a 90.29% decolorization of indigosol blue dye.

This study supports SDG 6 (Clean Water and Sanitation) by providing an eco-friendly bioremediation solution that effectively reduces the toxicity of batik dye wastewater, thereby helping to preserve water resources. Additionally, the study contributes to SDG 12 (Responsible Consumption and Production) by promoting a sustainable approach using local bacteria for industrial waste treatment. This solution is not only cost-effective but also encourages more responsible production practices in creative industries such as batik.

There were no significant differences in the ability of bacterial isolates or consortia to degrade blue and red dyes, or between natural dyes (*Indigofera*, *Caesalpinia*) and synthetic dyes (methyl, naphthol, remazol, indigosol). This demonstrates that the bacteria exhibited consistent efficiency across various types and colors of dyes.

These findings underscore the robust enzymatic frameworks of these bacteria, enabling them to degrade multiple dye compounds under diverse environmental conditions. This study concludes that these bacterial isolates and consortia hold substantial potential for practical implementation in the bioremediation of batik dye waste, providing a cost-effective and environmentally friendly solution to tackle the challenges associated with batik dye waste.

Further research can focus on testing bacterial consortia on complex industrial effluents, such as those from textiles or manufacturing, to assess their effectiveness under real-world conditions. This can include optimizing factors like pH, temperature, and salt concentration to enhance performance. Additionally, developing bacteria with specific capabilities through microbiological engineering could help degrade more complex toxic compounds. Evaluating the environmental impact and safety of degradation by-products is also crucial to ensure the method is safe and sustainable. This approach offers a practical solution for environmentally friendly waste management.

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Fabrication of Loofah Sponge as an Effective Natural Chromium Sequestrant

Santhiya Jayakumar^{ID} and K. J. Sharmila^{†ID}

Department of Biotechnology, Dr. M.G.R. Educational and Research Institute, Maduravoyal, Chennai-600095, Tamil Nadu, India

[†]Corresponding author: K. J. Sharmila; sharmila.ibt@drmgrdu.ac.in

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ABSTRACT

Industrialization is key to a nation's progress, but exposure to industrial effluents containing heavy metals can cause deadly diseases; therefore, mitigation measures must be applied to safeguard both human beings and the environment. In this study, loofah sponges, a natural heavy metal sequestrant, were modified with sodium hydroxide, acetic acid, sequestering, and wetting agents and showed increased mechanical strength with strong interfacial bonding with the composite materials than untreated loofah sponge. Characterization techniques, including ASTM-D570, FTIR, SEM, SEM-EDX, TGA (thermogravimetry analysis), XRD (X-ray diffraction spectra), and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) were employed to investigate the SSGP-pretreated loofah sponge. The FTIR and SEM examination revealed the removal of waxes and contaminants and heavy metal adsorption on the loofah sponge noted in the SEM-EDX image. The ICP-OES analysis revealed that the pretreated loofah sponge adsorbed about 39 mg/g of chromium. This study proved that pretreated loofah sponges adsorbed about 12.4% more chromium than untreated loofah sponges. Therefore, a pretreated loofah sponge, an environmentally based green technology, can be utilized as an effective biocarrier for heavy metal-contaminated effluents. This study supports the Sustainable Development Goals of clean water and sanitation and recommends using a modified loofah sponge for large-scale applications.

INTRODUCTION

Public Health Services in different countries reported the exposure of hazardous excess metals to significant numbers of people in different ways (Cotruvo 2017). The high atomic density and weight are properties of recalcitrant heavy metals that make them quite toxic, even at low concentrations, when released into the environment. Heavy metals were released into the environment after industrialization as untreated or partially treated effluents, which were discharged into the water stream. Due to their constant and bioaccumulation properties, heavy metal sets constantly accumulate in the environment (Saxena et al. 2024, Vidhya et al. 2023). Heavy metals are naturally released into the environment through the leaching of rock and soil into water streams or by human activities, such as the discharge of untreated wastewater directly into rivers (Rezooqi et al. 2021). According to the US EPA, lead, cadmium, and chromium are designated as the top-priority contaminants of major public concern (Faheem et al. 2020). In general, heavy metals are carcinogenic and mutagenic, and they also induce cell apoptosis. Therefore, to mitigate the effects of such heavy metals, natural methods must be taken into account. The advantages and disadvantages of conventional processes are depicted in Fig. 1.

Although advantages are present in conventional processes (Agarwal et al. 2006), the disadvantages outweigh the benefits, making biosorption a glimpse of the future (Kanamarlapudi et al. 2018). Naturally, chromium is predominantly encountered in its oxidation states Cr (VI) and Cr (III) (Goddeti et al. 2020),

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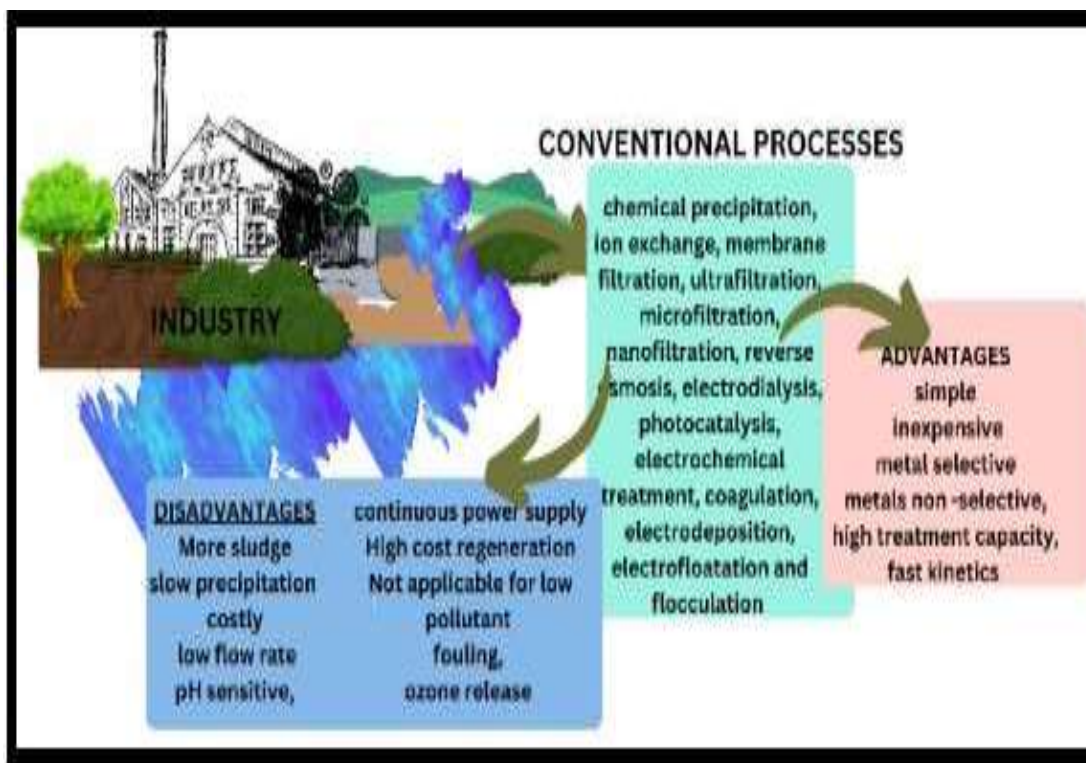


Fig. 1: Advantages and disadvantages of conventional process.

where Cr (VI) showed several times more toxicity, as it is present in industrial effluents that are discharged in tones, so chromium mitigation must be propelled as it is carcinogenic and mutagenic. The loofah sponge (LS) is obtained from the dried fruit of *Luffa cylindrica* (L.), a member of the Cucurbitaceae family, and features a polyporous structure. When dried, it forms a natural mat that can serve as a bio-adsorbent for treating industrial effluents. Asia, Africa, and tropical America are the main cultivation spots for loofah plants (Oboh & Aluyor 2009). The LS has a higher strength, stiffness, and energy absorption capacity and contains cellulose (62%), hemicelluloses (20%), lignin (11.2%), extractives (3.2%), and ash (0.4%), with functional groups that adsorb metals from various sources. Over the past decade, LS has been explored less as a source of wastewater treatment than other fibers; therefore, this research investigated LS as a heavy metal sequestrant for wastewater treatment. This study evaluated the potential of chemically SSGP-pretreated loofah (treated with sodium hematophosphate, sodium hydroxide, glycerol, and potassium permanganate) as a heavy metal sequestrant for the treatment of industrial wastewater. Unmodified loofah adsorbed about 34.7 mg/g of chromium, while SSGP-pretreated LS adsorbed about 39 mg/g of chromium.

MATERIALS AND METHODS

Fiber Collection

Dried LS in sizes between 18 and 22 cm was chosen and collected from a local market in Perambur, Chennai, Tamil Nadu. It was washed thoroughly with distilled water to remove dirt and soil, then dried and cut down to a size of 2 cm for use.

Fiber Modification Process

Washing and pre-sterilization: The 0.85% saline solution was used to remove dirt and biofilms. The loofah was then cut into 2 cm height and pretreated with chemical treatments.

Alkali treatment: To increase the tensile strength of LS, 4% NaOH was used to treat LS at 120°C for 4 hours using a hot plate stirrer. Then washed and dried in a hot air oven at 120°C.

Acid treatment: LS was treated with 2% acetic acid at room temperature for 30 min and washed with distilled water.

Neutral salt treatment: LS was treated with 0.6% potassium permanganate for 5 min at room temperature and washed thoroughly till a neutral pH was reached.



Fig. 2: (a) LS (b) cross-section of LS (c) cut piece of LS (d) alkaline, acid treated LS (e) KMnO₄ treated LS (f) SSGP-pretreated LS.

Sequestering agent: LS treated with 2 % (2 g/l) of sodium hematophosphate at 50°C for 3 hours and washed with distilled water.

Wetting agent: LS was further treated with 30% glycerol for 30 min and washed again with distilled water and 70% ethanol several times to remove dirt and scum, then dried in a hot air oven at 160°C for 4 h and used. The LS and its treatment are depicted in Fig. 2.

The fabricated LS were modified using sodium hydroxide, acetic acid, potassium permanganate (Hallad et al. 2019), and sodium hematophosphate. The inorganic cyclophosphate sodium hematophosphate increases permeability (Sampaio et al. 2022) and removes surface impurities, acting as both a surfactant and a sequestrant (Karim et al. 2024). It was used in conjunction with alkali and potassium permanganate, which enhances the strength of the fiber. The tenacity of the fibers increases, elongation of the fibers occurs (Wetaka et al. 2016), and delignification of the fibers occurs (Ghali et al. 2009), which enhances the shelf life of the fibers. Acetic acid replaces water, aiding in the plastination of fiber (Dhir et al. 2020), and shifts the pH to neutral due to alkali treatment. Glycerol decreases surface tension and reduces the propensity of molecules to stick together (Karim et al. 2024).

Physicochemical Characterization of Loofah Sponge

ASTM-D570 test: The ASTM D570 procedure is used for interpreting the adsorption rate of untreated and SSGP-pretreated LS. Approximately 10 × 0.5 × 0.1 mm LS were immersed in a separate container containing distilled water for one hour at room temperature. Samples were removed at 15-minute intervals and kept between filter sheets under weights. LS water absorption percentages were calculated by weighing the filter paper using a digital balance machine (Tanobe et al. 2014).

$$\text{Water Absorption \%} = \frac{\text{weight after immersion} - \text{weight before immersion}}{\text{weight before immersion}} \times 100$$

FTIR: The positional status of LS chemical functional groups was ascertained by a Fourier Transform InfraRed spectrophotometer (FTIR, Shimadzu IRTRACER 100, Japan). The untreated and treated LS, each at about 1 mg, was crushed and mixed with a 200 mg dosage of potassium bromide at room temperature and 65% humidity. The interaction of infrared light with untreated and SSGP-pretreated LS produces its molecular fingerprint in the wavenumber range of 400–4000 cm⁻¹ at a scan rate of 32 with a resolution of 2 cm⁻¹.

XRD: The crystalline and amorphous structures of untreated and treated LS were investigated by X-ray Diffraction (XRD, X'Pert PRO MRD, PANalytical, Netherlands) at a 50 mA current and 40 kV voltage. The untreated and treated LS diffraction measurements were scanned from 5 to 100 (2θ) with a 0.9° min⁻¹ scanning rate at 25°C.

TG analysis: Thermogravimetry (TG) was performed on untreated and treated LS using a thermogravimetric analyzer (TGDTA, NETZSCH, STA 449F5, Germany) with a heating rate of 30°C/10.0°C/min to 700°C.

SEM (SEM, AMETEK): The surface morphological property of the loofah fiber was observed using scanning electron microscopy (SEM, AMETEK, United States), with an imaging mode at 2 micrometers and a working distance of 20 mm. The loofah fibers were prepared using an aluminum carrier precipitation method and then covered with a thin gold film under a high vacuum at a 15 kV voltage.

SEM EDX: The gold-sputtered loofah fibers were analyzed

for their different elements to determine their strength and distribution using a scanning electron microscope (SEM) equipped with an energy-dispersive X-ray (EDX) detector (EDX AMETEK BV, Tilburg, Netherlands).

ICP-OES: The samples were acid-digested and analyzed for elemental characterization using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Agilent Technologies).

Chromium Removal By Loofah Sponges

Approximately 1 gram of LS was added to 100 mL of 200 ppm chromium-containing conical flasks and incubated for 6 days at 120 RPM and 37°C. The filtrate was then removed, and the sample was analyzed for chromium presence using inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis (Agilent Technologies, USA).

RESULTS

Water Adsorption Test

The LS was cut into 10 x 0.5 x 0.1 mm and used for the adsorption test. The LS showed increased adsorption with an increase in contact time. The absorbency of untreated and SSGP-pretreated LS showed 123% and 114% adsorption within 15 min, respectively, with a further increase in adsorption noted. The highest adsorption rates were 132% and 137% for untreated and SSGP-pretreated fiber, respectively, noted within 24 hours. SSGP-pretreated LS has more water adsorption capacity than untreated LS, so it has great efficiency in absorbing the contaminated effluents that benefit the contamination removal as well as heavy metals removal.

FTIR

FTIR results indicate that sodium hematophosphate-sodium hydroxide, glycerol, and potassium permanganate treatments were perfectly incorporated into the fiber, and chemical modifications shifted peaks of LS from a higher wavelength to a lower wavelength. The FTIR peaks show the chemical complexity nature of untreated LS and SSGP-pretreated

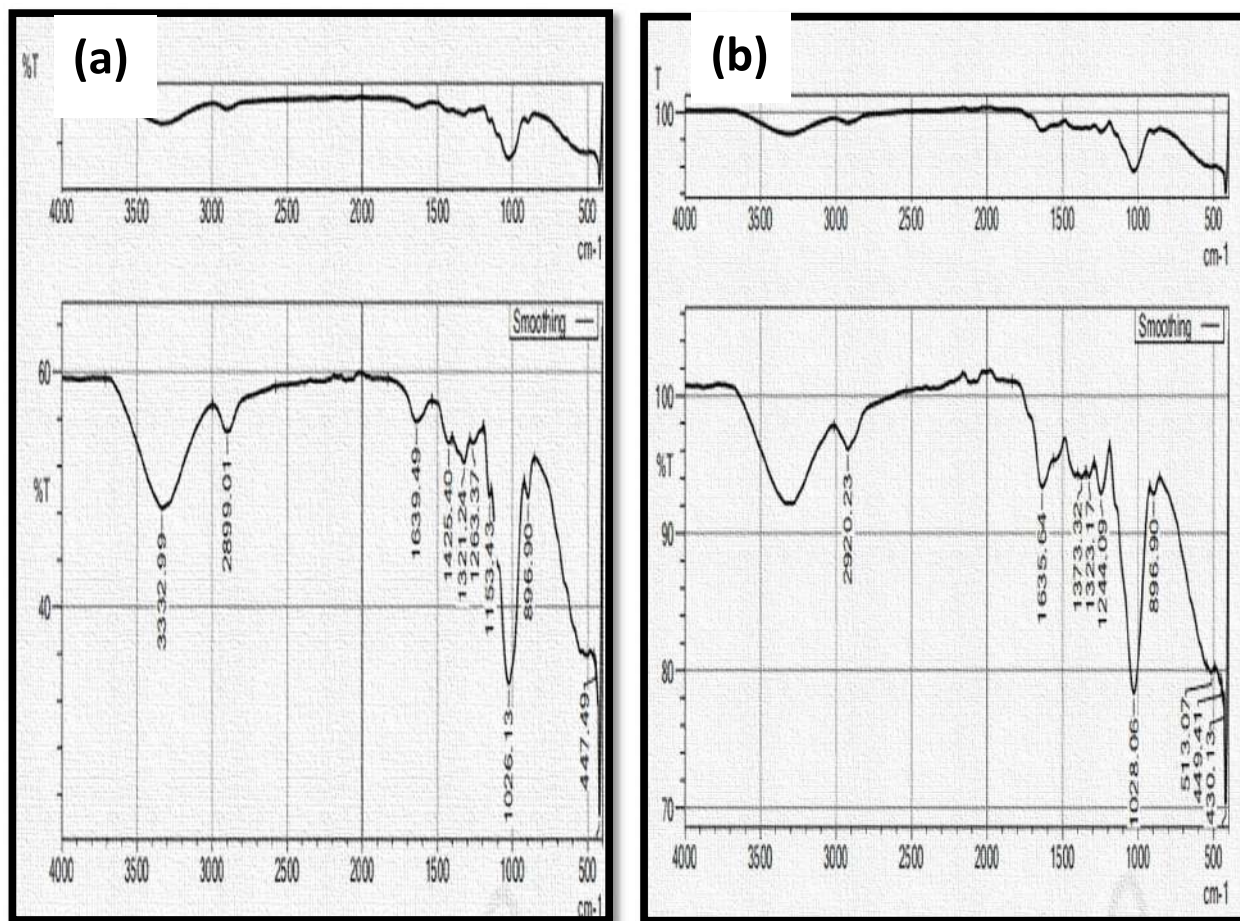


Fig. 3: FTIR spectra peaks of (a) untreated, (b) SSGP-pretreated LS.

Table 1: Chemical stretches and bending distributions and peak locations of Luffa fiber.

Untreated	Treated	Chemical stretching	Reference
420	420	Aryl disulfides S-S stretch	(Coates, 1996)
430	—	C-Cl stretching vibration	(Balachandran et al. 2011)
449	447	449 cm ⁻¹ (PO ₄ bond) to aryl disulfides: S-S stretch.	(Coates, 1996; Ramdan et al. 2017)
513	-	C-H bending vibrations.	(Jeya et al. 2018)
897	897	C-O-C stretching, β -glycosidic linkage.	(Rahman & Khan 2007)
1028	1026	Pectin-OH bond stretches more in SSGP-pretreated LS than in untreated alkyl and aryl halides. In untreated loofah fibers, C-H stretches happened at higher points.	(Mollah et al. 2023)
1244	1153	C-O stretching (1244 cm ⁻¹) to C-O-C bond (1153 cm ⁻¹).	(Rusu et al. 2008; Saito & Iwata 2012)
1323	1263	NO ₂ stretches (1323 cm ⁻¹) shifts to C-H stretching at 1263 cm ⁻¹ in the fingerprint region-pyranose ring.	(Thombare et al. 2023, Zhang et al. 2018)
1373	1321	Phenol O-H bending (medium) of the untreated material shifts to alkane C-H stretch (methyl and methylene groups of cellulose, hemicellulose, and lignin).	(Lakshmanan et al. 2018)
1636	1425	The alkene C=C symmetric stretch (medium) in untreated fibers is shifted to CH ₂ bending, which is hidden in fingerprint regions.	(Sentharamaikkannan et al. 2019)
2920	1639	The alkane CH ₂ asymmetrical stretching (2920 cm ⁻¹ , medium bond) of waxes in loofah fibers shifts to the alkene C=C stretch (medium) in SSGP-pretreated fibers.	(Zhang et al. 2018)
3333	2899	The sharp alcoholic O-H stretch (med) of untreated samples shifts to the alkane C-H stretch (med, which is present in lignin, cellulose, and hemicellulose methyl and methylene groups).	(Zeng et al. 2019))
3758	3333	Pectin, sharp alcoholic O-H stretching of untreated fibers (α - cellulose) shifts to sharp alcoholic O-H stretch (med stretching (lignin).	(Karim et al. 2023)

LS, as depicted in Fig. 3. Functional groups and chemical stretching involved in pretreated and SSGP-modified LS at

a wavelength between 400 and 4000 cm⁻¹ were shown in Table 1. The untreated sample, with bond frequencies at 430

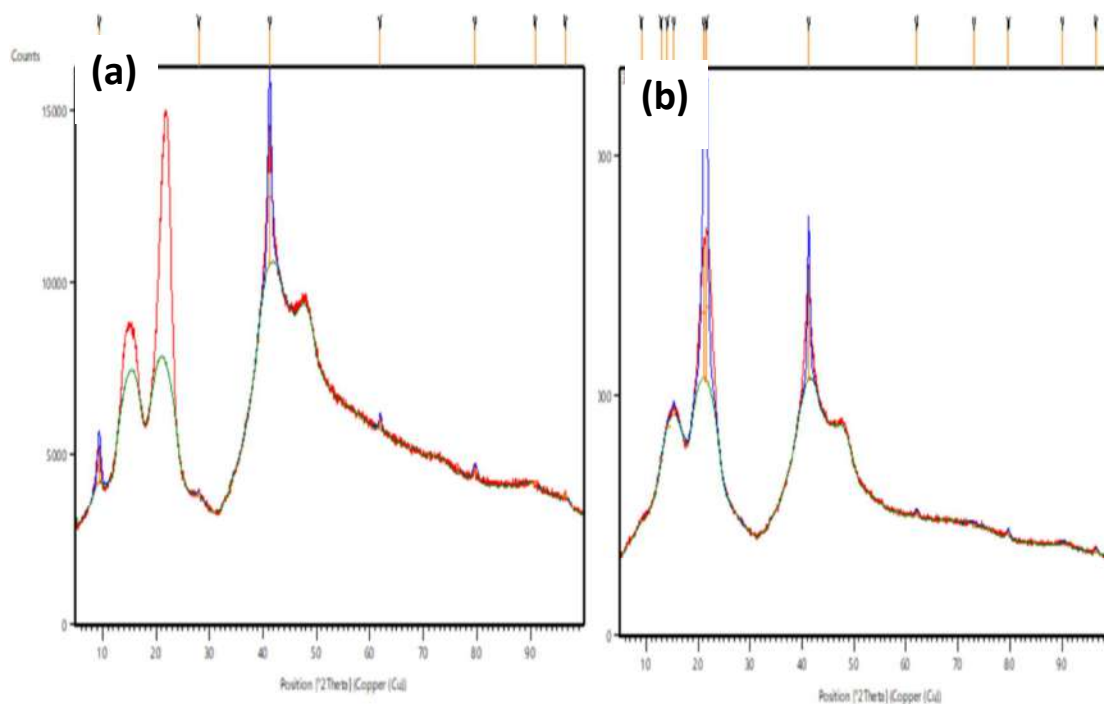


Fig. 4: (a) XRD graph interpretation; (b) XRD-SSGP-pretreated; (c) XRD-untreated LS.

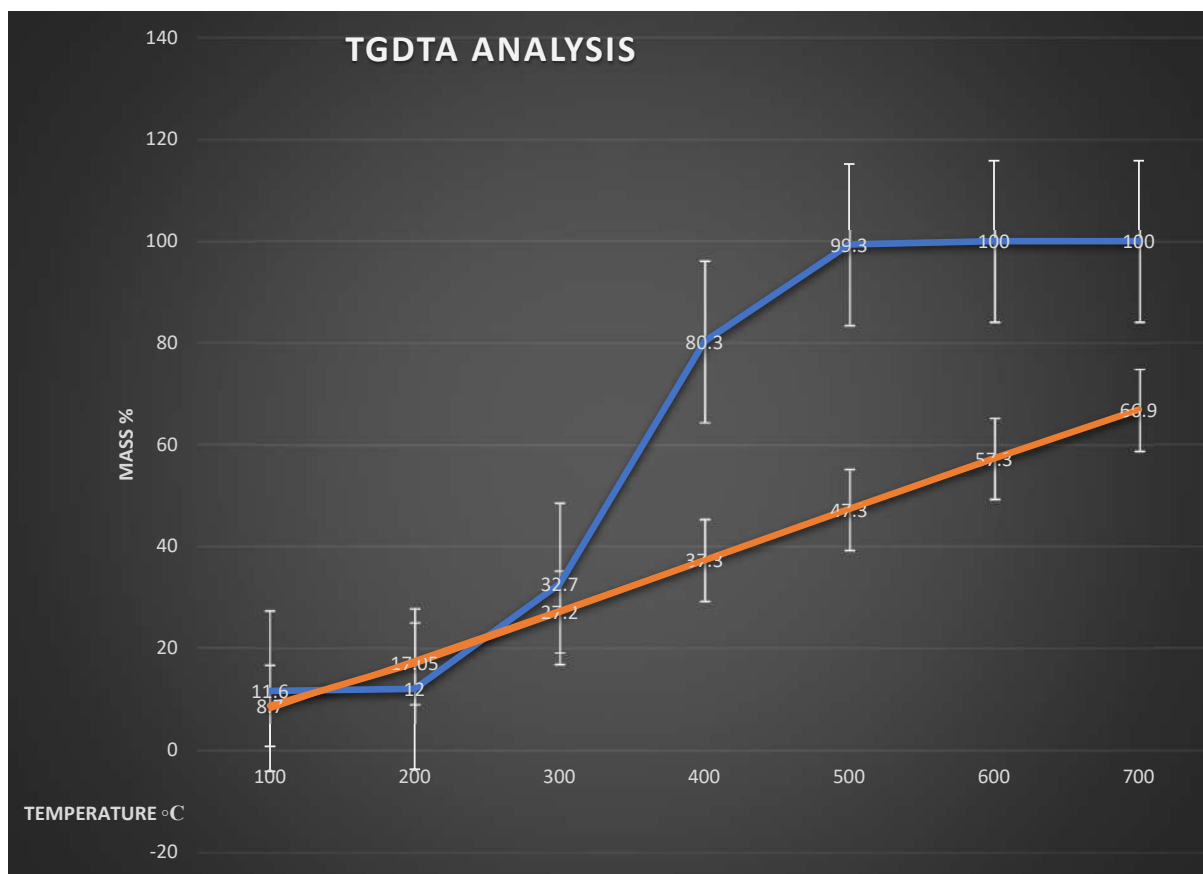


Fig. 5: TGDTA analysis of untreated and treated LS.

cm⁻¹ and 513 cm⁻¹, denotes highly polar covalently bonded C-Cl stretching and C-H bending vibrations, respectively, which are not present in the treated sample. The chemical modifications show an increase in bond dissociation energy with increased heat, breaking bonds to simpler forms or free bonds, which helps sequestrate heavy metals to LS.

XRD

Sharp and strong peaks indicate the crystallinity property of both LS samples; SSGP-pretreated LS shows increased tensile strength. The pattern of untreated loofah exhibits a broad peak at $2\theta = 21.6^\circ$ and a small peak at 21.1° , 17.9° . The pattern of SSGP-pretreated loofah exhibits a broad peak at $2\theta = 41.24^\circ$, 27.9° , and a small peak at 9.37° (Fig. 4).

The characteristic peaks at 9.3° were not present in untreated LS. The 41.27° peak shifts to 41.24° , and the 27.6° peak shifts to 27.9° with higher relative intensity. The increase in crystallinity index shows high tensile strength and microstructural regularity of SSGP-pretreated LS. Therefore, chemical modifications in LS increased the crystalline index, resulting in modified LS that exhibits higher strength, wear

resistance, and toughness, as well as ductility, compared to untreated LS.

TG/DTA

Thermogravimetric analysis was performed using a thermal analyzer (NETZSCH STA 449F5, Germany) for both untreated and treated LS at a heating rate of 10 K/min and a nitrogen flow rate of 250 ml/min in the temperature range of 30-700°C (Matter, Hassan, Elfaramawy, & Esmail, 2024).

Table 2: TGDTA analysis of LS.

Temperature °C	TGDTA Untreated %	TGDTA SSGP-pretreated %
100	11.6	8.7
200	12	17.05
300	32	27.2
400	80	37.3
500	99.3	47.3
600	100	57.3
700	100	66.9

The result shows that at temperature 700°C, only 66.9% mass loss occurred in SSGP-pretreated LS, but untreated LS mass loss happened within 600°C, showing the modification treatment by SSGP greatly strengthened SSGP-pretreated LS, and as the sturdiness is increased in SSGP-modified LS, it can be reused more than untreated LS Fig. 5, Table 2.

SEM

The morphological properties of untreated and treated fiber at 300 X magnification were captured using Scanning Electron Microscopy images (FEI Quanta 600 FEG ESEM, USA). The change in surface morphology, removal of waxes, and surface impurities on surface visualized in SEM images. The waxes and impurities on the loofah surface prevent water adsorption in untreated LS. After chemical treatment, a surface free from waxes, dirt, and impurities was visible in SEM images (Fig. 6).

SEM-EDX

SEM with energy dispersive X-ray spectrometry (SEM-EDX; FEI Quanta 600 FEG ESEM, USA) elemental analysis showed the presence of the Crystal lattice structured chromium heavy metal presence in the surface of both untreated and SSGP modified LS (Fig. 7). The weight and atomic percentage of up to 26.82% and 30% were noted in the surface of LS, which shows free bonds sequestered the heavy metal on the LS surface as well as layers of LS. Therefore, the modified LS demonstrates the chromium adsorption capability across all layers of LS.

ICP-OES Analysis

The ICP-OES analysis utilizes argon plasma to excite the HM-containing solution, emitting light with a wavelength proportional to the elements. The analysis showed that untreated loofah adsorbed only 34 mg/g of chromium, but SSGP-pretreated LS adsorbed about 39 mg/g of chromium, which is 12.4 % more than untreated LS. Thus, LS modification has increased chromium adsorption at the surface and in the layers, as confirmed by the ICP-OES analysis.

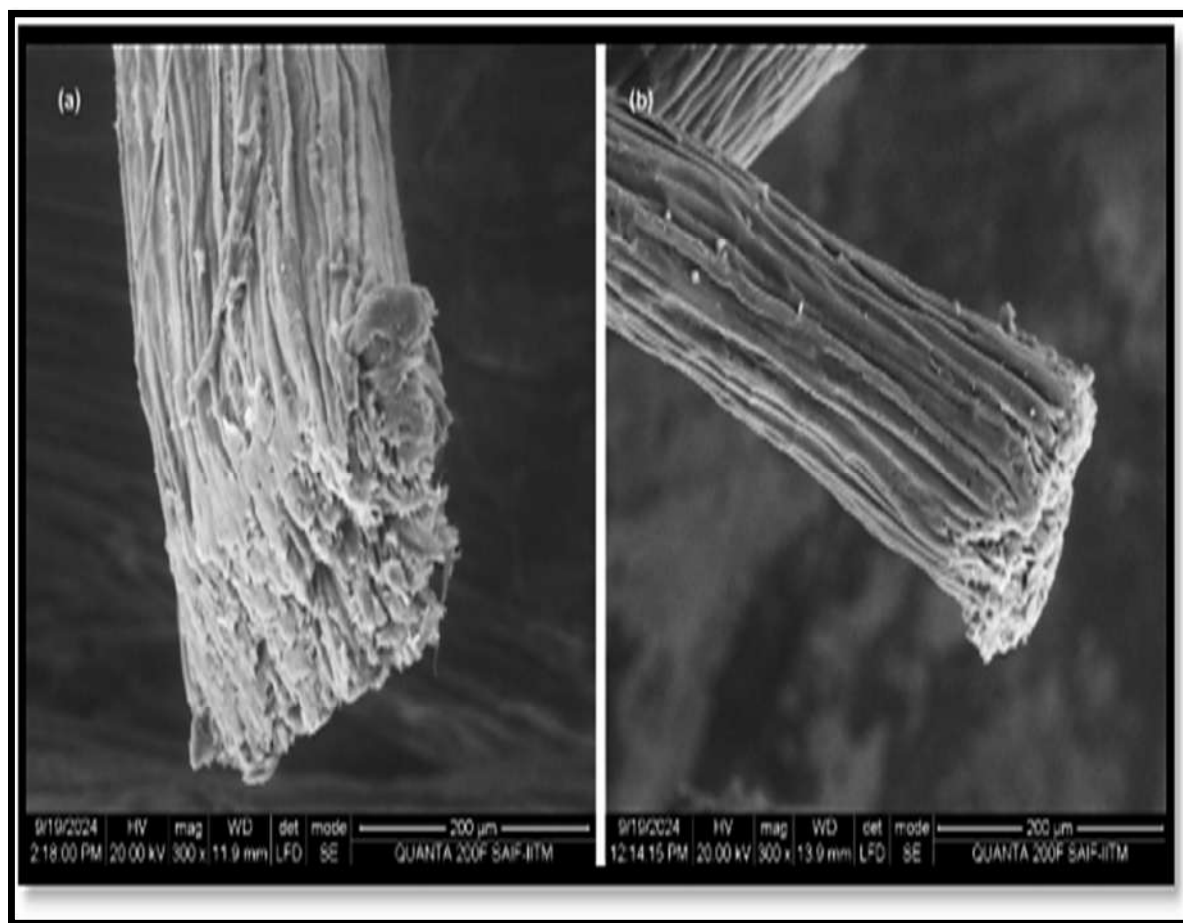


Fig. 6: SEM images of (a) untreated; (b) SSGP-pretreated.

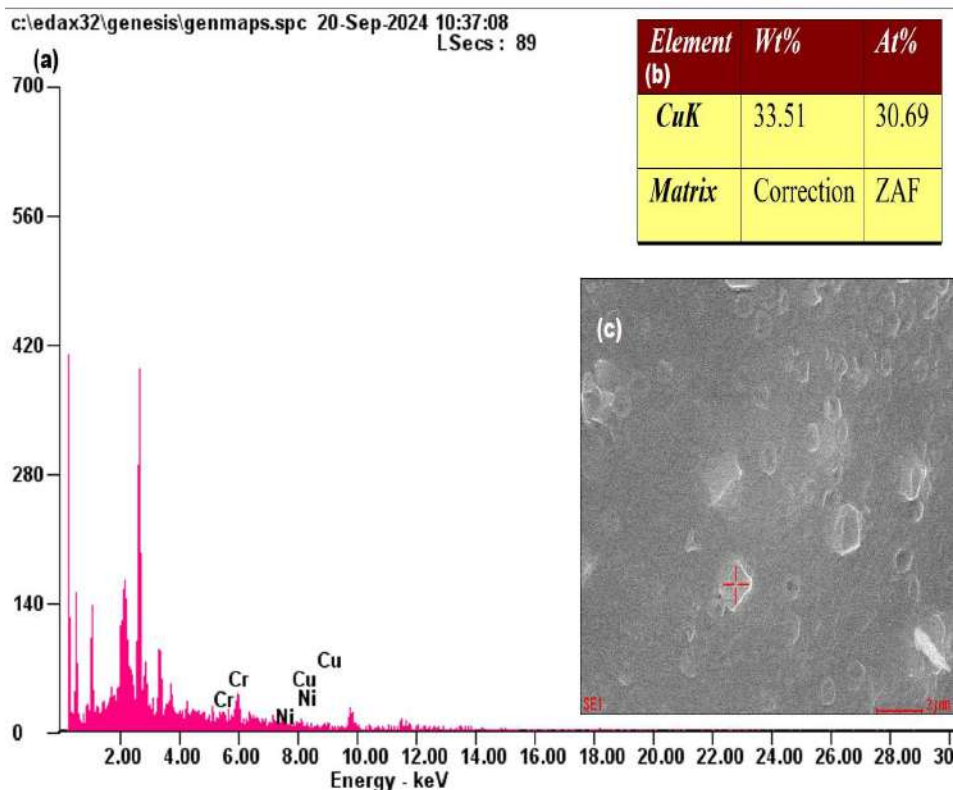


Fig. 7: SEM-EDX of elemental chromium.

DISCUSSION

The conventional treatment process is harmful to our environment as it uses chemicals in large amounts, which makes the process expensive. Additionally, conventional approaches have several disadvantages, as discussed in Fig. 1, making the secondary pollutants discharged during these treatments a significant threat to our environment. The treated water must be further treated to remove these chemicals, which makes the process unacceptable. The natural bioremediation of heavy metals is achieved through various approaches, including the use of activated carbon, biological organism-based heavy metal biodegradation, and bioremediation using natural fibers. The activated carbon-based bioremediation results showed that activated carbons from different sources exhibited varying results and demonstrated specificity in adsorption. The activated carbon process requires a significant amount of materials as its source, but only a very small amount is produced at the end of pyrolysis, which can even lead to air pollution. The activated carbon cannot be implemented on a large scale because the source varies, and the result also varies. Additionally, pyrolyzing tons of sources daily raises significant concerns, making large-scale implementation

unapproachable. The biological-based treatment is a great source of bioremediation, but growing microbes daily requires a good source of amendments, which makes the process expensive. Additionally, environmental contamination is a possibility, and the failure of the approach is also a concern. Implementing one organism is not possible, so different micro-organism-based bioremediation must be studied as the optimized heavy metal degradation is possible only at optimized conditions, which is specific for different organisms, so implementing on a large scale requires a bioreactor for propelling certain organisms at certain conditions, and using organisms that is not present in that habitat may cause an imbalance in the environment. In certain studies, genetically modified organisms are also used, so ethical reasoning is also present in this approach, so this technique has a lot of lagging in large scale implantation. The LS natural adsorbent is cost-effective, and locally available materials can be implemented as it is inexpensive. And makes this technique approachable. This technique can be combined with existing technology to make the process more cost-effective or used as a novel approach. This technique doesn't require new plant construction, as it can be implemented in any existing treatment plant, and it is also easy to train professionals for this approach. The collected samples were

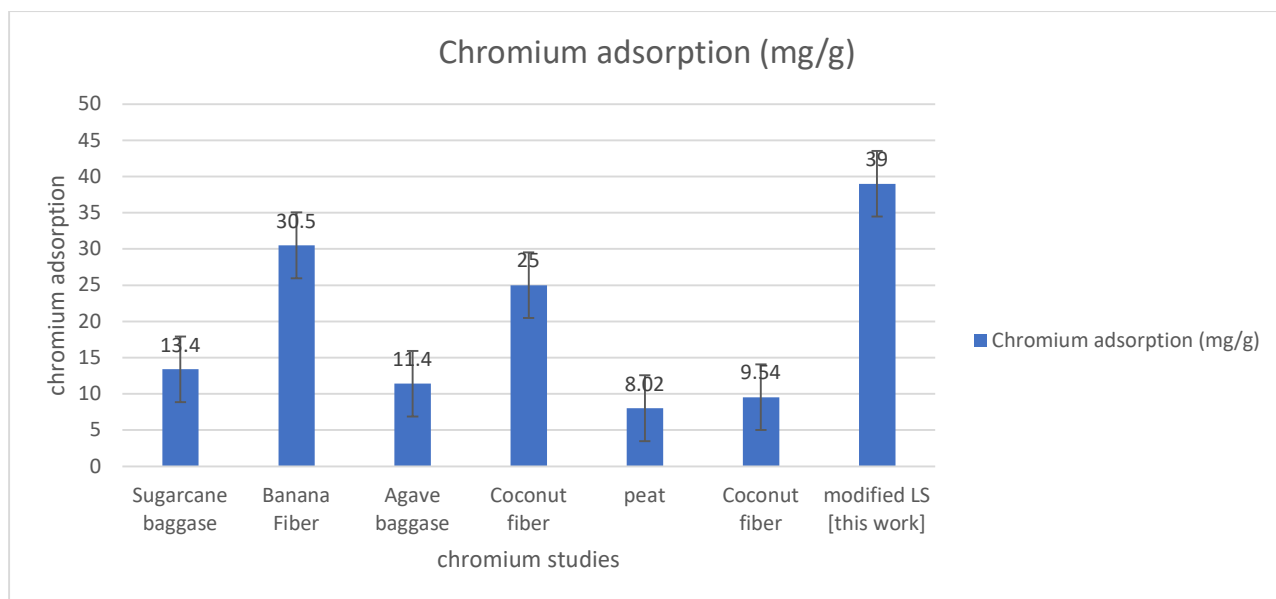


Fig. 8: Chromium adsorption by natural fiber.

cut in 2 cm size and modified with SSGP. LS is composed of 55–90% of cellulose, 8–22% of hemicellulose, 10–23% lignin, extractives >3.2%, ash, and others 0.4% (Chen et al. 2019), as the impurity blocks the water adsorption, alkali treatment is done (Begum et al. 2021). The SSGP-pretreated LS exhibited approximately 137% increased water adsorption with an increase in contact time (Tanobe et al. 2014). FT-IR spectra peaks at a wavelength between 400 and 4000 cm^{-1} in untreated and SSGP-pretreated LS show a change in functional groups, and certain wavenumbers were not present in modified LS, which shows incorporation of SSGP, lower bond energy, and also change in chemical stretching is also noted in modified LS as mentioned in Fig. 3, Table 1. An increase in tensile strength and crystallinity index was noted, with sharp and strong peaks in modified LS (Mohanta & Acharya 2016). Additionally, more peaks, such as $2\theta = 9.37^\circ$, demonstrated the robustness of the modified LS (Goddeti et al. 2020). The thermogravimetric analysis of both LS and the SSGP-modified LS shows that they withstand approximately 40% of the sample, even at 700°C (Matter et al. 2024). The SSGP-pretreated LS exhibited a clear surface morphology, with water adsorption impeding the presence of waxes and impurities (Goddeti et al. 2020, Matter et al. 2024). Additionally, chromium metal was noted on the LS surface (Mohanta & Acharya 2016). The ICP-OES analysis (Kotelnikova et al. 2024, Zeng et al. 2019) proved the adsorption status of LS, SSGP-modified LS about adsorbed 39 mg/g of chromium, which is more than previously reported studies with sugarcane bagasse (Sharma & Forster 1994), banana fiber (Begum et al. 2020), agave

baggase (Bernardo et al. 2009), coconut fiber (Franguelli et al. 2019), peat and coconut fiber (Kaszycki et al. 2004) and more depicted in Fig. 8.

The modified LS was reported to be copper sequestrant in our previous study (Jayakumar & Sharmila 2024). This study recommends the environmentally based green technology of using SSGP-pretreated LS as a superior option for chromium sequestration, which can be applied in large-scale applications. Thus, the SSGP-pretreated LS can be utilized as a source of heavy metal sequestration in industrial effluents contaminated by chromium and can also be employed at metal-contaminated sites for enhanced chromium sequestration.

Potential Challenges of Large-Scale Application

Although using LS for secondary treatment can be a novel approach or combined with conventional methods, approving new technology by the public, as well as government and private bodies, takes considerable time.

LS is seasonal and requires a significant amount of time to grow and harvest, so fibers must be switched or stocked for future use.

Future Research

In the future, the SSGP treatment must be tested for other fibers, as per the local availability of India as well as other countries, for converting the conventional approach, which produces the secondary pollutants as the result of treatment, to a natural fiber-based green approach for the betterment

of one's country's agriculture sector as well as reducing contamination in the environment using agriculture based natural fibers such as LS. The SSGP-LS was tested only for copper and chromium sequestration; therefore, further research is needed to investigate the removal of other heavy metals and pollutants, such as dyes, as well as contaminants like sulfate, nitrate, total dissolved solids, and total suspended solids.

CONCLUSIONS

This study demonstrates that novel SSGP-pretreated LS is an effective adsorbent for heavy metals. The sodium hemetaphosphate, glycerol, potassium permanganate, acetic acid, sodium hydroxide modified, and SSGP-pretreated LS showed effective chromium adsorption; about a 12.4% increase in efficiency of chromium adsorption was noted. The FTIR results proved the successful incorporation of SSGP in pretreated LS, and XRD results showed SSGP-pretreated LS is more crystalline and sturdier than untreated LS. SEM images showed that the chemical treatments significantly removed the impurities and waxes present in LS, which prevents water adsorption. The SEM-EDX results showed the efficiency of untreated and SSGP-pretreated LS as chromium adsorbent; the crystalline lattice structure of chromium was observed in LS. The ICP-OES analysis proved the chromium adsorption capacity of SSGP-pretreated LS, with a 12.4% further increase in adsorption noted. The SSGP-pretreated adsorbent exhibits good chromium adsorption, approximately 39 mg.g⁻¹, making it suitable for use as a heavy metal sequestrant in heavy metal-contaminated industrial discharges and sites that receive sludge with heavy metals. So, the SSGP-modified LS can be used as a strong metals sequestrant. This study utilized simulated heavy metals for treatment; therefore, future research should test real-time tannery effluent to observe the SSGP-LS efficiency in sequestering metals, as well as other contaminants.

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Ibuprofen Pollution in the Environment: A Critical Review of Sources, Physicochemical Properties, Ecotoxicological Implications, Human Health Risks, and Bioremediation Technologies

Ali Mohsen Mohammed[†] , Aalaa Fahim Abbas  and Haider Mashkoor Hussein 

Al-Qadisiyah University, College of Science, Al Diwaniyah, Iraq

[†]Corresponding author: Ali Mohsen Mohammed; nnb893505@gmail.com

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ABSTRACT

Ibuprofen (IBU) is increasingly recognized as a significant category of emerging micro-pollutants that infiltrate aquatic ecosystems. IBU possesses a significant capacity to inflict ecological harm, adversely affecting both ecosystems and the health of humans and animals. The primary contributors to the environmental presence of IBU encompass the pharmaceutical manufacturing sector, wastewater treatment plants (WWTPs), hospital effluents, and agricultural byproducts. The degradation of IBU is contingent upon various factors, including its chemical and biological persistence, physicochemical properties, and the methodologies employed for its treatment. A multitude of techniques has been employed to mitigate its detrimental effects, involve adsorption, coagulation, bioremediation (constructed wetlands (CWs), membrane bioreactors (MBRs), microalgal-based systems), advanced oxidation processes (AOPs), membrane filtration systems (including reverse osmosis, nanofiltration, and microfiltration), as well as photocatalytic methods, among others. The exploration of more innovative and effective technologies aimed at IBU degradation necessitates a thorough investigation and should be specifically tailored for cost-efficiency and scalability. Additionally, the assessment of green and eco-friendly alternatives for IBU, characterized by attributes such as negligible bioaccumulation, minimal persistence, environmental compatibility, and low or no toxicity, is equally essential. Bacterial degradation mechanisms constitute a highly promising alternative for the biodegradation of IBU, especially through the application of meticulously chosen strains that have been isolated from contaminated environments.

INTRODUCTION

Water is indispensable for all forms of life and should never be underestimated, even though water blankets more than 71% of the Earth's surface, only approximately 1.2% of it is considered potable. This restricted availability underscores the necessity of clean water for maintaining a healthy way of life (Ranjith & Rajeev 2023). Regrettably, various substances like industrial chemicals, personal care products, pesticides, plastic debris, synthetic hormones, and pharmaceuticals have been discharged by rapidly advancing industries and urban areas, leading to the contamination of a significant portion of drinkable water sources and impacting water quality as well as aquatic life (Parolini & Binelli 2012, Show & Halder 2024, Yuniarto & Hadibarata 2024).

Over the past two decades, developed nations have observed an unparalleled increase in pharmaceutical consumption attributed to the expanding global population, demographic aging, and enhanced investments in health care infrastructure. In the year 2001, worldwide expenditures on pharmaceuticals amounted to 390 billion dollars, which escalated to 1.2 trillion dollars in 2018 and are estimated to reach 1.5 trillion dollars by the year 2023 (IQVIA 2019).

Pharmaceutical compounds are frequently identified as significant emerging pollutants within aquatic ecosystems globally (Samal et al. 2022). Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), for instance, paracetamol, naproxen, and ibuprofen, are among the most widely used pharmaceuticals and consequently, the most significant pollutants. The existence of NSAIDs contamination poses a significant concern within ecosystems due to its extensive magnitude, complex nature, as well as toxic potential (Rastogi et al. 2021, Pawłowska et al. 2023). Ibuprofen (IBU) is a widely familiar anti-inflammatory agent with high rates of human consumption, often prescribed for relieving fever, headaches, toothaches, and more (Mazaleuskaya et al. 2015, Chopra & Kumar 2020, Yilmaz & Goktas 2023). IBU constitutes one of the substances suggested as option 1 for inclusion in the catalog of individual priority compounds within the European Union's initiative for a novel directive revising (Document 52022PC0540, 2022). Anthropogenic activities are accountable for the escalation in the concentration of IBU within the environment. Concentrations of IBU observed in natural aquatic environments vary from 37 ng.L⁻¹ to 30 µg.L⁻¹, whereas in wastewater systems, concentrations fluctuate between 406 ng.L⁻¹ and 2.11 mg.L⁻¹ (Thalla & Vannarath 2020, Rastogi et al. 2024).

However, improper disposal, industrial facilities, WWTPs, veterinary treatments, among others, also contribute to the introduction of IBU into various environmental compartments. After ingestion by humans and animals, the substance is not entirely metabolized and subsequently eliminated from the body. The enzymes found in humans and animals facilitate the conversion of this substance into a variety of metabolic products. The resultant excretion product encompasses both IBU and its associated metabolic byproducts, which may exhibit greater toxicity than the original compound. Subsequent to excretion, these substances are introduced into WWTPs, lakes, rivers, soil, groundwater, and oceans, among other environments. Additionally, they are consumed by plants and aquatic organisms. Consequently, this process facilitates the incorporation of these substances into the food web (Jan-Roblero & Cruz-Maya 2023, Chopra & Kumar 2020). Some recognized harmful influences associated with heightened exposure to IBU encompass cellular and genetic impairment, disruption of enzymatic equilibrium, reduction in body length and ovum production, deterioration of overall health status, and modifications in hematological parameters across various organisms. Owing to its widespread presence and bioaccumulation within aquatic food structures, eradicating IBU from water bodies has become an urgent issue for researchers (Du et al. 2016, Wang et al. 2016, Rastogi et al. 2024). IBU's environmental persistence varies when

compared to other NSAIDs. For instance, a study comparing the photodegradation rates of IBU and ketoprofen in aqueous solutions revealed that IBU degrades more slowly under light exposure, suggesting a higher environmental persistence relative to ketoprofen (Szabó et al. 2011). In addition, biodegradation studies have demonstrated that IBU is more amenable to microbial breakdown than diclofenac. Using activated sludge systems, it was found that IBU degrades at a faster rate, pointing to its lower persistence in wastewater treatment processes compared to diclofenac (Peng et al. 2019). In contrast, paracetamol exhibits lower environmental persistence compared to IBU due to its higher rate of biodegradation (Quintelas et al. 2020).

The issue of pharmaceutical contamination, particularly that of IBU, is complex because of the insufficient accessibility of effective strategies for capturing, extracting, or eliminating these compounds in a controlled and efficient manner. Conventional wastewater treatment processes are inadequate for eliminating pharmaceutical residues. Furthermore, the diverse chemical composition of pharmaceuticals presents a significant challenge in developing targeted treatment technologies. However, a variety of advanced technologies are available for IBU removal, including advanced oxidation processes (AOPs), membrane processes, membrane bioreactors (MBRs), coagulation-flocculation, UV/H₂O₂, electronic oxidation, adsorption, Fenton and Fenton-like oxidation, O₃/UV, and sonolysis (Chopra & Kumar 2020, Rastogi et al. 2024, Singh et al. 2022).

These methods have some limitations, such as operational costs, sludge generation, pre-treatment requirements, operability, reliability, and efficiency. However, many toxicological studies have shown that the intermediate byproducts generated during advanced chemical treatments can exhibit higher toxicity compared to the initial compound. Additionally, these oxidation technologies often have a significant environmental impact due to high energy consumption (Quero-Pastor et al. 2014, Chopra & Kumar 2020, Show & Halder 2024). As a result, IBU biodegradation is emerging as a promising substitute for removing IBU contamination in water bodies. The biodegradation method offers a number of benefits compared to conventional, physical, and chemical techniques, including being a cost-effective, natural process, capable of operating under various circumstances. Microorganisms metabolize IBU, producing carboxylated-ibuprofen and hydroxyl-ibuprofen as byproducts of the biodegradation process (Chopra & Kumar 2020, Show & Halder 2024, Lara-Moreno et al. 2024).

The objective of this review was to conduct a comprehensive evaluation of the properties, pathways, ecological and human

health effects, and possible bioremediation approaches of IBU. IBU has been selected as a focus pollutant owing to its widespread detection in aquatic ecosystems at elevated concentrations and its known ecotoxicological effects. Previous studies on IBU pollution have primarily focused on its environmental presence, human health impacts, and traditional removal methods. However, many of these studies fail to comprehensively explore the mechanisms of IBU biodegradation, particularly the role of specific bacterial strains and their genetic components. Additionally, there is limited discussion on the integration of biological degradation methods with engineering and physical systems to enhance efficiency. While some research has explored IBU detection methods, the development and application of advanced and more sensitive techniques for IBU quantification in environmental samples remain insufficiently addressed.

Furthermore, studies often overlook detailed analyses of the main sources contributing to IBU pollution, such as pharmaceutical industries, hospital waste, and domestic sewage. This review addresses these gaps by providing a thorough analysis of IBU's structure, its effects on humans and living organisms, and advanced methods for its removal, including bacterial degradation. It also highlights the latest detection techniques for IBU and examines major environmental sources responsible for its release. Additionally, the review delves into the genetic factors responsible for IBU biodegradation and evaluates the influence of environmental parameters such as temperature, pH, and heavy metals on bacterial efficiency. By combining biological and engineering approaches, this work presents a holistic framework to tackle IBU contamination more effectively than previous studies.

MATERIALS AND METHODS

This systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency and accuracy in the selection and analysis of relevant studies. The following steps were followed in accordance with PRISMA:

1. **Protocol:** A search protocol was developed before the review to ensure consistency in study selection. The main objective was to evaluate the impact of ibuprofen contamination and its removal methods. Inclusion and exclusion criteria were predefined to focus on studies directly related to ibuprofen degradation and bacterial removal processes.
2. **Inclusion/Exclusion Criteria:** Studies were included if they focused on IBU's degradation, physicochemical properties, bacterial removal methods, and environmental impact. Studies were excluded if they did not provide

experimental data, focused on unrelated pollutants, or lacked peer-reviewed status.

3. **Search Strategy:** A comprehensive search was conducted in databases including PubMed, Scopus, and Google Scholar using keywords such as ibuprofen degradation, bacterial removal of ibuprofen, environmental contamination, bioremediation methods of ibuprofen, and more. The majority of studies were published in the last five years.
4. **Study Selection Process:** After searching, a total of 170 studies were identified. Following the inclusion and exclusion criteria, 20 studies were excluded for reasons such as unrelated topics or methodological issues. Ultimately, 150 studies were included in the final review.
5. **Quality Assessment:** The quality of the included studies was evaluated based on their peer-reviewed status and methodological rigor. Preference was given to studies with clear experimental designs and robust data reporting.
6. **Data Synthesis and Analysis:** Data from the selected studies were synthesized qualitatively, focusing on the methods of ibuprofen removal and the environmental implications. A comparative analysis was performed to highlight the most effective removal techniques.
7. **Discussion of Limitations:** While this review followed systematic review principles, some limitations include focusing on recent and relevant studies, which may have excluded some older research or studies that didn't focus directly on research items.

THE STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF IBUPROFEN

IBU is a highly popular over-the-counter medicine, ranking third globally (Murdoch & Hay 2015, Xu et al. 2018, Marchlewicz et al. 2017b). IBU, with the molecular formula $C_{13}H_{18}O_2$, is chemically described as 2-(4-isobutylphenyl) propanoic acid, which encompasses an aromatic ring with isobutyl substituents and propanoic acid, alongside two enantiomeric forms (R and S) (Chopra & Kumar 2020, Show & Halder 2024). The name "ibuprofen" is derived from its structural components: "ibu" for isobutyl, "pro" for propionic acid, and "fen" for phenyl (Show & Halder 2024). IBU exhibits low water solubility and significant mobility in aqueous environments; the main physicochemical characteristics of IBU are presented in Table 1.

THERAPEUTIC USE, CONSUMPTION AND HUMAN METABOLISM OF IBUPROFEN

IBU is a prevalent medicine for both human and animal

health and is involved in the World Health Organization WHO “Essential Drug List”; it possesses anti-inflammatory, analgesic, and antipyretic influences. It is available in a variety of dosage forms, such as capsules, tablets, granules, oral suspensions, creams, suppositories, drops, injections, and gels. IBU is a widely used medication across many societies for various medical purposes. In the United States, the United Kingdom, and Poland, people consume a significant amount of IBU each year, at around 300, 162, and 58 tons, respectively (Ferreira et al. 2023, Marchlewicz et al. 2015, Jan-Roblero & Cruz-Maya 2023). Recent years have seen an escalation in IBU use across numerous Nordic countries. Nevertheless, Norway and Denmark have a lower consumption rate compared to other Nordic countries, with figures approximating 2.0 and 1.15 tons annually, in that order (Jan-Roblero & Cruz-Maya 2023, Hudec et al. 2012).

IBU is utilized in the therapeutic management of osteoarthritis, migraines, cancer, gout, rheumatoid arthritis, pericarditis, fever, neuritis, muscle soreness, neuropathic pain, dysmenorrhea, and postoperative pain management (Ivshina et al. 2021, Chopra & Kumar 2020, Xu et al. 2018, Yılmaz & Goktas 2023). IBU hinders the activity of cyclooxygenase, an enzyme that catalyzes the biotransformation of arachidonic acid into cyclic endoperoxides, subsequently leading to the formation of thromboxanes and prostaglandins, both of which are recognized as key mediators in the inflammatory process. The inhibition of cyclooxygenase, along with the subsequent synthesis of prostaglandins, leads to a reduction in the secretion of inflammatory agents

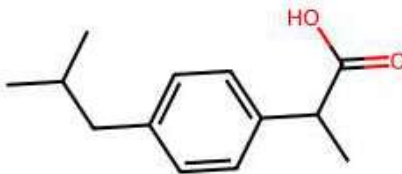
and mediators, thereby impeding the nociceptor activation (Upadhyay et al. 2021, Jan-Roblero & Cruz-Maya 2023, Parolini et al. 2011).

Humans metabolize IBU through two distinct pathways: conjugation and hydroxylation. Conjugation involves reactions with glucuronic acid, sulfates, or glutathione (Marchlewicz et al. 2015). The oral administration of IBU leads to its rapid distribution across the human organism, with a considerable fraction (99%) associating with plasma albumin (Marchlewicz et al. 2015). Only a small portion (1-8%) of IBU leaves the body unchanged after it is taken. The majority undergoes a two-step process for excretion: first, the body oxidizes IBU in phase one metabolism, then, in phase two, these modified molecules are conjugated, often with glucuronic acid, for easier excretion. Roughly 14% of the total IBU dose is excreted as a glucuronide conjugate. Carboxyhydratopic acid (CA-IBU), carboxyibuprofen (CA-IBU), and Hydroxyibuprofen (OH-IBU) are the primary metabolites of IBU found in humans (Aissaoui 2017, Aissaoui et al. 2017). In the environmental context, glucuronide-conjugated ibuprofen has the potential to undergo hydrolysis, thereby liberating free IBU (Murdoch & Hay 2015, Marchlewicz et al. 2015). Fig. 1 illustrates the proposed pathway for IBU metabolism in the human body.

SOURCES OF IBUPROFEN (EMERGING POLLUTANT) IN THE ENVIRONMENT

IBU production has been estimated at thousands of tons

Table 1: Physicochemical characteristics of IBU (Majeed et al. 2018, Ferreira et al. 2023, Akay et al. 2021)

Item	Ibuprofen
Structure	
Formula	$C_{13}H_{18}O_2$
Molecular weight [g.mol ⁻¹]	206. 28
CAS no.	15687-27-1
Density [g.cm ⁻³]	1.03
Pk_a	4.52
Log K_{ow}	3.97
Log K_d [pH 7.5]	1.00-1.78
Water solubility[mg.L ⁻¹](at [20 °C])	21
Boiling point [°C]	157
Melting point	75-77.5

¹ pK_a : dissociation constant, K_d : solid-water distribution coefficient, K_{ow} : octanol-water partition coefficient.

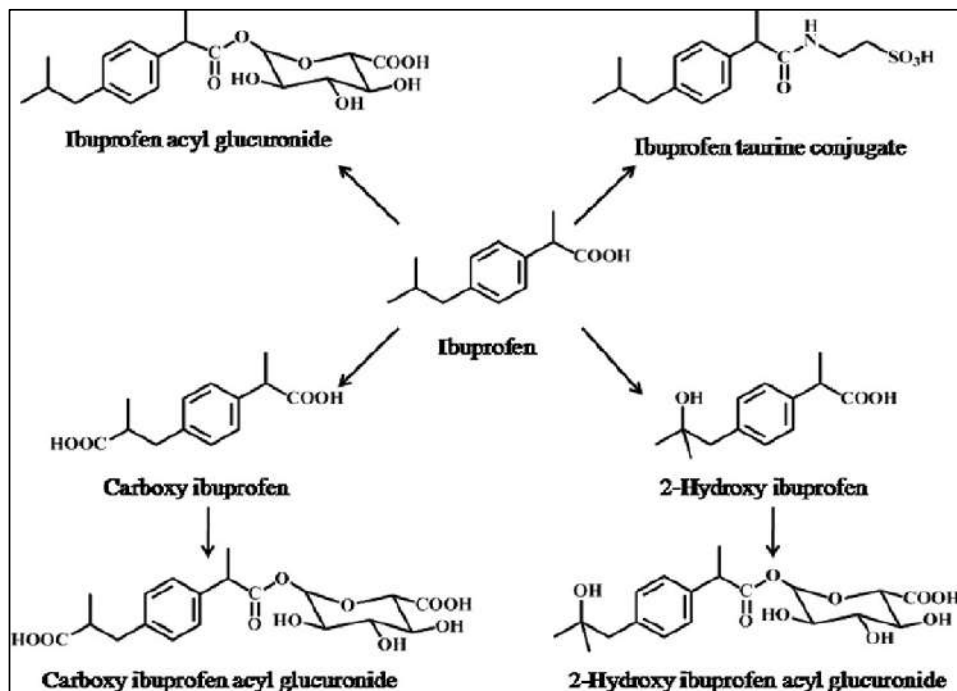


Fig. 1: Metabolic Fate of Ibuprofen in the Human Body (Aissaoui 2017).



Fig. 2: The Entrance of IBU into the Environment by Different Pathways.

annually, and it has been found in concentrations oscillating from ppt to ppb in the environment (Ma et al. 2018). Small amounts of IBU have been detected in water bodies, soil, sediment, and even agricultural fields (Parolini et al. 2011, Dolu & Nas 2023). It is mostly discharged into the ecosystem via wastewater owing to its well-known utilization, inadequate degradation within the human body, and highly

stable molecule, as well as the inappropriate disposal method of expired and unused IBU (Sadutto et al. 2021, Chopra & Kumar 2020, Bashaar et al. 2017). Additionally, IBU ends up in the environment through other pathways, such as homes, veterinary clinics, drug factories, and hospitals, as shown in Fig. 2 (Wiest et al. 2018, Ebele et al. 2017).

IBU is introduced into the soil primarily through its incorporation in veterinary medicine, as animals excrete ibuprofen metabolites into the soil. The organic waste can then be turned into sewage sludge or manure-based fertilizers, which have the potential to infiltrate or disperse within agricultural fields. Consequently, the presence of ibuprofen within the soil enables its mobilization, facilitating its migration towards the groundwater (Arpin-Pont et al. 2016). Finally, obtaining IBU without a medical prescription is a prevalent occurrence, resulting in its metabolites entering municipal wastewater via the excretion of feces or urine by individuals. Alternatively, unused or expired ibuprofen is directed towards municipal landfills in certain instances, infiltrating the soil as an environmental contaminant (Jan-Roblero & Cruz-Maya 2023, Marchlewicz et al. 2015). As a result, IBU is considered an emerging contaminant because it is ever-present in surface, underground, treated wastewater, drinking water, and soils (Aus der Beek et al. 2016, Chopra & Kumar 2020, Singh & Suthar 2021).

EFFECT OF IBUPROFEN ON HUMAN HEALTH AND ORGANISMS

IBU is related to several harmful consequences for human well-being. Notably, daily and excessive consumption could cause life-threatening influences, for example mild heart failure, liver failure, kidney problems, renal toxicity, metabolic acidosis, ulceration and gastrointestinal bleeding, hepatic injury, and severe hypersensitive responses and other health disorders for human bodies (Mathias et al. 2018, Volans 2015, Khalil et al. 2020, Rahman et al. 2024). A surplus intake of IBU can induce renal dysfunction by impeding cyclooxygenase, which results in a reduction in the production of renal vasodilators (Volans 2015). Additionally, cutaneous unfavorable responses like generalized bullous fixed drug eruption have been associated with the ingestion of IBU, underscoring the significance of overseeing and addressing such reactions (Bhanja et al. 2020). The safe dose of IBU for humans as a medication depends on medical consultation, the type and severity of the illness, and the patient's age (Lackie et al. 2019). However, regarding the safe exposure to IBU from contaminated water, no specific guidelines or safe exposure limits have been established by the World Health Organization (WHO) or the United States Environmental Protection Agency (EPA). Furthermore, there are currently no studies that have defined or set safe exposure limits for humans regarding the consumption of IBU-contaminated water, as IBU is considered an emerging pollutant. Both the WHO and EPA have raised concerns about the long-term accumulation of such emerging pollutants and their potential adverse effects on human

health. Further research is needed to evaluate the potential health risks of such exposure.

The high lipophilicity of IBU, with a log K_{ow} value of 3.97, is attributed to the incorporation of a 2-methylpropyl group and the lack of extra oxygen atoms. This characteristic aids IBU to efficiently permeate biological membranes and reveal an important distribution within living beings, with a value of 0.18 l/kg (Czyrski 2019, Majeed et al. 2018, Oliveira et al. 2015). Accordingly, IBU tends to bio-accumulate in marine (Ericson et al. 2010, Mezzelani et al. 2018) and freshwater (Xie et al. 2015, Parolini 2020) molluscs, fish (Pyhälä & Zandaryaa 2017, Xie et al. 2019), mammals (Richards et al. 2011), plants (Pi et al. 2017), and undergo biomagnification in food chains (Xie et al. 2015, Richmond et al. 2018). Upon accumulating in vertebrates and invertebrates, IBU elicits harmful effects, such as DNA damage, oxidative stress, inhibition of specific enzyme actions (such as protein nitration), lipid peroxidation, and disruption of mitochondria (Sánchez-Aceves et al. 2021, Žur et al. 2018, Parolini et al. 2011, Ortiz de García et al. 2014). A severe impact is elicited in living beings following exposure to elevated levels of IBU ($>100 \text{ mg.L}^{-1}$). Within the concentration spectrum of 10 to 100 mg.L^{-1} , it has the potential to induce non-lethal consequences. The typical IBU concentration found in ecosystems falls within the bracket of 0.2 to $8.0 \text{ } \mu\text{g.L}^{-1}$ (Parolini & Binelli 2012, Jan-Roblero & Cruz-Maya 2023).

Extended ecological exposure to IBU in aquatic organisms may result in persistent and severe consequences, such as genotoxic and cytotoxic impacts, elevated oxidative stress in cells, and detrimental effects on various aspects, including growth rate, reproductive functions, and behavioral patterns. Furthermore, there is a proposition that within living organisms, IBU could undergo biotransformation, leading to the formation of intermediary substances possessing greater toxicity compared to IBU alone (Jan-Roblero & Cruz-Maya 2023).

The harmful effects of IBU have been studied by using biological models, such as zebra mussels (*Dreissena polymorpha*). Exposure to IBU for seven days in zebra mussels resulted in the disruption of enzymatic activities and lipid peroxidation (Gonzalez-Rey & Bebianno 2012), as well as long-term exposure to IBU caused cellular damage, chronic effects, and elicited genetic (Jan-Roblero & Cruz-Maya 2023, Parolini et al. 2011). Research by Bartoskova et al. 2013 demonstrated that ibuprofen exposure triggered a considerable increase in glutathione peroxidase activity, a crucial antioxidant enzyme, in a manner dependent on the dosage, accompanied by a reduction in malondialdehyde levels, a biomarker of lipid peroxidation. Moreover, Parolini

& Binelli's 2012 work underscored that the exposure of zebra mussels to a combination of NSAIDs, including ibuprofen, induced oxidative stress, leading to modifications in enzyme activities and DNA fragmentation, suggestive of genotoxic impacts. Additionally, Schmidt et al. (2014) observed that exposure to diclofenac and ibuprofen resulted in distinct protein expression in marine mussels, impacting proteins associated with the response to oxidative stress, thereby reinforcing the interference with enzymatic activities and oxidative stress prompted by IBU in non-target organisms.

Several investigations have been dedicated to examining the negative impact of IBU on various freshwater fish types. Research conducted by Hodkovicova et al. (2022) identified that exposure to IBU led to notable alterations in the renal systems of *Oncorhynchus mykiss*, such as hyalinosis, modifications in the expression of heat shock protein 70, and heightened oxidative stress levels. Furthermore, the medication also impacted the hepatic function of the fish, leading to dystrophy, inflammatory responses, and cellular congestion. Also, Martyniuk et al. 2022 found that within bivalve mollusks (*Unio tumidus*), the exposure to IBU concentration of 0.8 g.L^{-1} over a period of 14 days resulted in a decrease in protein carbonyl levels, a decline in integrity of lysosomes within the digestive glands, and the activation of cholinesterase enzyme. Additionally, Wang et al. (2016) detected that the exposure of *Daphnia magna* to IBU levels in natural environments resulted in a marked reduction in both the overall quantity of eggs and broods for each female as well as body size.

In alternative occurrences, the existence of IBU at levels in nature had adverse effects on tadpoles. A study by Aliko et al. (2021) reported that IBU negatively affected the growth of tadpoles by causing a substantial postponement in their metamorphosis duration and a decrease in body mass, as well as, an examination of blood for mutagenic activity indicated a notable rise in the occurrence of nuclear and cellular irregularities in tadpoles of the species *Bufo bufo*. Ibuprofen's damaging impacts extend beyond animals to plants. The study of Wijaya et al. 2020 on cowpea (*Vigna unguiculata*) showed reductions in shoot/root length, leaf area, weight, chlorophyll, carotenoids, minerals (Mg and K), and antioxidant activity, interestingly, calcium (Ca), manganese (Mn), and sodium translocation increased, along with stress markers like hydrogen peroxide and enzymes.

Limited data exists on the adverse impacts of IBU and its metabolites, as well as other NSAIDs, on some processes, including nitrogen fixation, the induction of oxidative stress within microbial populations, and perturbation of cellular membrane integrity (Palyzova' et al. 2020, Guzik et al. 2019). Recent studies have revealed that IBU can contribute

to the dissemination of antibiotic resistance by facilitating the acquisition of exogenous antibiotic resistance genes (Alav & Buckner, 2023, Wang et al. 2020). This phenomenon is associated with IBU-induced bacterial competence, oxidative stress distinguished by an excessive generation of reactive oxygen species, and heightened permeability of the cell membrane (Ivshina et al. 2021). These mechanisms enable bacteria to acquire resistance genes more easily, leading to the spread of multidrug-resistant strains. The emergence of resistant bacteria in aquatic environments poses risks to water safety, as standard treatment processes may not fully eliminate resistant strains. This contributes to higher infection rates, treatment failures, and increased healthcare costs (Lajqi Berisha et al. 2024, Adekanmbi et al. 2023).

Additionally, the presence of these resistant bacteria disrupts ecosystems, threatening both environmental and human health, as well as affecting biodiversity and the health of various species (Asif et al. 2024). Prior investigations highlighted the influence of IBU on bacterial communities in freshwater environments. Hodkovicova et al. 2022 reported that the exposure to IBU altered the intestinal microbiota of fish (*Oncorhynchus mykiss*), leading to increased growth of Gram-positive bacteria and subsequent changes in bacterial ratios of the Fusobacteria/Firmicutes. In addition, Liu et al. 2022 detected in the systems of activated sludge that ibuprofen considerably altered microbial variety and bacterial community structures. For instance, several denitrifiers (Hyphomicrobium and Denitratisoma) improved notably, whereas Nitrospira considerably reduced. Also, distinct IBU responses were observed in different phylogenetic bacterial populations, such as a reduction in Proteobacteria and a rise in Chloroflexi.

The IBU structure endows its S-(+)- and R-(-)-ibuprofen enantiomers with distinct pharmacological activities and toxicological profiles, for instance, R-(-)-ibuprofen exhibits stereo-selectivity that inhibits hepatic mitochondrial beta-oxidation in rodent models, nevertheless, both S-(+)- and R-(-)-ibuprofen result in a moderate inhibition of mitochondrial respiration. The S-(+)-ibuprofen enantiomer demonstrates a lack of toxicity towards chondrocytes and synoviocytes. Empirical investigations indicate that S-(+)-ibuprofen possesses superior anti-inflammatory properties compared to R-(-)-ibuprofen, accompanied by reduced toxicity, enhanced clinical efficacy, and diminished variability in therapeutic outcomes. Furthermore, S-(+)-ibuprofen contributes to the mitigation of cancer progression and offers preventive measures against the onset of neurodegenerative diseases (Jan-Roblero & Cruz-Maya 2023, Gliszczynska & Sánchez-López 2021). IBU, a racemic mixture, was found in various aquatic environments, including the Guadalquivir

River basin. Both enantiomers, S-(+) and R-(-), have been found in water and sediment systems, with the R-(-) form exhibiting faster degradation (Qu et al. 2021, López-Serna et al. 2013). Aquatic organisms exhibit differential responses to IBU enantiomers. For instance, rainbow trout metabolize them slowly, while zebrafish exposed to IBU displayed stereoselective alterations in biomarkers. Additionally, ibuprofen enantiomers have varying toxicities to algae and bacteria (Jan-Roblero & Cruz-Maya 2023).

ANALYSIS AND DETECTION OF IBUPROFEN IN THE ENVIRONMENT

The detection and analysis of pharmaceutical compounds (PhCs), such as IBU, are crucial for comprehending the properties of their bulk substances and byproducts (Singh et al. 2022). Various methodologies for PhCs detection, including titrimetry, electrochemistry, chromatography, and spectroscopy (Femina Carolin et al. 2021, Qarah & El-Maaiden 2023).

In general, the analysis of PhCs involves a series of crucial steps. The sampling process is essential to collect representative samples from water, wastewater, or soil for analysis (Sapingi et al. 2023). Subsequently, extraction procedures like solid-phase extraction (SPE) are commonly employed to isolate and concentrate PhCs from complex matrices, offering advantages over traditional liquid-liquid extraction methods (Badawy et al. 2022). Concentration and cleaning of the extracted samples are vital to increase the sensitivity and accuracy of the analytical measurements, with SPE offering various sorbent technologies for improved selectivity and efficiency (Badawy et al. 2022). Finally, chromatographic methodologies, including liquid chromatography (LC) and gas chromatography (GC), are essential for separating and quantifying PhCs in the samples, ensuring accurate identification and measurement of these composites across diverse environmental media (Badawy et al. 2022, Gulhane et al. 2022, Sapingi et al. 2023).

Recent advancements in combining GC or LC with mass spectrometry (MS) have enabled researchers to detect IBU at much lower concentrations in surface and groundwater. For example, Salgado et al. (2020) used a gas chromatography-mass spectrometry (GC-MS) system in their investigation to elucidate the metabolic products through the IBU biodegradation process within the reactors.

One ongoing area of research in environmental analysis focuses on exploring innovative methods for analyzing IBU. For instance, high-performance liquid chromatography (HPLC) has been utilized to ascertain IBU concentrations in environmental samples with exceptional sensitivity and accuracy (Salgado et al. 2020, Kelani et al. 2023). One

study highlighted the utilization of HPLC combined with mass spectrometry (HPLC-MS) for evaluating the residual eco-toxicity of IBU degradation products in wastewater, indicating that the number and abundance of these products correlate with toxicity level (Hussain et al. 2024).

Recently, the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) has surfaced as an innovative and efficacious methodology for the identification of IBU and other pharmaceutical compounds within environmental matrices. This analytical method involves solid phase extraction (SPE) followed by LC-MS/MS, which allows for the simultaneous detection of multiple emerging contaminants at ultra-trace concentrations in different media, including water environments (Ramadan et al. 2024, Yao et al. 2023). Likewise, analytical techniques such as High-Performance Liquid Chromatography with Diode Array Detector coupled with Tandem Mass Spectrometry (HPLC-DAD-MS/MS) and High-Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) have been utilized by Salgado et al. 2020 to identify and evaluate the metabolic products that are produced in bioreactors during the IBU biodegradation. These methodologies are crucial for understanding the metabolic pathways that govern the degradation process, as they enable the comprehensive analysis of intricate mixtures of metabolites.

IBUPROFEN REMOVAL BY BIOTRANSFORMATION/BIODEGRADATION MECHANISM

An ecologically sustainable option for pharmaceutical removal involves the biodegradation by microorganisms, for example, bacteria (bioremediation) (Wu et al. 2024). The primary processes engaged in the biotransformation of the majority of pharmaceuticals are hydroxylation and oxidation catalyzed by cytochrome P450 (CYP). Hydroxylation refers to the addition of a hydroxyl group to a radical or ion, typically by replacing hydrogen. This assertion was corroborated by the reduction in pharmaceutical concentrations when microbial cultures are exposed to 1-aminobenzotriazole (an inhibitor of CYP) (Singh et al. 2022, Hata et al. 2010). The capability of microorganisms to break down xenobiotic compounds is attributed to possessing functions akin to mammalian CYP2C9, CYP3A4, and others. These isoforms of cytochrome P450 play a major role in drug metabolism in humans. Purified laccase also catalyzes the biotransformation of certain pharmaceuticals; nevertheless, the degradation capabilities of laccase are limited to a specific subset of pharmaceuticals, such as ciprofloxacin and naproxen (Singh et al. 2022).

Besides hydroxylation, microbial pharmaceutical degradation also employs other mechanisms like decarboxylation (carboxyl group elimination),

dehydrogenation (hydrogen removal), demethylation (methyl group removal), dealkylation (alkyl group removal), acetylation (introduction of an acetyl group), dechlorination (chlorine elimination), acetate group detachment, hydroxyl and glucuronide group loss, and ester group cleavage. Various enzymes are observed to be upregulated during the degradation process, suggesting their potential involvement in the microbial breakdown of pharmaceuticals (Singh et al. 2022).

The biodegradation of IBU predominantly relies on the particular microbes utilized for biodegradation. Factors such as pH, optimal temperature, appropriate xenobiotic concentrations, and an additional carbon source required for microbial growth significantly influence the efficiency of degradation. IBU contains phenylacetic acid (PAA) in its fundamental composition. The chemical composition of IBU provides strong resilience against biodegradation, attributable to the presence of an aromatic ring featuring branched substitutions located at the para position. Aromatic ring-containing compounds typically exhibit greater resistance to degradation compared to aliphatic compounds. As a result, the structural characteristics of IBU render it resistant to degradation by microorganisms. Despite its physicochemical properties suggesting high mobility in aquatic settings, experimental evidence indicates that IBU's persistence is lower than that of other pharmaceuticals,

illustrating its degradability (Jan-Roblero & Cruz-Maya 2023, Parolini et al. 2011).

Generally, IBU biodegradation proceeds through multiple pathways depending on the microorganism involved. Thus far, some IBU biodegradation pathways using bacteria have been clarified. The main process involves hydroxylation, either by binding to coenzyme A (CoA) or direct trihydroxylation of the aromatic ring (Jan-Roblero & Cruz-Maya 2023, Murdoch & Hay 2013). Various investigations on the biodegradation of IBU via bacteria demonstrated the hydroxylation on its side chains (isobutyl and propanoic) or aromatic ring. Hydroxylation of the isobutyl chain by aliphatic monooxygenases results in the formation of 2-hydroxyibuprofen or 1-hydroxyibuprofen, which are subsequently dihydroxylated by acyl-CoA synthase to yield hydroquinone byproducts. Similarly, hydroxylation of the propionic acid chain results in the addition of CoA to the carboxylic group of propionic acid by acyl-CoA synthase, generating ibuprofen-CoA, which is further hydroxylated by dioxygenases and deacetylated to create p-isobutylcatechol. An alternative biotransformation route includes reducing the carboxyl group of propionic acid to generate ibuprophenol, which is then acetylated to form ibuprophenol-acetate. Lastly, the hydroxylation of the aromatic ring of IBU by aliphatic monooxygenases produces trihydroxyibuprofen, leading to destabilization and cleavage

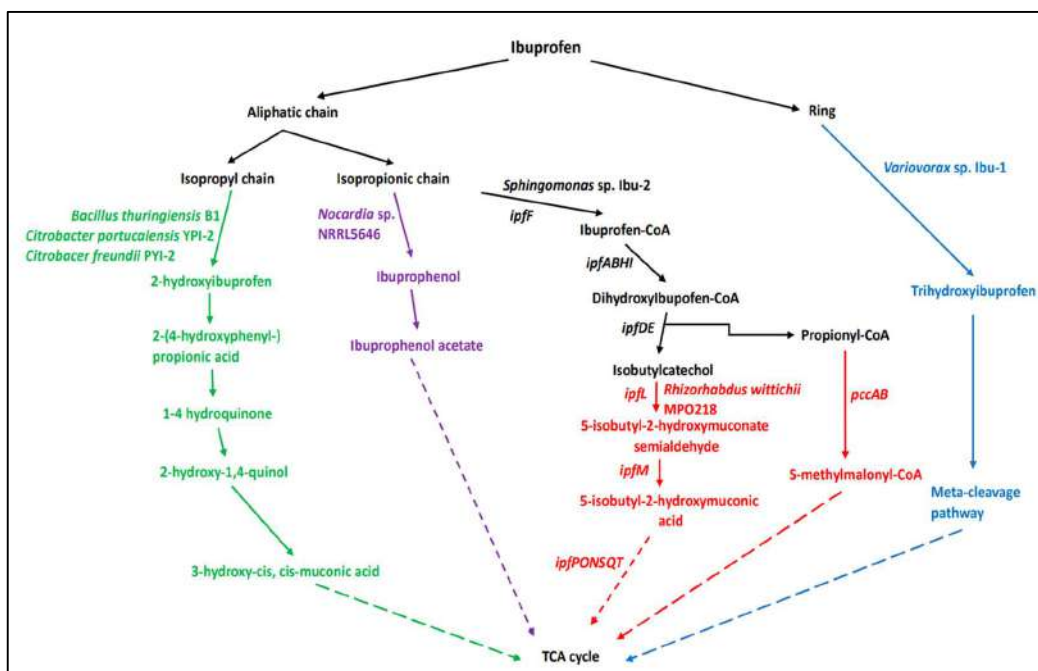


Fig. 3: The bacterial biodegradation of ibuprofen. Colored lines symbolize the metabolic pathways employed by individual bacterial genera. Dotted lines indicate the existence of additional steps within the biodegradation process (Jan-Roblero & Cruz-Maya 2023).

by the meta-cleavage pathway as shown in Fig. 3 (Murdoch & Hay 2013, Jan-Roblero & Cruz-Maya 2023).

IBUPROFEN-DEGRADING BACTERIA

Although bacteria exhibiting biodegradation potential towards ibuprofen have been recognized in recent years, the quest for novel strains with unique properties persists, employing diverse methodologies aimed at acquiring new data and understanding relevant to applied bioremediation.

The initial documentation of microbial IBU biotransformation was presented by Chen & Rosazza 1994 concerning *Nocardia* sp. NRRL 5646, which utilized IBU as the exclusive energy and carbon source, resulted in the formation of intermediates such as IBU acetate, ibuprofenol, and benzoic acid byproducts. Besides the investigations conducted by Chen and Rosazza, all efforts to elucidate the bacterial catabolic route of IBU have relied on the reports carried out by Murdoch and Hay in 2005 and 2013. These studies unveiled the microbiological biodegradation process of IBU through *Sphingomonas* sp. Ibu-2 strain isolated from a wastewater treatment plant. The bacterium exhibited the capacity to degrade IBU by enzymatically removing the propionic acid chain, resulting in the formation of catechols or methylcatechols like isobutylcatechol. Similar transformations were observed with other PAA derivatives, including 2-phenylpropionic acid, 3- and 4-tolylacetic acids, and 2-(4-tolyl) propionic acid.

Biochemical and genetic analyses conducted on *Sphingomonas* sp. Ibu-2 led to the determination of its IBU degradation constituents. The establishment of a comprehensive DNA library for *Sphingomonas* sp. Ibu-2, which was inserted into fosmids, facilitated the discovery of the *ipfABDEFHI* operon consisting of seven genes involved in the biodegradation route of IBU and other PAA compounds. The functionalities of the operon genes were elucidated: *ipfA* and *ipfB* genes encode two dioxygenase subunits targeting the aromatic ring, the *ipfD* gene encodes a sterol carrier protein X thiolase, and the *ipfF* gene encodes a CoA ligase enzyme. Nonetheless, the role of the protein produced by the *ipfE* gene remains undisclosed. Furthermore, *ipfH* and *ipfI* genes encode a ferredoxin reductase and aromatic dioxygenase, respectively (Fig. 3). Upon the identification of these genes, it was postulated that the mechanism of IBU biodegradation in *Sphingomonas* sp. Ibu-2 unfolds as follows: the enzyme CoA ligase (*ipfF*) conjugates CoA to IBU, the multicomponent oxygenase *IpfABHI* subsequently dihydroxylates ibuprofen-CoA to generate 1,2-cis-diol-2-hydroibuprofen-CoA, *IpfD* and *IpfE* then decompose this compound to yield 4-isobutylcatechol and propinyl-CoA (Kagle et al. 2009, Murdoch & Hay 2013, Murdoch &

Hay 2005). Presently, the pathway of IBU biodegradation is believed to consist of dual phases. The initial (upper) phase involves the production of 4-isobutylcatechol and propinyl-CoA (Aguilar-Romero et al. 2021, Murdoch & Hay 2013, Murdoch & Hay 2005). The subsequent (lower) phase encompasses the cleavage of the IBU ring, producing substances that enter the tricarboxylic acid (TCA) cycle.

IBU biodegradation by *Patulibacter* sp. I11 was solely noticed in the existence of tryptone and yeast extract. Through the utilization of proteomic analysis, the proteins responsible for the biodegradation of ibuprofen were successfully identified. These proteins encompass acyl-CoA synthetase, a protein containing a Rieske (2Fe-2S) iron-sulfur cluster, enoyl-CoA hydratase, and ATP-binding cassette (ABC) transporter proteins. Specifically, the protein featuring a Rieske (2Fe-2S) iron-sulfur cluster is acknowledged for its role in the initial oxidation of the aromatic ring, while enoyl-CoA hydratase facilitates the hydroxylation of double bonds subsequent to ring union. Furthermore, ABC transporters are actively engaged in the transportation of compounds (Almeida et al. 2013a). The identification of these proteins strongly implies their potential involvement in the IBU biodegradation with the bacterium *Patulibacter*.

The biotransformation of IBU to trihydroxyibuprofen through the meta-cleavage route was demonstrated in the *Variovorax* sp. Ibu-1 strain isolated from activated sludge by Murdoch and Hay (Murdoch & Hay 2015). In contrast to the known degradation *ipf* route of the Ibu-2 strain, which involves coenzyme A ligation followed by dioxygenation and deacetylation steps leading to isobutylcatechol formation, the *Variovorax* sp. Ibu-1 strain directly trihydroxylates the aromatic ring of ibuprofen to produce a substrate for ring cleavage. The presence of 3-fluorocatechol, a recognized inhibitor of the meta-ring fission enzymes pathway, resulted in the detection of only poly-hydroxylated IBU metabolites. This study marked the first instance of utilizing the inhibitor 3-fluorocatechol to accumulate short-lived catecholic intermediates. These same metabolites were found in sewage sludge polluted with IBU, indicating that the meta-ring fission pathway is likely the primary catabolic route for ibuprofen decomposition in the environment (Toyama 2010, Murdoch & Hay 2015).

Marchlewicz et al. (2017a) recently delineated a new pathway for the degradation of ibuprofen in the Gram-positive bacterium, *Bacillus thuringiensis* B1(2015b). The substantial activity of aliphatic monooxygenases, along with phenol and hydroquinone monooxygenases, confirmed the hydroxylation of both the aromatic ring and aliphatic chain of IBU, resulting in the establishment of various intermediates, such as 2-hydroxyibuprofen,

2-(4-hydroxyphenyl)-propionic acid, 1,4-hydroquinone, and 2-hydroxyquinol (Fig. 3). The product of acyl-CoA synthase, 1,4-hydroquinone, might undergo additional transformation via hydroquinone monooxygenase to yield 2-hydroxy-1,4-quinol, which exhibits a favorable binding affinity for hydroxyquinol 1,2-dioxygenase, an enzyme implicated in the ortho-cleavage of the aromatic ring, ultimately leading to the formation of 3-hydroxy-cis, cis-muconic acid. The biodegradation of IBU by *Bacillus thuringiensis* B1(2015b) can be augmented by co-contaminants. Notably, the addition of phenol and benzoate as co-contaminants enhanced the efficiency of IBU biodegradation by *Bacillus thuringiensis* B1(2015b) (Marchlewicz et al. 2017a, Marchlewicz et al. 2017b). Furthermore, the bacterial strain *Bacillus siamensis* DSI-1, isolated from wastewater treatment facilities, exhibits considerable efficacy in the bioremediation of ibuprofen concentrations present in effluent (Chopra & Kumar 2022).

Likewise, the bacterial strain *Rhodococcus cerastii* IEGM 1278 represents an additional bacterium capable of biodegrading IBU, converting it into decarboxylated and hydroxylated derivatives. It is noteworthy that the presence of IBU induced a transition from single- to multi-cellular forms, as detailed by Lvshina et al. 2021.

A novel mechanism for enzymatic-nonenzymatic coupling degradation was postulated, utilizing the widespread marine *Pseudoalteromonas* sp. This newly proposed IBU degradation pathway involves the initiation of degradation through exposure to extracellular reactive oxygen species, leading to the formation of the intermediate 4-ethylresorcinol, which is subsequently broken down by intracellular enzymes. The treatment of IBU with extracellular hydroxyl and superoxide anion radicals, along with hydrogen peroxide, results in the generation of 4-ethylresorcinol. Subsequently, 4-ethylresorcinol is enzymatically degraded intracellularly until complete mineralization, facilitated by enzymes such as 4-hydroxyphenylpyruvate dioxygenase, homogentisate 1,2-dioxygenase, long-chain acyl-CoA synthetase, acetyl-CoA acyltransferase, and enoyl-CoA (Li et al. 2022).

In recent times, *Sphingomonas wittichii* MPO218 exhibited the capability to biodegrade IBU, employing it as its exclusive source of carbon. Subsequent investigations revealed that the genomic structure of this strain comprised a singular circular chromosome alongside two circular plasmids. Furthermore, this plasmid demonstrated conjugative properties, facilitating the horizontal transfer of the IBU degradation capacity to *Sphingopyxis granuli* TFA, thereby indicating that gene transfer is a crucial factor within

Table 2: Summary of IBU Biodegradation by Bacteria.

Bacteria	Isolation place	Reference
<i>Sphingopyxis granuli</i> RW412	River	Aguilar-Romero et al. 2021
<i>Sphingobium yanoikuyae</i>	WWTP	Balciunas et al. 2020
<i>Bacillus thuringiensis</i>	Soil	Marchlewicz et al. 2016
<i>Patulibacter</i> sp. Strain L11	WWTP	Almeida et al. 2013a
<i>Gordonia amicalis</i> EU266486.1	WWTP	Almeida et al. 2013b
<i>Acinetobacter bouveti</i> JF681285	WWTP	Almeida et al. 2013b
<i>Bacillus siamensis</i> DSI-1	WWTP	Chopra & Kumar 2022
<i>Microbacterium paraoxydans</i>	Pharmaceutical wastewater	Show et al. 2023
<i>Variovorax</i> Ibu-1	WWTP	Murdoch & Hay 2015
<i>Patulibacter medicamentivorans</i>	WWTP	Salgado et al. 2020
<i>Nocardioides carbamazepini</i> sp. nov. CBZ_1T	Groundwater	Benedek et al. 2022
<i>Sphingomonas wittichii</i> MPO218	Sewage sludge	Aulestia et al. 2021
<i>Pseudoalteromonas</i> sp	Marine environment	Li et al. 2022
<i>Rhodococcus cerastii</i> IEGM 1278	Regional Specialized Collection of Alkanotrophic Microorganisms	Ivshina et al. 2021
<i>R. cercidiphylli</i> IEGM 1184	Regional Specialized Collection of Alkanotrophic Microorganisms	Ivshina et al. 2021
<i>Pseudoxanthomonas</i> sp. DIN-3	WWTP	Lu et al. 2019
<i>R. erythropolis</i> IEGM 501	Regional Specialized Collection of Alkanotrophic Microorganisms	Ivshina et al. 2021
<i>Paracoccus aminophilus</i> NR_042715.1	WWTP	Almeida et al. 2013b
<i>Patulibacter americanus</i> NR_042369	WWTP	Almeida et al. 2013b

bacterial communities engaged in biodegradation (Aulestia et al. 2021). A summary of IBU biodegradation by different bacteria is presented in Table 2.

FACTORS AFFECTING IBUPROFEN BIODEGRADATION

IBU biodegradation is influenced by various environmental factors that can impact both the metabolic pathways of degradation and the overall growth of microbial communities. For instance, elevated temperatures can stimulate metabolic rates, leading to increased biomass and potentially accelerated IBU degradation. Conversely, factors hindering microbial growth by inhibiting core metabolic processes may indirectly affect IBU breakdown. Understanding how environmental conditions influence ibuprofen biodegradation is crucial for predicting the degradation capacity of a given strain in natural settings (Marchlewicz et al. 2017a, Chopra & Kumar 2022).

Influence of pH on Ibuprofen Degradation

In case of the elimination of acidic pharmaceuticals, such as ibuprofen, the pH level is the critical variable that affects the rate of drug removal (Zembrzuska et al. 2019). pH represents a critical determinant that profoundly affects bacterial cell morphology, membrane properties, and microbial functionality (Sanguanpak et al. 2015). Furthermore, pH may exert influence over biosorption, toxicity, and the ionization states of pharmaceuticals found within environmental contexts (Boström et al. 2015). In specific scenarios, a diminished degradation rate may be associated with the ionic state of the molecule when situated at alkaline pH levels. In such circumstances, the surface of the bacterial cell acquires a negative charge, thereby resulting in diminished electrostatic interactions between negatively charged compounds and the binding sites on the surface of biomass. IBU, possessing a pKa of 4.52, exists in anionic form when the pH exceeds 6.5, with over 99% of the drug being in this state. Consequently, at pH levels of 7.2 and 8.0, variations in the efficiency of degradation will not correlate with distinct ionic species of IBU. An additional consideration is that the identified discrepancies could be pertinent to the active status of proteins involved in the biodegradation mechanism. At lower pH values (4.0–5.0), IBU exists in a neutral state and engages with the surfaces of bacteria (Aksu et al. 2004, Marchlewicz et al. 2017a). This may imply a more facile and rapid degradation process for IBU; however, it is important to note that this uncharged state can exhibit heightened toxicity to microorganisms (Boström et al. 2015).

Influence of Temperature on Ibuprofen Degradation

It is widely recognized that temperature constitutes one

of the most significant factors influencing biodegradation processes. In accordance with van't Hoff's principle, the rate of a chemical reaction is expected to increase by a factor of (2-4) for every increment of 10°C in temperature (Horel & Schiewer 2011, Marchlewicz et al. 2017a, Marchlewicz et al. 2016). As the temperature of water is raised, the polarity of water molecules decreases, consequently enhancing the solubility of hydrophobic organic compounds, including pharmaceuticals (Akay et al. 2017, Akay et al. 2021, Emire et al. 2017). The aqueous solubility of PhCs is crucial in determining their bioavailability and therapeutic efficacy, in addition to influencing their removal from wastewater and contaminated environments. IBU solubility was observed to increase with rising temperatures, attributable to the establishment of hydrogen bonds between the oxygenated groups present in IBU and the water molecules (Akay et al. 2021).

Nevertheless, within biological processes, the temperature effect on degradation mechanisms is considerably complex. This complexity arises from the interplay between temperature variations and cellular membrane function. Elevated temperatures may induce denaturation of proteins associated with cellular membranes, while excessively low temperatures can lead to increased viscosity of membrane phospholipids. Such changes may ultimately lead to an increase in the membrane rigidity, thereby obstructing transport processes within the cellular membrane (Akulava et al. 2024, Kim et al. 2013). Furthermore, temperature also impacts the metabolic processes and growth rate of bacteria, affecting their efficiency and community dynamics (Aguilar-Romero et al. 2021, Marchlewicz et al. 2017a).

Influence of Heavy Metals on Ibuprofen Degradation

In landfills, wastewaters, and various ecological settings, a multitude of contaminating agents are identified. The introduction of aromatic compounds, including non-steroidal anti-inflammatory drugs, into the environment is frequently associated with the occurrence of heavy metals, which may significantly affect biodegradation mechanisms. The impact of heavy metals on these mechanisms is contingent upon the specific type of metal and its chemical and physical states, such as separated-phase solids, soil-adsorbed species, colloidal solutions, soluble complexed species, or ionic solutes (Marchlewicz et al. 2017a). Environmental parameters, including pH, the cation exchange capability of the soil, the redox potential of the aqueous state, and the content of organic material, play a crucial role in determining the physical and chemical properties of metals (Olaniran et al. 2013). Additionally, the effect of metallic elements on biodegradative processes is fundamentally contingent upon their interactions with microbial entities (Marchlewicz et

al. 2017a). Consequently, the influences of the metal ions Co^{+2} , Cd^{+2} , Cu^{+2} , Hg^{+2} , or Cr^{+6} on *Bacillus thuringiensis* B1(2015b) have been ascertained (Marchlewicz et al. 2016).

Influence of Aromatic Compounds on the Degradation of Ibuprofen

Pharmaceutical contaminants exist concurrently in the environment alongside other pollutants, including aromatic compounds that infiltrate ecosystems as a result of both natural phenomena and anthropogenic activities (Kumar et al. 2022, Pierre et al. 2015). Due to their analogous chemical composition to IBU, these aromatic compounds possess the potential to activate enzymes that facilitate the degradation of IBU. Conversely, these aromatic compounds may engage in competitive interactions with IBU for binding sites within the enzyme's active site, thereby diminishing the degradation efficiency (Greń et al. 2010). A reduction in the rate of biodegradation may be attributed to the competitive interactions among reactants for the limited active sites of a single enzyme. Conversely, an augmentation in degradation efficiency could be attributed to the upregulation of shared enzymes involved in this degradation process. In instances where these degradative enzymes are components of distinct metabolic pathways, the observed increase in degradation rate may be ascribed to improved biomass proliferation in the presence of Nutrients, consequently leading to the synthesis of vital reducing equivalents (Nowak & Mrozik 2016, Wojcieszynska et al. 2011). In research carried out by Marchlewicz (2017a), it was discerned that phenol was the only aromatic compound that exhibited no significant effect on the IBU degradation. Concurrently, a reduction in phenol concentration was noted alongside the degradation of IBU, indicating that phenol may not have impeded the breakdown of its para-substituted derivatives (Nowak & Mrozik 2016).

BIOREMEDIATION APPLICATIONS FOR IBUPROFEN REMOVAL

Traditional WWTPs were typically not engineered to effectively eliminate pharmaceutical micro-pollutants present in aquatic systems (Couto et al. 2019, Priya et al. 2022). It required innovative methodologies for the elimination of these chemicals (Zhou et al. 2023, Varma et al. 2020). To mitigate the prevalence of these contaminants (such as IBU) within the ecosystem, a variety of biological approaches have been innovatively developed, e.g., membrane bioreactor (MBR), attached growth MBR, stabilization ponds, constructed wetlands (CWs), Immobilized Cells Bioreactors, and others (Tiwari et al. 2017, Gharibian & Hazrati 2022, Serna-Galvis et al. 2019).

Membrane Bioreactors (MBRS)

Membrane bioreactors (MBRs), which represent an integration of conventional biological methodologies (biodegradation using activated sludge) with advanced membrane filtration (MF), have been deemed suitable for wastewater treatment, exhibiting numerous advantages including a reduced spatial footprint, the production of superior-grade effluent, and diminished sludge generation (Nhut et al. 2020, Ren et al. 2022, Park et al. 2018). Membrane-based separation systems exhibited noteworthy efficacy in the elimination of pharmaceuticals, particularly in the context of antibiotics, NSAIDs, and others (Alshehri et al. 2022, Maryam et al. 2020). The amounts of degradable PhCs removed in MBRs are superior to that in activated sludge facilities, attributable to extended sludge retention times, which facilitate the development of different and diverse microbe populations within the MBR framework, high level of activated sludge, and enhanced microbial activity (Tiwari et al. 2017, Song et al. 2022).

Regarding mechanisms employed within MBRs, the elimination of pharmaceuticals encompasses both biodegradation and adsorption to sludge (Gharibian & Hazrati 2022). Microorganisms possess the capacity to alter the pharmaceutical structure either directly or indirectly through the production of specific enzymes. Both the transformation and degradation processes of PhCs via microbial communities, particularly bacteria, predominantly happen by two principal routes: co-metabolism (in which PhCs are partially transformed and/or decomposed without serving as a primary source of carbon), and mixed matrix growth/development (in which PhCs are completely mineralized and utilized as an energy and carbon source) (Wittich et al. 2023, Nguyen et al. 2024). The population of denitrifying bacteria within MBRs has exhibited a significant increase, accompanied by enhanced denitrification activities, which collectively contribute to the effective elimination of PhCs (Gharibian & Hazrati 2022). The activated sludge has shown considerable efficacy in adsorbing PhCs, thereby facilitating the elimination from aqueous phases (Nguyen et al. 2024).

MBRs exhibit numerous improvements in the treatment of wastewater, such as their exceptional efficacy in the elimination of a diverse array of PhCs (Gouveia et al. 2022, Wang et al. 2018). The implementation of MF enhances the effectiveness of water purification processes. Nevertheless, it is essential to acknowledge certain limitations associated with MBRs. Among these limitations is the phenomenon of membrane fouling, which poses a substantial challenge in the operation of MBR systems. Gradually, the buildup of microorganisms, particulates, and various substances on the

surface of the membrane can result in diminished permeability and operational efficiency. Furthermore, MBRs typically necessitate greater energy consumption in comparison to traditional wastewater treatment methodologies (Li et al. 2020a, Nguyen et al. 2024).

Constructed Wetlands

Constructed wetlands (CWs) have demonstrated significant efficacy in removing various emerging PhCs, including IBU, before their release and discharge to the environmental ecosystem (García et al. 2020, Khan et al. 2023). The CW represents an engineered approach that operates without the necessity for supplementary chemical additives and generates minimal sludge mass, thereby achieving benefits that include efficiency, economic, and environmental sustainability in the elimination of aquatic pollutants (Liu et al. 2019, Nguyen et al. 2023, Ky et al. 2020, Zhang et al. 2023). CW consists of a specially designed wetland pool in conjunction with immobilized microorganism plates, where the constructed wetland pool is stratified into five distinct layers arranged sequentially from the top to the bottom: a sand layer, a sand-gravel layer, an immobilized microorganism layer, an artificially mixed matrix layer, and a gravel layer (Stefanakis 2020).

The predominant processes involved in the elimination of PhCs in CWs can be encompassed by biodegradation, precipitation, plant uptake, substrate sorption, hydrolysis, and photolysis (Vo et al. 2018, Avila et al. 2017, García et al. 2020). Microbial-mediated degradation has assumed a pivotal role in the breakdown and elimination of PhCs inside the CWs system (Chen et al. 2019). The availability of specific kinds of plants or vegetation further influences the functional dynamics and composition of the microbiota (e.g., bacteria), thereby impacting their overall efficacy of treatment (Zhang et al. 2023). The filter materials utilized in CWs can also significantly contribute to PhCs removal, attributed to their unique physical and chemical characteristics that enhance sorption processes, biological activities, and precipitation within these systems. Furthermore, the elimination efficiency of various PhCs families (e.g., IBU) in CWs demonstrates variability that relates to their inherent characteristics, such as Kow coefficient and water solubility, among others (Nguyen et al. 2024). Elevated specific surface area (SSA), silanol (Si–OH) configurations, retention time (HRT), hydraulic loading rate (HLR), and a variety of microporous structures facilitate microbial adhesion and promote chemical adsorption, thereby enhancing the eradication of PhCs (Anderson et al. 2013, Al Falahi et al. 2022).

CWs exhibit significant benefits, such as environmentally sustainable, economically viable solutions characterized

by reduced maintenance and operational expenditures alongside low usage of energy (Ravikumar et al. 2022, Li et al. 2020b). Despite their numerous advantages, they are also associated with certain constraints, notably the requirement for extensive spatial allocation, prolonged retention periods, and the inadequate eradication of specific PhCs (Ravikumar et al. 2022, Nguyen et al. 2023).

Immobilized Cells Bioreactors

Immobilized cell bioreactors signify a significant advancement in bioprocessing technology, improving the efficiency and stability of biocatalysts across diverse applications. These systems employ immobilization methodologies to restrict microorganisms within a permeable polymeric gel matrix, wherein the immobilized microorganisms are subjected to polluted media in the gel matrix indirectly, thereby enhancing their reusability and mechanical robustness for the elimination of targeted pollutants, including IBU compounds, from wastewater (Lapponi et al. 2022, Das & Adholeya 2015). A range of techniques, such as encapsulation, entrapment, and covalent bonding, are utilized for cell immobilization, each presenting distinct advantages pertinent to specific biocatalytic processes (Lapponi et al. 2022).

Many studies have displayed that microorganisms residing in fixed systems exhibit superior efficacy in pollutant degradation, less sensitivity to adverse environmental fluctuations (like temperature and pH), as well as an enhanced growth rate compared to those in suspended cultures. Various fixed bioreactors were applied in the wastewater treatment, including but not limited to fluidized bed bioreactors (FBR), semi-fluidized bed bioreactors, packed bed bioreactors (PBR), anaerobic membrane bioreactors (AnMBR), and hybrid bioreactors (HBR). Bioactive beds within these systems are comprised of biocarriers that are densely populated with microorganisms (biomass). These biocarriers, distinguished by their porous and rough surfaces, facilitate the retention and attachment of a diverse microbial community (Mehrotra et al. 2021, Aissaoui 2017). However, research by Navrozidou et al. 2019 demonstrated that bioreactors utilizing immobilized cells are capable of efficiently eliminating IBU, attaining removal efficiencies as high as 98.4%. The existence of particular microbial communities that are adept at degrading IBU, such as *Novosphingobium* and *Rhodanobacter*, significantly enhanced the degradation process.

CONCLUSIONS

An elevation in the concentration of IBU in both domestic and hospital sewage systems is anticipated as a consequence of the population's utilization of this pharmaceutical for

the management of various infections and ailments. This phenomenon results in its release into the environment, thereby engendering significant ecological concerns.

Microbial degradation mechanisms represent a promising alternative for the biodegradation of IBU, particularly through the utilization of specifically selected strains isolated from contaminated habitats. Furthermore, comprehensive investigations into the biodegradation pathways are imperative for elucidating the fate of this compound, which includes an understanding of the genetic and enzymatic factors implicated in these biochemical processes.

The proliferation of microbial degradation research focusing on IBU, predominantly involving bacterial species, is noteworthy; however, the ecotoxicological implications of the biodegradation byproducts remain largely unexplored. Consequently, several areas necessitate substantial advancements, including the methodical surveillance of IBU along with its biodegradation byproducts within in vivo experimental settings. Additionally, the identification and implementation of strains exhibiting high efficiency for the advancement of applied biotechnological procedures must be prioritized. Moreover, evaluations concerning the cost-effectiveness and energy efficiency of various degradation methodologies should also be investigated.

To address the growing environmental pollution caused by ibuprofen (IBU), it is essential to implement comprehensive strategies. First, effective pharmaceutical waste management policies should be established, encouraging proper disposal practices in both households and healthcare settings to prevent improper release into the environment. Additionally, advancements in microbial biodegradation techniques, particularly using efficient bacterial strains, should be prioritized to improve the breakdown of IBU in wastewater. Further research is also needed to investigate the ecotoxicological effects of biodegradation byproducts, as their potential impacts on ecosystems and human health remain largely unexplored. Continuous monitoring of IBU concentrations and their degradation products in vivo is crucial to assess their long-term environmental and biological consequences. Finally, the evaluation of cost-effective and energy-efficient degradation technologies should be a key focus to ensure that these methods are both sustainable and economically viable for widespread application. By addressing these areas, it will be possible to reduce the ecological impact of IBU and contribute to the preservation of ecosystem health.

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Brassica juncea and Medicago sativa as Phytoremediators for the Removal of Chromium and Arsenic

S. Monisha† and S. P. Sangeetha

Department of Civil Engineering, Aarupadai Veedu Institute of Technology, Vinayaka Mission's Research Foundation, (Deemed to be University), Chennai, Tamil Nadu, India

†Corresponding author: S. Monisha; monisha.civil@avit.ac.in

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ABSTRACT

This work aims to examine the effectiveness of phytoremediation, a process that uses *Brassica juncea* (Indian Mustard) and *Medicago sativa* (Alfalfa) plants to remediate contaminated soil with Cr and Ar. An economical and ecologically appropriate way to remove, immobilize, and degrade contaminants from soil and water is through phytoremediation. With this experiment, plants can grow in a controlled environment with different Cr and Ar concentrations in soil and the addition of organic compost. This entails evaluating the plants' capacity to absorb metal, monitoring variations in the concentrations of metal in the plants' roots, stems, leaves, and seeds, and looking into how organic matter affects the efficiency of phytoremediation. The findings showed that plants accumulated large amounts of chromium and arsenic across all experimental plants, but the highest accumulation was observed in the root system, which suggested that the plants were involved in the process of rhizofiltration. The roots pick up much more of the metals than the aerial of the plant, including stems, leaves, and seeds, thereby minimizing metal translocation to the parts of the plant that can be ingested by animals and, in turn, humans. This is a fundamental criterion for phytoremediation for assurance of a safe and effective process. Overall, the present study underscores the ability of phytoremediation in the remediation of heavy metal-polluted soils, especially under the use of organic growing media. It has made me understand the usefulness of this method for the effective and efficient cleaning of the soil in comparison with traditional methods, which could benefit the environment and future cost savings. Further research should be concerned with field-scale experiences and examine the potential of phytoremediation approaches in the range of environmental conditions.

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INTRODUCTION

Impact of Heavy Metals: Chromium and Arsenic

Chromium and arsenic are hazardous elements that are dangerous for the sustainability of ecosystems as well as for human health. Arsenic is an element and a potent toxin, which can be of geogenic or anthropogenic origin, the former being sources including rocks and the latter being mining and industrial activities. Drinking water containing arsenic is likely to cause some diseases despite the dosage used in this analysis being relatively small, bearing in mind that arsenic-contaminated water may cause skin problems, cancer, and other developmental complications in a child. Arsenic was found to contaminate the groundwater, and this has led to many cases of arsenic poisoning in different parts of Asia, especially Bangladesh (Srivastava et al. 2021).

Several industries, for example, electroplating and leather tanning, produce chromium, some of which is the hexavalent chromium ion Cr (VI) that has been rated to be a severe pollutant (Kumar et al. 2018). The overall properties of this type of chromium make it dangerous to human health, especially because it is

poisonous and causes cancer. Chromium can be hazardous to human health depending on the dosage; especially through intake of water containing Chromium at high concentrations, one can develop respiratory diseases and skin diseases and have an increased tendency to suffer from cancer. Due to their endurance in the environment, arsenic and chromium are difficult to rehabilitate from water; hence, their removal is paramount to public health. The use of phytoremediation as an eco-friendly solution to this issue has been growing in popularity. To evaluate the impact of five different soil management techniques (cow manure, sewage sludge, leaf compost, vermicompost, and chicken manure) on the accumulation of Cd, Cu, Mo, Ni, Pb, and Zn in the leaves and pods of three different varieties of mustard (Black mustard, yellow mustard, and Mighty mustard), this study aims to investigate the efficacy of phytoremediation in agricultural settings (Nepal et al 2024). *Brassica juncea* was grown hydroponically to examine its morphological and physiological reactions to trichlorfon (TCF) and cadmium (Cd) stressors, as well as its capacity for phytoremediation. The results demonstrated that when the concentration of Cd rose, *B. juncea*'s biomass and root shape were strongly inhibited. Malondialdehyde and soluble protein levels, as well as superoxide dismutase activity, rose with the increased Cd concentration, while *B. juncea*'s chlorophyll fluorescence intensity and chlorophyll content dropped. TCF did not significantly affect these *B. juncea* morphological and physiological characteristics at varying concentrations. Under the co-exposure to Cd and TCF, the biomass and physiological state of *B. juncea* were mostly controlled by the Cd level. *B. juncea* may collect Cd in many ways. Zhang et al. (2022).

Water Quality and its Significance

The quality of water is very crucial for the survival of all the species on planet Earth. Get deterioration of agriculture, the health of people, and last but not least, the ecology of the planet. Potable water is therefore essential for man's existence, as diseases such as cholera and dysentery spread very fast through waterborne diseases (Prasad et al. 2021). Since most countries in the world today recognize clean water as a basic human right, the same governments at all levels must ensure that they strive as much as possible to ensure that the water supply meets or exceeds international standards.

Various pollutants that are capable of reducing the quality of water include discharge from agricultural and industrial activities and poor disposal of waste (Sharma et al. 2018). Forces such as chemicals, heavy metals, pesticides, and viruses can highly contribute to compromised water safety. The problem of the presence of heavy metals in the environment has recently stepped out of the background

and become one of the key problems. Some heavy metals, including arsenic and chromium, are more dangerous as they are not biodegradable and can stay in the body of an organism for many years and, therefore, pose an extended threat to future generations of both human and animal beings.

Characteristics of *Brassica juncea* (Indian Mustard)

Brassica juncea, commonly known as Indian mustard, belongs to the Brassicaceae family and originates from Central Asia. This annual herbaceous plant typically grows to a height of 40-150 cm. Its leaves are lobed with crenate or serrate margins; the basal leaves are stipulate and petioled, while the cauline leaves are exstipulate and sessile. Indian mustard produces small yellow flowers arranged in racemes. Its root system consists of shallow, fibrous roots supported by lateral branching underground stolons, which help the plant adapt to various soil textures. While it prefers light, well-drained soils, it is versatile and tolerates a range of soil types, including saline and alkaline conditions. Originally grown in mild climates, Indian mustard can also withstand drought and extreme heat. The plant is valued for its seeds, which are used to produce mustard oil and as a spice (Patel et al. 2019). Its leaves are consumed as greens or vegetables. Additionally, Indian mustard is utilized in bioremediation to remove heavy metals from soil. However, it is vulnerable to pests like aphids and cabbage worms and diseases such as downy mildew and blackleg.

Characteristics of *Medicago sativa* (Alfalfa)

Medicago sativa, commonly known as Alfalfa, belongs to the Fabaceae (Leguminosae) family and originates from the Mediterranean and southwestern Asia. It is an herbaceous perennial legume that typically grows to a height of 30-90 cm. Its trifoliate leaves consist of three oblong leaflets, with the terminal leaflet being longer than the two side leaflets (He et al. 2018). Alfalfa produces small, purple to blue flowers on racemes and has a well-developed, deep root system, which can extend over 5 meters, allowing it to withstand dry conditions. It thrives in well-drained, loamy soil with a neutral pH range of 6.5 to 7.5. This plant adapts well to the middle and southern latitudes, being both frost-tolerant and heat-resistant. Primarily grown as a fodder crop due to its high protein, vitamin, and mineral content, Alfalfa is also valuable for soil improvement through nitrogen fixation. However, it is susceptible to pests such as weevils and aphids and fungal diseases like root rot and anthracnose.

Concept of Phytoremediation Techniques

The primary idea of phytoremediation is the utilization of plants to absorb, accumulate, and purify pollutants

found in industrial effluent water and contaminated soil. Phytoremediation is a cost-effective and environmentally friendly technique.

Applications of the Phytoremediation Technique

Phytoremediation is a novel and environmentally sound approach that employs plants and their associated microorganisms to immobilize, transport, transform, and degrade contaminants from water and soil (Baltrenaite et al. 2017, Hemani et al. 2024). To elaborate, since this method capitalizes on plants' natural air purifying qualities, it is both affordable and eco-friendly. It is comprised of several stages, like rhizofiltration, phytoextraction, phytostabilization, and phytodegradation, all of which are designed for particular pollutants.

Phytoremediation is the process of using plants to eliminate heavy metals from the soil by letting the plants' roots take in the metal and transport it to their above-ground tissues. (Vijayant & Ananda Babu 2023). Phytostabilization is a technique that helps avoid the mobilization and bioavailability of contaminants to other organisms by fixing them onto the soils. While rhizofiltration is centered on the removal of pollutants from water, phytodegradation is more directed toward the breakdown of chemical substances (Sabreena et al. 2022). Phytoremediation deserves research because it is promising to become a great-scaled eco-technology (Panneerselvam & Shanmuga Priya 2023). It possesses the potential to restore the degraded landscape to better-optimized ecosystems in different settings, such as cities, farmland, and past mining areas. Since pollution control and environment conservation form part of the sustainable development agenda, this approach is commendable.

Though deployed mainly to increase the effectiveness of conventional phytoremediation technologies, a new approach collectively known as bio-remediation is gradually

incorporating technology such as genetic engineering and nanotechnology (Wang & Delavar 2024). Such breakthroughs open new possibilities to increase the intake of toxins that can be absorbed and ingested, therefore increasing the potential of phytoremediation as a way of eradicating heavy metal pollution (Qadir et al. 2021). Phytoremediation Techniques are depicted in Fig. 1.

Objectives of the Study

This research work aims at identifying and evaluating the viability of using phytoremediation to tackle the pollution posed by these two pollutants, namely Chromium and Arsenic, in the water and soil. Specific objectives include:

1. To assess the efficiency of plant species, such as *Brassica juncea* and *Medicago sativa*, for the remediation activity of heavy metals chromium and arsenic and enhancement of soil and water quality under controlled experimental conditions.
2. To identify the impact of heavy metal contamination on the health monitoring of plants, Morphological characteristics, and plant responses with different effluent doses.
3. To analyze the heavy metal uptake with and without organic amendments and also statistical analysis.
4. To analyze the impact on soil properties before planting and after harvest, the following phytoremediation.

Current Research on Phytoremediation Techniques

Some empirical evidence shows that phytoremediation works well in eliminating contaminants in ecosystems due to the increasing interest in the subject in the last two decades. Studies have revealed that certain species of plants can accumulate these metals to sets of measures that are acceptable (Guerra Sierra et al. 2021). Afterward, these plants can be collected, accumulated, and perhaps disposed of without any negative impact. Previous research, research, and experiments have shown that *Brassica juncea* possesses a high potential for phytoextraction of metals, including nickel, cadmium, and lead.

Studies on phytoremediation have also helped in identifying the absorption and detoxifying processes that metals involve at physiological and biochemical levels. Works of literature on root exudates and soil microbes show that these molecules increase the solubility and bioavailability of metals, thus increasing the uptake of these metals by plants. Yet, there is hope that with the application of new technologies such as plant genetic engineering, they can improve their Toxins retention capacity (Mohan et al. 2021).

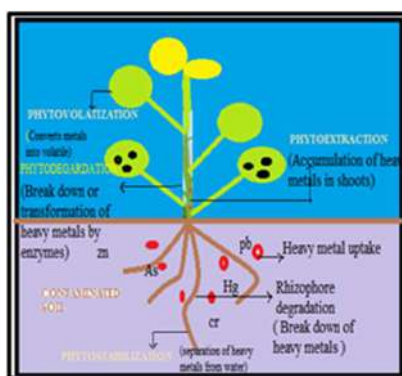


Fig. 1: Phytoremediation techniques.

Despite the progress made in recent years, the enhancement of the phytoremediation technologies for various pollutants and site conditions remains a difficult problem. The present study underlines the need for a better understanding of the mutual relation of soil, plants, and contaminants by focused studies site-dependent. The purpose of this study is to begin building upon future successful use of phytoremediation by conducting a review of prior research.

MATERIALS AND METHODS

Selected Industrial Wastewater

Some industries, for instance, polishing of metals, mining, and the manufacture of chemicals, release wastewater containing materials that are dangerous, for instance, arsenic and chromium. These two substances, therefore, have significant impacts on the well-being of human beings as well as the environment. General Pasteurization: the wastewater has a pH of about 7.2 ± 0.3 , an electrical conductivity of batch about $1200 \pm 50 \mu\text{S.cm}^{-1}$, the total dissolved solids concentration of batch of about $800 \pm 40 \text{ mg.L}^{-1}$, an Arsenic concentration is $30.5 \pm 1.5 \text{ mg.L}^{-1}$ and Chromium values $50.3 \pm 2.0 \text{ mg.L}^{-1}$ respectively (Farhadkhani et al. 2018). Table 1 shows the characteristics of selected industrial wastewater.

Selection Criteria for Indian Mustard and Alfalfa

Based on the characteristics of industrial effluent water for the investigations, two plant species were selected. *Brassica juncea*, also known as Indian mustard, and *Medicago sativa*, also known as alfalfa, were chosen for phytoremediation because they have high biomass yields, are resistant to toxic levels of heavy metals, also can concentrate chromium and arsenic, among other metals. Because of its rather large and sprawling root system *B. juncea* can efficiently take up nutrients and metals from the contaminated soil, and it has a rather high bioconcentration factor concerning chromium (Cr) and arsenic (As). *Medicago sativa* promotes soil stability due to a higher density of root system and performs nitrogen fixation that helps the transportation of metals through it

Table 1: Characteristics of selected industrial wastewater.

Parameter	Unit	Values
pH	-	7.2 ± 0.3
Electrical Conductivity (EC)	$\mu\text{S.cm}^{-1}$	1200 ± 50
Total Dissolved Solids (TDS)	mg.L^{-1}	800 ± 40
Arsenic (As) Concentration	mg.L^{-1}	30.5 ± 1.5
Chromium (Cr) Concentration	mg.L^{-1}	50.3 ± 2.0

(Zhang et al. 2019). Both these species are highly suitable for the long-term bioremediation of polluted sites as they are capable of growing in variable types of soil, grow very fast, and are also stress tolerating.

Soil and Plant Tissue Analysis

A preliminary study carried out with analysis of soil and plant tissue samples is important to reviewing and evaluating the progress of phytoremediation. Amendments and treatments can be studied by collecting soil samples at different time intervals to determine the heavy metal concentrations. To evaluate the effectiveness of different plant species, several samples of plant tissue are collected, and contaminated pollutant concentrations are measured. Usually, in order to provide more exhaustive analysis, it is possible to take samples of soil from different layers of the soil. The samples concerning the root, stem, and leaves are gathered to analyze the increase of metals and the fact of plants' activity (Yanitch et al. 2020). By following the method, in order to determine the levels of heavy metals, the soil and plant samples are first dried, ground, and digested, and then analyzed using highly sophisticated techniques like inductively coupled plasma mass spectrometry. Table 2 shows the collection of samples, sampling timing, preparation, and analysis methods selected for the investigations.

Seed Morphology Characteristics

***Brassica juncea* (Indian Mustard):** The seeds of *Brassica juncea* are round or slightly oval-shaped, small in size, typically ranging from 1.5 mm in diameter. Their color varies from dark brown to reddish-brown or black. The seed surface

Table 2: Sample and analysis methods for the investigations.

Sample Type	Sampling Time Points	Preparation Method	Analysis Method
Soil	Pre-treatment, Mid-treatment, post-treatment	Air-drying, Grinding, Sieving	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
Plant Tissue (Roots)	Pre-treatment, Mid-treatment, post-treatment	Washing, Drying, Grinding	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
Plant Tissue (Leaves)	Pre-treatment, Mid-treatment, post-treatment	Washing, Drying, Grinding	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
Plant Tissue (Stems)	Pre-treatment, Mid-treatment, post-treatment	Washing, Drying, Grinding	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)



Fig. 2: *Brassica juncea* seeds.



Fig. 3: *Medicago sativa* seeds.

has a glossy texture, enhancing its appearance despite its small size. Fig. 2 shows the collected *Brassica juncea* seeds for investigation purposes.

***Medicago sativa* (Alfalfa):** The seeds of *Medicago sativa* are small and round, with a kidney-like or reniform appearance. They typically measure between 1.5 mm to 1.8 mm in length. The seed color can range from light brown to yellowish-brown, and they have a smooth surface with a high-gloss luster. Fig. 3 shows the collected Alfalfa seeds for the investigation purposes.

Treatments and Amendments

Phytoremediation treatments often include organic and inorganic amendments applied in order to improve the properties of the soil and make available for plant uptake of heavy metals like arsenic and chromium (Patel et al. 2021). Typically, compost is the most common organic additives that increase microbial growth and cation exchange capacity, thus increasing metal plant uptake. To improve the dissolution of metals and increase the chances of plants absorbing the metals, the addition of compost increases the rate of plant growth and constitutes an efficient way of increasing the uptake of chromium and arsenic by hyperaccumulators such as *Medicago sativa* and *Brassica juncea* (Rather et al. 2022).

Characteristics of Composting

Composting characteristics are mainly classified into

physical, biological, and chemical properties. The plants are typically grown within the physical characteristics of composting are shown in Table 3. Physical parameters are the main support for plant growth of both *Brassica* and *Medicago*. Temperature and humidity are a key parameter determining the success of composting operations in the experiment can accurately assess metal uptake and growth impacts in *Brassica juncea* and *Medicago sativa*, ensuring repeatable and reliable results. Apart from the physical characteristics, environmental conditions also should be monitored during the investigation. For phytoremediation techniques, compost can be beneficial for supplying the nutrients for plant growth and establishment and reduces the solubility of heavy metals from the industrial effluent.

Statistical Analysis for the Phytotechniques

To identify statistically significant differences among values, the content of plant tissues (taproot, lateral root, stem, and leaf) was analyzed using one-way ANOVA and post hoc Tukey's test ($p < 0.05$). ANOVA was used to analyze the relationship between plant height and growth. The use of statistics helps the researcher to quantify the parameters of relations between variables, which include the association between plant species and heavy metal concentration in the soil. It is used in deciding whether or not some observed patterns were really high or really low purely by chance. It becomes relevant here to quantify such results to justify the feasibility of phytoremediation approaches and the extent of metal adsorption by *Medicago sativa* and *Brassica juncea*.

A multiple comparison test is carried out to find out which of the treatments offered in the experiment are significantly different after a significant result is obtained from the ANOVA. It is always used to compare all pairs of treatments while at the same time controlling for Type I errors; it's called Tukey's Honestly Significant Difference (HSD) test. In this regard, following the analysis of variance by ANOVA to determine the variation in metal uptake across treatments, Tukey's HSD test would be used in comparing the chromium and arsenic accumulation in the roots, stems, leaves, and seeds of *Brassica juncea* and *Medicago sativa* across the various treatment codes. This procedure assists in determining which of the amendments or conditions

Table 3: Physical characteristics of compost.

S. No.	Compost physical characteristics	Range
1.	pH	
2.	Temperature	22-28°C
3.	Humidity	45-60%
4.	Moisture content	40-60%
5.	Organic content	35-55%

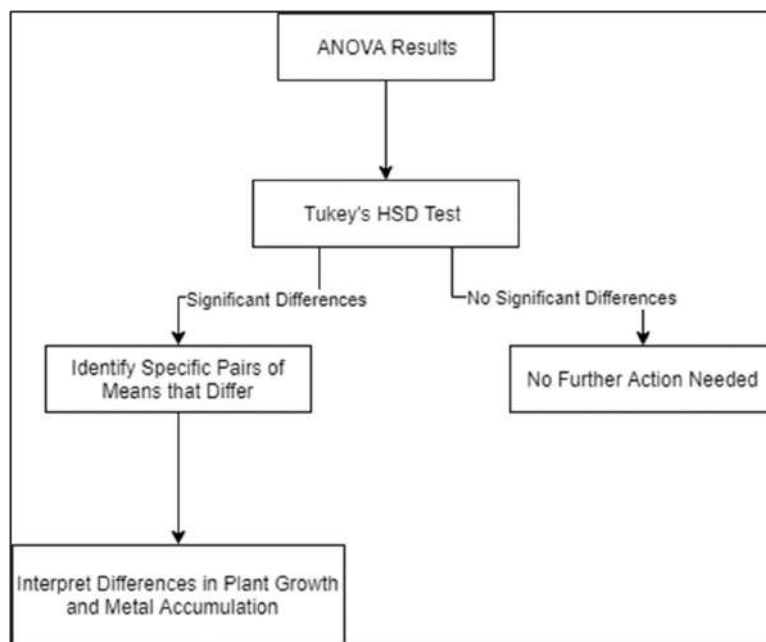


Fig. 4: Statistical analysis of the phyto-technique results.

enhances or reduces the efficiency of phytoremediation. Fig. 4 shows the statistical analysis of the phyto-techniques results.

Phytoremediation Experiment Details

Experimental design and setup: The experimental design involved planting seeds of Indian mustard (*Brassica juncea*) and alfalfa (*Medicago sativa*) in pots treated with industrial wastewater collected from a tanning industry in India. Additionally, composted organic matter was added to the soil to improve its efficiency in filtering various metals of interest. Each pot was then treated with solutions containing different concentrations of chromium and arsenic.

Quality and morphological analysis of plant samples: Throughout the experiment, the physical characteristics of the crops, including biomass, height, and leaf count, were recorded (Atabaki et al. 2020). Every two weeks, the chlorophyll content was measured using the SPAD meter; weekly measurements were made of the number of leaves, shoot length, and root growth. It also helps assess how well plants are doing in terms of growth and health at different pollution levels. (Kumar et al. 2018)

Plant sample analysis: Samples of the plants were taken. The meticulous handling did not do any damage to the root system. To remove dirt particles, deionized water was used to clean the plants. After that, the samples were divided into four groups: seeds, stems, leaves, and tree roots. To get a consistent weight, the samples were cleaned, let to

air dry, and then baked at 70°C. To ensure there was no contamination, these dried samples were subsequently crushed into a fine powder. The powdered samples were set aside for additional analysis and put in sealed vials.

Measurement of chromium and arsenic concentration in plant organs: Thus, the levels of arsenic (As) and chromium (Cr) in plant tissues were measured using inductively coupled mass spectrometry (ICP-MS) (Heitland et al. 2017). The components were broken down under carefully controlled settings in a microwave digestion facility using a 3:1 nitric oxide to hydrogen peroxide ratio. ICP-MS was used to analyze the digested solutions and determine the concentration of Cr and Ar in the roots, stems, leaves, and seeds of each plant.

Data interpretation: The results obtained at the end of the experiment show that the process of phytoremediation helped to decrease the level of heavy metals in the soil, especially chromium and arsenic. The means obtained for the metals are significantly higher in the root system than in stems, leaves, and seeds, which indicates that plants have used rhizofiltration, whereby metals are taken up and stored mainly in the root system without translocation to the aerial parts. This is considered advantageous in phytoremediation because it prevents the likelihood of metals being ingested by other creatures and making their way through the food chain or affecting wildlife by consuming the tissues of the plants.

Specifically, the use of organic compounds significantly increased the efficiency of metal absorption. Chromium and

arsenic levels were higher in plant tissues grown on soils supplemented with compost, suggesting that these substances increased the solubility and availability of heavy metals. By improving soil structure conformation and enhancing plant metal tolerance while preventing phytotoxicity symptoms, the amendments help plants grow.

RESULTS AND DISCUSSION

Monitoring of Plant Health

To maximize the absorption of metals and plant health, it is necessary to monitor the condition and development of *Medicago sativa* and *Brassica juncea* during phytoremediation. Biotic parameters, which include plant growth, chlorophyll content in leaves, biomass produced, and root elongation, are recorded at different intervals of time. Bi-weekly evaluation is done in terms of the height and biomass of plants that may be hampered by heavy metal stresses (Jan et al. 2024). Further, the chlorophyll index is checked every two weeks by a SPAD (Soil Plant Analysis Development) meter to measure photosynthesis activity. Some of the typical tests performed include chromium and arsenic content from soil and plant tissue samples, respectively. Thus, to improve the outcomes of remediation and to support the well-being of plants, it is necessary to control such factors and make necessary changes to the types of added soil and watering frequency. Table 4 shows the parameters and frequency of measurement and measurement method.

Morphological Characteristics

We evaluated seed morphology by measuring the length and diameter of the seeds. A digital caliper was used to measure

the length of each seed. The diameter of Indian mustard seeds is 1.5 mm, while the length of alfalfa seeds ranges from 1.5 mm to 1.8 mm.

Plant Growth and Response to Varying Effluent Doses

The impact of arsenic and chromium on plants was observed based on the changes in development in various concentrations of both metals in the soil (Saud et al. 2022). Fifty to 100 percent concentrations affected the plants negatively by decreasing their overall height, the number of leaves, and dry matter accumulation. However, with the addition of organic matter such as compost, some of the adverse consequences were offset since this improved plant growth, particularly at low levels of contaminants. Table 5. shows the effect of contaminant concentration on plant growth.

Accumulation of Chromium and Arsenic in Indian Mustard and Alfalfa

Consistent with previous findings, this study also showed that the level of metal accumulation was higher in root > stem > leaves > seed parts. Table 6 indicates the Accumulation of Cr and Arsenic in various parts of *Brassica juncea* (mg/kg dry weight) and is depicted in Fig. 5. The statistical analysis using one-way ANOVA for Accumulation of Cr and As in various parts of *Medicago sativa* of concentration of 50% of average value is 30.9%, standard deviation value is 33.5 and the F-ratio value is 6.77544. The p-value is .035272. Also, the statistical analysis using one-way ANOVA for Accumulation of Cr and As in various parts of *Medicago sativa* of concentration of 100% of average value is 35.8%, standard deviation value is 40.9, and the F-ratio value is 6.1133. The p-value is .042676.

Table 4: Parameter and frequency of measurement and measurement method.

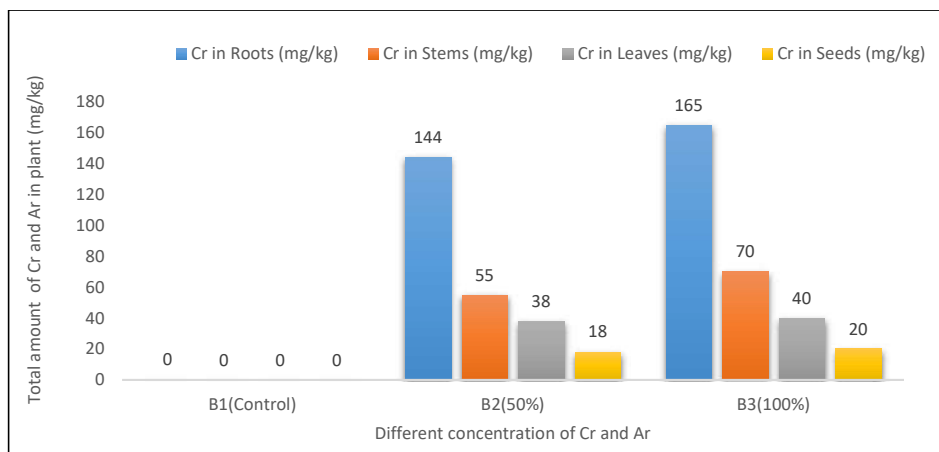
Parameter	Frequency of Measurement	Measurement Method
Plant Height	Weekly	Measuring Tape
Leaf Chlorophyll Content	Bi-weekly	SPAD Meter
Biomass Production	Weekly	Dry Weight Analysis
Root Development	Monthly	Root Excavation and Imaging
Metal Concentration (Cr, As)	Monthly	ICPMS

Table 5: Effect of contaminant concentration on plant growth.

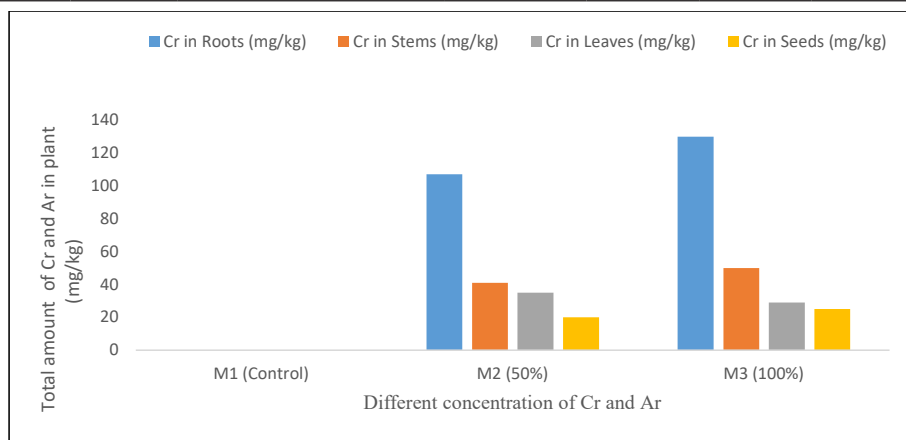
Plant species	Dose	Average Plant Height (cm)	Biomass (g)
<i>Brassica juncea</i>	B1 (Control)	120 ± 5	95 ± 4
	B2 (50%)	105 ± 4.5	82 ± 3
	B3 (100%)	90 ± 3.8	73 ± 2.5
<i>Medicago sativa</i>	M1 (Control)	15 ± 3.2	60 ± 3
	M2 (50%)	12 ± 2.5	45 ± 2
	M3 (100%)	10 ± 2.5	38 ± 2

Table 6: Accumulation of Cr and Arsenic in various parts of *Brassica juncea* (mg/kg dry weight).

Treatment Code	Cr in Roots (mg/kg)	Cr in Stems (mg/kg)	Cr in Leaves (mg/kg)	Cr in Seeds (mg/kg)	Ar in Roots (mg/kg)	Ar in Stems (mg/kg)	Ar in Leaves (mg/kg)	Ar in Seeds (mg/kg)	Average	SD
B1(Control)	0	0	0	0	0	0	0	0	0	0
B2(50%)	144	55	38	18	29	13	7	6	38.75	45.7
B3(100%)	165	70	40	20	34	12	9	8	44.75	52.8

Fig. 5: Accumulation of chromium and arsenic in all parts of *Brassica juncea*.Table 7: Accumulation of Cr and Arsenic in various parts of *Medicago sativa* (mg/kg dry weight).

Treatment Code	Cr in Roots (mg/kg)	Cr in Stems (mg/kg)	Cr in Leaves (mg/kg)	Cr in Seeds (mg/kg)	Ar in Roots (mg/kg)	Ar in Stems (mg/kg)	Ar in Leaves (mg/kg)	Ar in Seeds (mg/kg)	Average	SD
M1 (Control)	0	0	0	0	0	0	0	0	0	0
M2 (50%)	107	41	35	20	25	8	6	5	30.9	33.5
M3 (100%)	130	50	29	25	30	9	7	6	35.8	40.9

Fig. 6: Accumulation of chromium and arsenic in all parts of *Medicago sativa*.

It was established that the roots took in far higher levels of chromium and arsenic than any other parts of the plant that grew above the ground. The organic amendments in the soil improved metal accumulation in the plants, most

notably on their roots. Table 7 indicates the accumulation of Cr and arsenic in various parts of *Medicago sativa* (mg/kg dry weight) and is depicted in Fig. 6. The statistical analysis using one-way ANOVA for Accumulation of Cr and As in

various parts of *Medicago sativa* of concentration of 50% of average value is 38.759%, standard deviation value is 45.7, and the F-ratio value is 5.75488. The p-value is .047541. Also, the statistical analysis using one-way ANOVA for Accumulation of Cr and As in various parts of *Medicago sativa* of concentration of 100% of average value is 45.7%, standard deviation value is 52.8, and the F-ratio value is 5.73639. The p-value is .047811.

Comparison of Statistical Values *Medicago sativa* and *Brassica juncea*

To assess statistically significant variations in values, one-way ANOVA was used to analyze all of the data. Measurements of tissues (leaf, stem, and seeds) were

examined. One-way ANOVA $p < 0.05$. A one-way ANOVA with repeated measures was utilized to examine how plant height and growth in both plant species comparison of statistical values *Medicago sativa* and *Brassica juncea*. For plant species 1, *Brassica juncea* concentration of 50% of average value is 38.75%, the standard deviation value is 45.7, and the F-ratio value is 5.75488. The p-value is .047541, and the concentration of 100% of the average value is 44.7%, the standard deviation value is 52.8, and the F-ratio value is 5.73639. The p-value is .047811, and plant species 2 *Medicago sativa* of concentration of 50% of average value is 38.9%, the standard deviation value is 33.5, and the F-ratio value is 6.77544. The p-value is .035272, and the concentration of 100% of the average value is 35.8%,

Table 8: Chromium and arsenic concentration in roots vs. aboveground parts (mg/kg dry weight).

Plant species	Concentrations	Plant Part	Chromium (Cr) (mg/kg)	Arsenic (As) (mg/kg)
<i>Brassica juncea</i>	Control	Below ground	0	0
		Above ground	0	0
	50%	Below ground	144±5	29±1
		Above ground	111±3	26±3
	100%	Below ground	165± 6	34±1.2
		Above ground	130±3	29±4
<i>Medicago sativa</i>	Control	Below ground	0	0
		Above ground	0	0
	50%	Below ground	107±4	25±0.8
		Above ground	96±3.2	19±0.9
	100%	Below ground	130±5	30±0.9
		Above ground	104±4	22±0.7

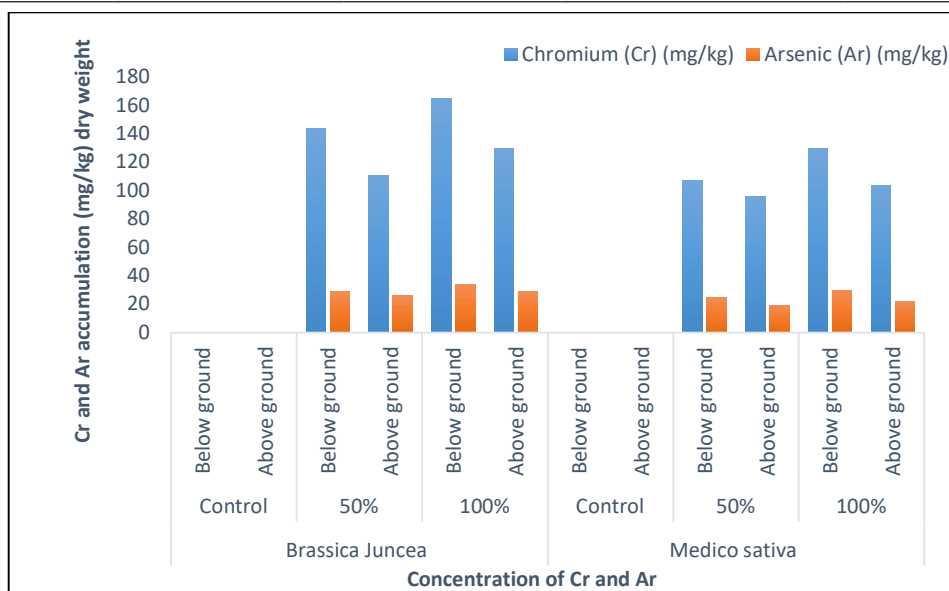


Fig. 7: Chromium and arsenic concentration in below-ground vs. above-ground parts.

the standard deviation value is 40.9, and the F-ratio value is 6.1133. The p-value is .042676. *Brassica juncea* takes a higher growth accumulation of Ar and Cr.

Comparison of Roots vs. Aboveground Accumulation

Chromium and arsenic mobility were higher in the root tissues as compared to the aboveground tissues of the plant. Such differences are usually observed in phytoremediation work where roots are known to be the main location of metal accumulation and first depot (Ullah et al. 2021). In this experiment, the roots had much higher levels of both metals, which can be attributed to the fact that they are in direct contact with the tainted soil and are responsible for taking in water and nutrients, where dissolved metals usually follow.

Nevertheless, the concentration of metals in the stems, leaves, and seeds was found to be comparatively lesser than that in the roots. Little transport of the heavy metals from the root to the above-ground portion was observed, meaning while the plant is good at the uptake of the contaminants, most of the metal is tied to the roots. Indeed, this implies that the role of the plant in phytoremediation is mainly in rhizofiltration (the absorption of contaminants through the roots without transport to other parts of the plant). Table 8. indicates the Chromium and Arsenic Concentration in Roots vs. Aboveground Parts (mg/kg dry weight) and is depicted in Fig. 7.

This comparison shows that plant roots can accumulate metals much more efficiently and are, therefore, useful in the phytoremediation of soils, in which it is essential to minimize the movement of metals to other parts of the plant that are edible or can be harvested.

Biological and Anatomical Factors Influencing Metal Uptake

Several factors related to biological and anatomical aspects of the plant have a direct bearing on its ability to uptake and store heavy metals. This is made possible by the root morphology, which has a highly developed surface area and also penetrates deep into the soil to ensure maximum contact with the contaminated soil, hence increasing the absorption of the metals (Akcin et al. 2018). Through root surface area and root exudates, which are substances secreted by the root tip, the absorption efficiency of the plant is enhanced by increasing the root or absorbing area and modifying the chemistry of the nutrient-avoiding layer about the root tips to increase the solubility and mobility of the metals, thus making them more absorbable.

Another factor is the ability of the plant to transport proteins that are located within root cells. These proteins assist in the transport and localization of heavy metals from

the root cells or membrane. Some of the metals are stored in the cell walls after they have been taken in by the plant, and this is effective in avoiding their transportation to other parts of the plant. Also, the cell walls of roots are much thicker as compared to stems, and this increases the availability of binding sites in the root system, hence increasing the uptake of heavy metals.

Additionally, the plant's ability to tolerate metals, including the synthesis of phytochelatins, small molecules that chelate to heavy metals and allow the sequestration of these metals into vacuoles, thus rendering them less toxic and permitting the plant to exist within metal-contaminated soils. These biological processes explain the plant's efficiency in growing in contaminated soil, further accumulating metals and not getting metal stress.

Soil Organic Matter Estimation

Sample Collection and Preparation

To measure changes in soil organic matter, soil samples were taken from experimental pots containing plants at the beginning, middle, and end of the trial. Using a soil auger, soil samples were taken at depths of 0 to 15 cm. To eliminate debris and standardize the samples, the soil was air-dried, crushed, and sieved to a size of 2 mm after collection. Following processing, these samples were placed in airtight receptacles to facilitate additional analysis of their organic matter composition.

Walkley-Black Methodology

The Walkley-Black method, one of the most widely used techniques for estimating soil organic carbon, was employed to evaluate the organic matter content of the soil samples. This technique involves oxidizing the organic carbon in the soil sample by adding a standard solution of potassium dichromate and sulphuric acid. To determine the amount of organic carbon, ferrous sulfate is used to treat the excess dichromate. Since the estimated organic matter content of the soil is around 58% carbon, a factor of 1.724 is used to calculate the percentage of organic matter.

Data Interpretation and Analysis

This made it possible to compare the amount of organic matter in the soil throughout the phytoremediation experiment with the Walkley-Black method's percentage of organic matter. To ascertain how *Medicago sativa* and *Brassica juncea* affected the amounts of soil organic matter, the data was analyzed. To determine if the plants had an impact on the pace of degradation or had added biomass that contributed to the buildup of SOM, comparisons were done at the beginning, middle, and conclusion of the study. Variance analysis was one of the results examined in this

Table 9: Metal uptake with and without organic amendments (mg/kg dry weight).

Treatment	Chromium (Cr) (mg/kg)	Arsenic (As) (mg/kg)
Without Amendments (100%) (Brassica juncea)	165 ± 6	34 ± 1.2
With Compost (Brassica juncea)	175 ± 7	38 ± 1.8
Without Amendments (100%) (Medicago sativa)	130 ± 5	30 ± 0.9
With Compost (100%) (Medicago sativa)	140 ± 5	35 ± 1.8

field to ascertain variations in organic matter across different treatment settings. This contributed to the understanding of the general outcome of phytoremediation on the environment in enhancing the quality of the soil.

Impact of Organic Amendments on Metal Uptake Efficiency

The incorporation of organic matter such as compost significantly influenced the efficiency of uptake of the metals by the plants (Zulfiqar et al. 2023). Organic amendments enhanced the physical as well as the chemical associated with soil fertility, including the organic matter fraction and the cation exchange capacity (CEC). By enhancing this property, better penetration of the metals in the root of the plant was made possible since they were made more bioavailable in the soil.

Amendments such as compost also bring in a population of organisms that catalyze the nutrient changes in the soil concerning heavy metals, making them bioavailable. On the other hand, given its high surface area and pore volume, where metals are adsorbed in forms that are more bioavailable to the plant roots. This enhanced the accumulation of metals, especially in the roots of the plants, without a high level of metal stress that would reduce the growth of the plant.

From the data presented in Table 9, it is evident that with the application of organic amendment, the overall absorption and accumulation of chromium and arsenic in the plant tissues was boosted. It was also evident from the results that better metal uptake efficiency resulted in improved growth response and health of the plants, further stressing the role of organic amendments in enhancing phytoremediation processes. Fig. 8 shows the treatment of Cr and Ar uptake with and without Organic Amendments.

Impact on Soil Properties

In phytoremediation, it is important that the treatment process is monitored so that it can assess the overall impact of phytoremediation besides the growth of plants and the removal of toxins from the soil. This is because during the process of phytoremediation, information on changes in levels of heavy metal concentrations, organic matter content, pH, and soil quality as a whole is monitored. Some of the plants include *Medicago sativa* and *Brassica juncea*, which alter the pH of the soil besides having an influence on the health of the soil and the availability of metals (Zhang et al. 2022). The toxicity of hexavalent chromium to soil microbial processes concerning soil properties and aging time. Also, these plants contribute to the content of the organic matter in the soil due to the release of root exudates and the decomposition of biomass. To know the remediation process in the best possible way, the changes regarding these characteristics should be tracked. This would allow the evaluation of metal removal effectiveness as well as enhancement of the soil quality. Table 10. shows the impact on soil properties.

Data Collection and Analysis

Evaluating the success of established phytoremediation programs requires information related to the program. An

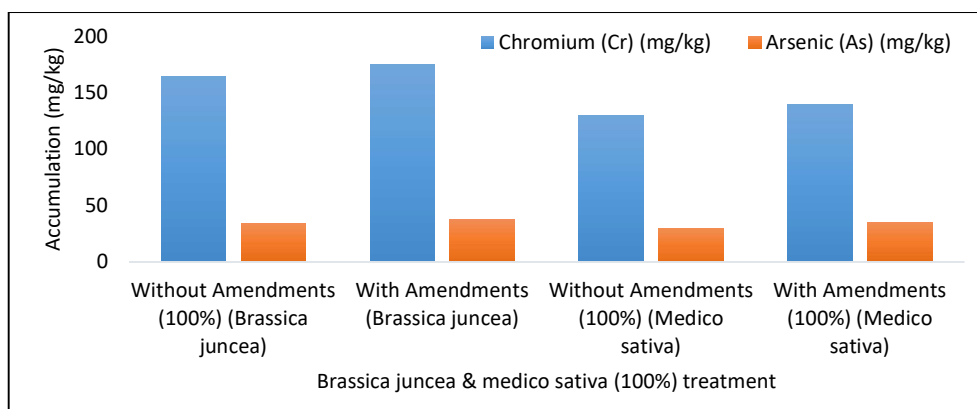


Fig. 8: Treatment of Cr and Ar uptake with and without organic amendments (mg/kg dry weight).

Table 10: Impact on soil properties.

Parameter	Initial Value	Final Value (Post-Experiment)
Soil pH	6.8±0.2	6.5±0.3
Organic Matter Content	3.5±0.2 %	4.0±0.2 %
Moisture Content (Soil)	25±2%	29±2%
Cation Exchange Capacity	15±1 cmol/kg	16±1 cmol/kg

indication of the accumulation and intake of heavy metals, especially chromium (Cr) and arsenic (Ar), in tissues of the root, stem, leaf, and seed of plants is indicated from the concentration levels of the tissue (Ansari et al. 2021). The information from gathered samples is useful for the purpose of comparing various treatments concentrating on increasing the yield of valuable metals. *Medicago sativa* and *Brassica juncea* have been exposed to various treatments to investigate the effect of the treatments on the bioconcentration of metals in the tissues of plants. This information entails a significant contribution to the optimization of plants to use in the process of operating for the decontamination of soil and to find out other treatments that would make the process of phytoremediation work more effectively.

Soil pH Determination

Collection and Preparation of Soil Samples

Three separate times were used to gather soil samples from

the experimental pots: before planting, during the trial, and after harvest. The results were extremely exact because the samples were taken in a grid pattern at depths of up to 15 cm. After that, stones and other undesirable particles were removed from the soil by air drying it, crushing it with a mortar and pestle, and sieving it through a 2 mm screen. For pH testing, the previously prepared soil samples were put in different, marked containers.

pH Measurement Technique

A 1:2.5 soil-to-water suspension method was used in the lab to assess the pH. A clean beaker was filled with 10 grams of dirt and 25 milliliters of distilled water. After thoroughly mixing the two solutions to incorporate all of the chemicals, the mixture was let to stand for half an hour. A calibrated digital pH meter was used to measure the mixture's pH after the solution had been added to bring it down to 10. Every sample was measured three times, and the mean value was calculated to increase measurement reliability. This is a good method for estimating soil pH, which determines the status of metals in relation to their bioavailability and plant growth. Soil pH before planting and after harvest data are indicated in Table 11. and depicted in Fig. 9.

Comparison of Results with Other Phytoremediation Studies

The findings in this study are in concordance with studies

Table 11: Soil pH before and after planting and harvest.

Plant species	Treatment Code	Soil pH Before Planting	Soil pH After Harvest
<i>Brassica juncea</i>	B1(Control)	6.5	6.7
	B2 (50 %)	6.3	6.5
	B3 (100%)	6.4	6.6
<i>Medicago sativa</i>	M1 (Control)	6.5	6.7
	M2 (50%)	6.3	6.8
	M3 (100%)	6.4	6.6

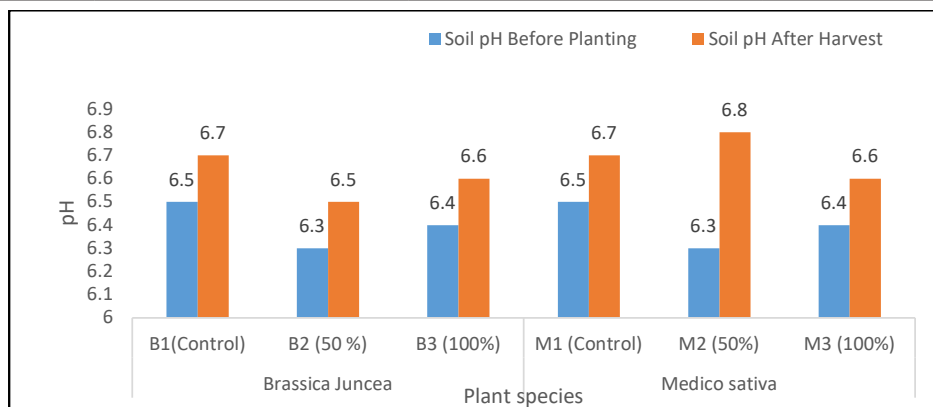


Fig. 9: Soil pH before planting and after harvests.

done on the efficiency of metal uptake in hyperaccumulator plants. Several studies have supported this by showing the effectiveness of the plant in absorbing and accumulating heavy metal, especially in the root system (Sajad et al. 2020). These results affirmed the enhanced capabilities supported by organic amendments, which have been demonstrated by other research on the effects of organic amendments on the efficiency of bioremediation.

The result of this study suggests that phytoremediation can be a viable technique for cleaning up contaminated soils with heavy metals like chromium and arsenic. The trends presented in the above results indicate that the plants, especially when combined with organic matter such as compost, have the potential to uptake some of these metals, hence lowering the total contamination level in the soil.

This study has the most important implication of bolstering the position that organic amendments can improve the efficiency with which these metals are taken up by plants. The increased CEC and SOM in the soils enabled the plant to absorb a higher amount of metals without any inhibition of growth rate. The present study's result also supports earlier studies, which demonstrate the importance of organic amendments in enhancing phytoremediation results. The root structure and the transport proteins found in the plants found the biological and anatomical ability of plants to accumulate heavy metals in roots to be important. (Ullah et al. 2021). This makes the selected plant species very suitable to be used for remediating contaminated soils, especially where it is necessary that the metal does not translocate to the above-ground tissues, hence preventing polluting the food chain.

Discussion on Statistical Analysis for Growth performance of *Brassica juncea* and *Medicago sativa*

In conclusion of statistical analysis, the current paper reveals the fact that dangerous heavy metals like arsenic and chromium that are found in water are very hazardous to human health. However, using indigenous plant species in phytoremediation comes out as a very viable solution. The purpose of this research is to identify the efficiency of different plants in order to perform a better study on how optimum phytoremediation can be achieved. Improving knowledge of these processes, as well as the practices that can support their application, can help to restore water and soil, or in other words, it contributes to the protection of the environment using statistical analysis tools like one-way ANOVA, the comparison of growth performance of three different concentration samples are selected and analyzed. Observed values are analyzed with the static tool, and the growth performance of *Brassica juncea* and *Medicago sativa* are detailed below:

All of the data were analyzed using a one-way ANOVA to see whether any differences in values were statistically

significant. Measurements of tissues (leaf, stem, and seeds) were studied. $p < 0.05$ for one-way ANOVA. Plant height and growth were compared between the two plant species, *Brassica juncea*, and *Medicago sativa*, using a one-way ANOVA with repeated measurements. For plant species 1, the F-ratio is 5.75488, the standard deviation is 45.7, and the 50% average concentration of *Brassica juncea* is 38.75%. The F-ratio is 5.73639, the standard deviation is 52.8, the p-value is 0.047541, and the concentration of 100% of the average value is 44.7%. There is a p-value of 0.047811. *Medicago sativa* plant species 2 had an average concentration of 38.9% at 50%, a standard deviation of 33.5, and an F-ratio of 6.77544. The F-ratio is 6.1133, the standard deviation is 40.9, the concentration of 100% of the average value is 35.8%, and the p-value is 0.035272. A p-value of 0.042676 is found. *Brassica juncea* accumulates more Ar and Cr throughout time.

Large-scale Applications and Potential Ecological Impacts - *Brassica juncea* and *Medicago sativa*

Brassica juncea and *Medicago sativa* plant species are cheaper, friendly to the environment, and easy to manage metal pollution in industrial areas when used with organic soil amendments. The methods hitherto employed for undertaking soil remediation include physical removal by digging or the use of chemicals, both of which are expensive and environmentally unsustainable. On this side of the argument, the process of phytoremediation appears to be an environmentally friendly technique with prospects of further application.

Environmental Management

The results obtained in the course of this study have a number of implications for the implementation of phytoremediation and other related environmental initiatives. This study establishes how *Medicago sativa* and *Brassica juncea* have the potential to uptake heavy metals such as lead, cadmium, and zinc from contaminated earth and, in the process, present an economical and efficient method of soil remediation.

Practical Applications

Utilization in contaminated sites: The results reveal the prospect of these plant species for utilization in contaminated agricultural land, industrial waste, and polluted city areas (Sharma et al. 2018). As such, the process does not disrupt the ecosystem as much as when people employ excavation and various chemical treatments. Environmental managers can, therefore, incorporate the use of such plants whenever the level of contamination is average to draw down the costs of cleaning the soil.

Advancement in green technologies: This study indicates that phytoremediation with *Medicago sativa* and *Brassica juncea* catalyzes the current trends for green approaches. Because they can bind metals without synthetic chemicals, they can now be marketed as environmentally friendly technologies for clean-up; thus, they are suitable for future green projects.

Potential for reforestation and land reclamation: These species can be put into the reforestation programs, especially in the post-mining or post-industrial sites with high concentrations of heavy metals. This effectively means that the plant can be useful in the restoration of damaged soil, which will, in turn, be useful for reforestation projects since it can spur the growth of vegetation and push for complete rehabilitation of affected ecosystems.

Incorporation into environmental policies: Based on the study's results, regulatory bodies, and environmental agencies should formally include the process of phytoremediation as a strategy for managing the environment. This could culminate in the setting of rules and policies concerning the utilization of plant-based remediation, hence encouraging industries to undertake environmentally friendly remedial methods.

Enhancement of remediation programs: Therefore, phytoremediation can effectively provide an affordable solution for dealing with other remediation programs, especially in scenarios where other repair strategies may be economically unfeasible and destructive to the environment.

Sustainable development goals (SDGs): The two plants used in phytoremediation, that is, *Medicago sativa* and *Brassica juncea*, affect more than one of the SDGs, including sustainable management of water resources and life on land.

It is recommended that large-scale field trials and research on phytoremediation involving *Medicago sativa* and *Brassica juncea* be conducted, focusing on exploring the long-term viability of using these plant species in large-scale applications, as well as assessing their potential ecological impacts. Such trials are essential for testing the effectiveness of various phytoremediation techniques, allowing researchers to gather valuable data on the suitability of these methods across different soil types and climatic conditions. This research could significantly enhance understanding and improve the practical application of phytoremediation strategies in diverse environmental contexts.

Thus, based on the practical findings of the current study, it is appropriate to state that the plant is efficacious in remediating a huge amount of material and contaminated soils and water bodies, particularly the industrial stretch (Prasad et al. 2021). This is because the plant is able to accumulate an amount of metal in its root, which is

rather useful in the remediation of soil contaminated with chromium and arsenic. Further, it is evident that the practical application of organic amendments, such as compost, is very advantageous for the process of phytoremediation due to the outcome of the current study. Other than improving the quantity of organic matter and adjusting the pH of the soil, they aid plant growth and give the plant a chance to absorb more of the metal.

Phytoremediation in Industrial Areas

There are significantly more green belt regions at the specific industry site. Depending on the water's features and industrial effluents, the plant species should be chosen, and phytoremediation techniques can be used in those specific locations. Through the careful selection of plant species that are appropriate for certain pollutants and the integration of many remediation processes, phytoremediation provides a sustainable approach to contamination management. Different pollution levels can be effectively treated with this approach. Incorporating community stakeholders into the process also increases support and improves the overall efficacy of remediation initiatives. In the end, this method turns polluted areas into healthy ecosystems by fostering environmental resilience and regeneration.

CONCLUSIONS

This study was useful in proving that the selected plant species is very efficient in phytoremediation, which was supported by the capacity to accumulate and deposit a respectable amount of chromium and arsenic within the root structures. As mentioned in the experiment, it is evident that the plant can adapt to the contaminated environment: the plant has good growth and biomass production in a lower concentration of contaminants. The study identified that the major source of heavy metals is the root system, where the authors discovered high levels of chromium and arsenic, especially in the root, as compared to the stems, leaves, and seeds. Furthermore, the incorporation of organic matters like compost enhanced the efficiency of total metal uptake. Regarding these amendments, it was possible to state that they were beneficial in improving the soil's properties for crop growth, increasing the organic matter content, and slightly decreasing the soil's pH to enhance the availability of heavy metals. As such, the results of this study showed that plants that have been treated with organic amendments have higher accumulations of chromium and arsenic in tissues than plants that were not treated with organic amendments. The results to up the effectiveness of phytoremediation and health and growth responses of plants under contaminated environments, implication for applied phytotechnology:

A multiple successional species approach to the use of organic amendment has been recommended, noting that this increases and boosts the overall health of the plant and growth performance.

Recommendations for Future Research Directions

Subsequent research should, however, focus on increasing the utilization of this plant species in various areas. It is essential there should be further studies of phytoremediation made in the field instead of the laboratory, as this study will help to realize the efficiency of the plant in natural ways where several factors, such as climate and types of soil, may influence the plant. Additional studies should also be conducted to identify how the efficiency of the plant in the uptake of the metals can be enhanced through genetic manipulation. Thus, phytoremediation could be even more effective and more used if only it had a higher tolerance to higher contaminant levels and better accumulation ability.

Recommendations for Policymakers and Environmental Practitioners

For policymakers and environmental practitioners, several steps can be taken to advance the application of phytoremediation:

When these recommendations are applied, it will be possible for policymakers and practitioners to contribute significantly to enhancing the application of phytoremediation as a typical environmental bioremediation process for ecological restoration and improvement of human health.

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Oxidized Acidic Biochar and *Pseudomonas aeruginosa* Enhanced Phosphorus Availability and Growth Performance of Transplanted Maize in Acid Soil

Hachib Mohammad Tusar¹, Md. Kamal Uddin^{1†} , Shamim Mia^{2,3}, Susilawati Kasim¹, Samsuri Bin Abd. Wahid¹, NorAsrina Sairi⁴ and Komariah⁵

¹Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

²School of Life and Environmental Sciences, The University of Sydney, Sydney-2015, Australia

³Department of Agronomy, Faculty of Agriculture, Patuakhali Science and Technology University, Dumki, PatuaKhali, Bangladesh

⁴Department of Chemistry, Faculty of Science, Universiti Malaya, Kuala Lumpur, Malaysia

⁵Department of Soil Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia

†Corresponding author: Md Kamal Uddin; mkuddin@upm.edu.my

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ABSTRACT

Maize plays a vital role in enhancing food security, particularly in regions facing agricultural challenges such as poor soil conditions, erratic rainfall, and limited access to resources. It can be advantageous for smallholder farmers in developing countries, where it can enhance productivity on limited land and under suboptimal soil conditions. One of the potential means for improving crop yield under suboptimal soil conditions, such as acidic soils, is the application of soil amendments. However, the combined effects of functionalized biochar (a pyrogenic carbon) and microbes on phosphorus (P) bioavailability and plant growth performance are still not well understood. This study investigates the optimization of transplanted maize growth in acidic soil through the application of rice husk biochar (RHB) that was oxidized with 10% hydrogen peroxide and inoculated with *Pseudomonas aeruginosa*, a phosphate-solubilizing bacterium. The oxidized biochar's pH was adjusted to 6.2 to enhance its effectiveness in challenging soil conditions. Soil properties and maize performance were determined using a pot culture. Results showed that the combined use of 10% oxidized RHB and *Pseudomonas aeruginosa* significantly increased P availability and phosphatase enzyme activity by 435% and 418%, respectively. Additionally, 10% Oxidized RHB treatment, microbes treatment and combination of biochar and microbes treatment showed yield increment 52%, 51% and 313% respectively, demonstrating the effectiveness of the treatment in improving soil fertility and crop productivity. This improvement in yield might have occurred due to an increase in soil pH, P bioavailability, and a reduction in Al toxicity since there were significant positive relationships between yield and soil pH and available P and a negative relationship with available Al concentration. These findings underscore the potential of integrating oxidized biochar and beneficial microbes, *Pseudomonas aeruginosa*, to enhance crop performance in acidic soils.

INTRODUCTION

Maize (*Zea mays*) has been an integral component of global agri-food systems since its domestication around 9,000 years ago. In recent decades, worldwide maize production has significantly increased, driven by growing demand, technological innovations, higher yields, and the expansion of cultivated areas (Erenstein et al. 2022). Transplanting involves relocating a plant from one site to another. This method is often used to establish crops when direct seeding is less viable due to unfavorable conditions (Sardar et al. 2020). Additionally, research findings indicate that transplanting cereals can help them adapt to unpredictable weather conditions, enhance their efficiency in using radiation and water, and suppress weed growth (Biswas 2020). Using transplanting as a strategy in maize cultivation helps achieve optimal plant density, improves crop stands, and ultimately enhances yield. This

technique also lowers nutrient requirements and shortens the crop's growth period (Sardar et al. 2020).

Soil pH plays a critical role in agricultural productivity because it directly affects the availability of essential nutrients to plants (Barrow & Hartemink 2023). In tropical regions, frequent rainfall, and high temperatures typically lead to the loss of essential cations, resulting in heavily weathered, acidic soils that pose a significant challenge for maize cultivation (Hasbullah et al. 2020).

Low soil organic matter is a major issue in arid and semi-arid regions. The increasing preference for inorganic fertilizers over organic alternatives has greatly contributed to the reduction of soil organic content, leading to adverse effects on soil health and fertility (Hussain et al. 2024). Organic amendments enhance the soil's physical, chemical, and biological properties, leading to improved soil fertility and increased crop yields (Eid et al. 2017).

Biochar, a carbon-rich material produced from waste biomass through pyrolysis, is gaining popularity for its ability to neutralize soil acidity while improving soil fertility and health (Tusar et al. 2023). It has gained worldwide attention due to its broad applications in enhancing soil quality and boosting soil productivity (Wang et al. 2017). When incorporated into the soil, biochar can modify the soil's texture, pore size distribution, and bulk density, leading to enhanced aeration and increased water-holding capacity (Hussain et al. 2020). Biochar has liming properties (Feigl et al. 2012). It enhances the cation exchange capacity of soil by raising its pH, modifying the availability of nutrients, and preventing nutrient loss through leaching (Fidel et al. 2017). Acidic soils frequently have limited phosphorus availability because phosphorus (P) tends to bind to soil particles, making it inaccessible for plant absorption (Muchoka 2021).

The natural solubilization of mineral phosphates is a key process carried out by various microorganisms, collectively referred to as phosphate-solubilizing microorganisms (PSM). Among these, bacteria are the most common and effective agents in dissolving mineral phosphates in natural environments compared to other types of microorganisms (Paul & Sinha 2017). For ensuring agricultural safety and sustainability, plant-growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* spp. Offer an excellent alternative to synthetic fertilizers. These eco-friendly and cost-effective biological applications serve as viable bioinoculants without posing a threat to soil biota (Das Mohapatra et al. 2024).

Phosphorus is a vital nutrient for plant growth and development, fulfilling multiple key functions in plant nutrition (Malhotra et al. 2018). Plants require phosphorus for various essential processes, including growth, sugar and starch utilization, photosynthesis, nucleic acid formation, and

cell division. A lack of P can impede overall plant growth and delay crop maturation (Gurmu 2023). Acid soils that bind significant amounts of phosphorus are typically medium-to fine-textured (Sanchez & Uehara 1980). Phosphorus is a highly reactive element that readily forms insoluble complexes with cations such as aluminum (Al) and iron (Fe) in acidic soils (Oburger et al. 2011). Research has demonstrated that phosphate-solubilizing microorganisms and different bacterial strains can greatly enhance phosphorus release from biochar, thereby increasing phosphorus availability in the soil and promoting plant growth (Rossati et al. 2023). Oxidized biochar creates a stable environment for the colonization and activity of phosphate-solubilizing bacteria (PSB), improving their survival and longevity in the soil (Ouyang et al. 2023).

Limited research has been conducted comparing the effects of rice husk biochar and *Pseudomonas aeruginosa* on transplanted maize specifically in acidic soils. Our study provides a tailored solution by leveraging the soil-amending properties of biochar alongside the biological action of *Pseudomonas aeruginosa*. Unlike conventional biochar applications, our work employs oxidized rice husk biochar treated with 10% hydrogen peroxide. This specific functionalization increases the surface properties and enhances the biochar's ability to adsorb and subsequently desorb phosphorus. While phosphate-solubilizing bacteria have been studied independently, our research uniquely demonstrates the combined effects of *Pseudomonas aeruginosa* with oxidized biochar. This combination would not only boost P solubilization but also enhance microbial activity, resulting in improved nutrient cycling and better soil health metrics such as pH balance and reduced aluminum toxicity. By using locally available rice husk biochar and a well-characterized bacterial strain, our approach is both cost-effective and environmentally sustainable. This holistic approach might demonstrate the long-term benefits of the treatments on soil health, going beyond the immediate yield improvements typically emphasized in other studies. Thus, our research is novel and impactful in overcoming the dual challenges of nutrient scarcity and soil acidity with significant implications for agriculture.

MATERIALS AND METHODS

Experimental Site

The pot trial was conducted in the new glasshouse, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor. The experimental site was located at 2°98'36.6" N (north) latitude and 101°73'81.9" E (east) longitude with an elevation of 56.8 m above sea level on the west coast of Peninsular Malaysia. The local climate was hot, humid tropics, and the

Table 1: Physical and chemical properties of oxidized RHB.

Treatments	Elemental composition [%]							Specific Surface Area (SSA)	
	C	H	N	S	O	C:H	O: C	BET Surface area [m ² .g ⁻¹]	Pore diameter Å
CBC	31.18±0.66a	2.11±0.05a	2.07±0.05b	1.78±0.07b	61.74±0.21c	14.78±0.05b	1.98±0.05c	278.52±0.92a	15.82±0.12d
5% OBC	24.67±0.83b	1.17±0.03b	3.66±0.05a	1.75±0.03b	66.81±0.33b	21.09±0.10a	2.71±0.08b	235.16±1.63b	16.97±0.03c
10% OBC	25.05±0.53b	1.15±0.01b	4.01±0.01a	0.98±0.02c	65.04±1.66bc	21.87±0.30a	2.60±0.01b	203.78±1.58d	38.09±0.16a
15% OBC	18.29±0.56c	1.16±0.01b	3.19±0.68ab	2.26±0.04a	71.56±0.83a	15.83±0.37b	3.92±0.08a	216.3±1.11c	24.95±0.01b
P value	<0.0001*	<0.0001*	0.0185*	<0.0001*	0.0006*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

C= Carbon, H= Hydrogen, N= Nitrogen, S= Sulpher, O= Oxygen

average minimum temperature was around 25°C, the average maximum temperature was 36°C, relative humidity was 79% during the whole experiment.

Rice Husk Biochar Collection, Activation, and Characterization

In this pot experiment, rice husk biochar (RHB) was utilized, produced from locally sourced feedstock in Malaysia. The biochar was collected from Sendi Enterprise (Sungai Burong, Selangor, Malaysia) and was produced by a pyrolysis process at 300°C. To measure the pH of the rice husk biochar, a 1:2.5 ratio of air-dried biochar to distilled water was used, using a pH meter following the method by (Ahmedna et al. 2000). The biochar was oxidized using 5%, 10%, and 15% H₂O₂, and the pH was adjusted $\approx$ to 6.2. Total elemental composition was determined using a CHN analyzer (LECO, Tru Spec Micro CHNS Analyzer, Germany). For elemental composition, 1 mg of the biochar samples was weighed into tin cups, and the mass was recorded. The total elemental composition was determined using a CHN analyzer (LECO, Tru spec® Micro). All samples were measured in triplicate.

Surface functionality was characterized using Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR, model Spectrum- 100, PerkinElmer). The surface area and micro-pore volume were determined by the Brunauer-Emmett-Teller method (Brunauer et al. 1938), which is the most well-known technique for the accurate determination of the surface area of carbonous materials. Briefly, the material is typically ground or sieved to ensure a uniform particle size and then degassed to remove any adsorbed gases or contaminants. The biochar samples were degassed at 300°C for 24 h under vacuum. By analyzing the amount of gas adsorbed as a function of pressure, the specific surface area and micro-pore volume were calculated using a mathematical model, the Brunauer-Emmett-Teller (BET) equation. The micro-pore volume was calculated using additional methods like the BJH method, which analyzes the distribution of pore sizes within the material (Barrett et al. 1951). The physico-chemical properties of the oxidized RHB are given in Table 1.

Soil Collection and Preparation

Surface soil (0-20 cm depth) was collected from the Bungor soil series (Typic Paleudult; Order: Ultisol) at Taman Pertanian, Universiti Putra Malaysia, Puchong, Selangor (2°58'59.7" N latitude; 101°38'47.5" E longitude). The soil sample was air-dried, ground, and sieved to less than 2 mm before undergoing chemical characterization and subsequent treatment. The properties of the soil, including texture, pH, cation exchange capacity (CEC), and exchangeable cations, are detailed in Table 2.

Pot Trial

A two-factor experiment was conducted following a randomized complete block design (RCBD) with three replicates. The treatment included a) biochar application-fresh and oxidized biochar with one control and b) microbial inoculation- *Pseudomonas aeruginosa* (Agkt 1) and with a control. Treatments were administered in plastic containers (38 cm in height, 32 cm in diameter, and 30 cm in depth) filled with 20 kg of soil. Each container had three holes drilled at the bottom to allow leachate to drain out. Moisture content was monitored and maintained using a portable moisture

Table 2: The physical and chemical properties of the initial soil.

Properties	Soil
Textural Class	Sand- 69.27%, Silt- 2.28% Clay-28.44% (Sandyclay loam)
pH	4.40±0.01
CEC [mmol.kg ⁻¹]	7.11± 0.03
Available P [mg.kg ⁻¹]	1.70± 0.05
Total C [%]	1.03± 0.02
Total N [%]	0.03± 0.02
Total S [%]	0.01±0.02
Exchangeable K [mmol.kg ⁻¹]	0.06±0.01
Exchangeable Ca [mmol.kg ⁻¹]	0.19±0.01
Exchangeable Mg [mmol.kg ⁻¹]	0.32± 0.02
Exchangeable Al [mmol.kg ⁻¹]	0.78± 0.04

meter (FieldScout TDR 150 Soil Moisture Meter). Biochar was mixed into the top 15 cm of the soil two weeks before maize seeds were sown. Four seedlings were transplanted at a depth of 2 cm in each pot and thinned to one healthy seedling per pot after 7 days of emergence. N-P-K fertilizer was applied in each pot recommended by Kashiani (2012), with application rates of: urea (4.87 g.pot^{-1}), triple superphosphate (3.49 g.pot^{-1}), and muriate of potash (2.64 g.pot^{-1}). The full dose of P and K fertilizer was applied as a basal dose one day before the seeds were sown. N fertilizer was applied in three equal splits on the 10th, 40th, and 65th day after sowing (DAS). In this experiment, the maize variety F1 hybrid sweet corn, a common variety used by Malaysian farmers and obtained from a local market, was used as the test crop. Plant management included manual weeding and pesticide application as needed to maintain experimental conditions. The experiment was conducted from January 2024 to April 2024 to assess the effects of bacterial inoculation on soil and plant health.

Preparation and Application of Bacterial Inocula

The bacterial culture of *Pseudomonas aeruginosa* (10^5 cfu.mL^{-1}) was utilized for soil microbial treatment. Bacterial strain was collected from the laboratory of microbiology, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia. The strains were initially sub-cultured in 100 mL Erlenmeyer flasks containing LB (Luria-Bertani) broth. These cultures were then shaken continuously for 24 h at 180 rpm and 28°C, following the method described by Jensen (1951). Bacterial concentration was measured using serial dilution techniques. Approximately 20 mL of the bacterial suspension was applied in two equal splits: one at the time of sowing (0 DAS - days after sowing) and the other at 20 DAS.

Soil Analysis

Determination of exchangeable K, Ca, Mg, and CEC in soil: Soil cation exchange capacity (CEC) was examined using the ammonium acetate shaking method (Rowell 2014) at pH 7. Five grams of the soil sample were placed into a Falcon tube. Subsequently, 50 mL of 1M ammonium acetate (NH_4OAc buffered at pH 7) was added to each tube, followed by shaking at 180 rpm for 30 min to facilitate cation exchange. Upon completion of shaking, the samples were centrifuged at 4000 rpm for 10 min to separate the soil particles from the solution. The filtered solution was kept to determine the concentrations of potassium (K), calcium (Ca), and magnesium (Mg) using an Atomic Absorption Spectrophotometer (AAS). Following the initial analysis, the soil in each tube, now saturated with ammonium ions, was passed through a three-times washing step with 50 mL of

95% ethyl alcohol to remove excess ammonium acetate. The washed soil was then mixed with 50 mL of 0.1N potassium sulfate (K_2SO_4) to facilitate the replacement of exchangeable ammonium ions by potassium ions. Subsequently, the process of centrifugation, filtration, and analysis of ammonium was performed using a segmented flow analyzer (AA 500).

Determination of Inorganic nitrogen in soil: Soil inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3\text{-N}$) was analyzed by extracting with 2M KCl (soil: solution, 1:5) (Nelson 1982). Briefly, 10 g of soil was taken into a Falcon tube, and 50 mL of 2M KCl was added while the suspension was shaken for 1 h. Ammonium and nitrate were analyzed using a segmented flow analyzer (AA 500) as discussed before (Nelson 1982).

Determination of available phosphorus in soil: Available phosphorus in the soil was determined using the Bray II method (Bray & Kurtz 1945). Two grams of air-dried soil (2.00 mm) were weighed into a 20 mL plastic vial and reacted with 14 mL of extracting solution (0.03 NH_4F and 0.1M HCl), then sealed with parafilm. The soil suspension was shaken for 45 seconds using the wrist inversion technique. The extract was filtered through Whatman No. 42 filter paper into a plastic vial. The final product was analyzed using a segmented flow analyzer (AA 500).

Determination of soil microbial population: Ten grams of fresh soil samples were used to determine the total microbial population using the spread dilution plate technique (Parkinson et al. 1971). Serial dilutions from 10^{-2} to 10^{-7} were prepared by sequentially transferring 1.0 mL of the sample into each test tube containing 9 mL of sterile distilled water (SDW). The samples were spread over the respective media using a sterilized bent glass rod. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 24-48 h for bacteria. The colonies formed were counted, and populations were calculated as colony-forming units (CFU) per mL solution.

Determination of soil phosphatase activity: Soil phosphatase enzyme activities were determined by using testing kits from Beijing Solarbio Science and Technology Co., Ltd (China). The catalog number of the soil alkaline phosphatase (S-AKP/ALP) Activity Assay kit was BC0280 (Guan & Wang 2023). To prepare the soil samples, 0.1 g of soil was placed in a vial. Then, 0.05 mL of toluene was added, and the mixture was shaken for 15 min. Next, 0.4 mL of reagent 1 was added to the vial, and the mixture was incubated at 37°C for 24 h. After incubation, 1 mL of reagent 2 was added, and the mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was carefully collected for further analysis. To prepare the supernatant solution, 50 μL of the collected supernatant was transferred to a new vial, and 100 μL of reagent 3 and 20 μL of reagent 4 were added. For the blank solution, 50 μL of reagent 1, 100 μL of reagent

3, and 20 μL of reagent 4 were mixed in a separate vial. For the standard solution, 50 μL of the supplied standard solution was combined with 100 μL of reagent 3 and 20 μL of reagent 4. Additionally, 830 μL of distilled water was added to the supernatant, blank, and standard solutions. All solutions were allowed to stand at room temperature for 30 min. After this incubation period, the absorbance of each solution was measured at 660 nm using a spectrophotometer.

Plant Performance Analysis

We examined the treatment effect of alkaline biochar on plant performance by measuring plant height, stem diameter, cob length, cob diameter, and yield using a measuring tape, vernier caliper scale, and weighing balance. After harvest, the plant parts (stem, leaves, and corn) were placed into envelopes and dried in an oven at 60°C for 72 h (Lija et al. 2014). The plant biomass was then recorded.

Measurement of SPAD Value by Using SPAD-502 Meter

Leaf greenness, serving as an indicator of chlorophyll content, was evaluated using a portable chlorophyll meter (SPAD-502, Konica Minolta, Inc., Tokyo, Japan). To ensure precision and consistency, SPAD readings were taken from fully matured leaves of each plant, with an average of three measurements per leaf (Yuan et al. 2016).

Root Measurement

After harvesting the plants from the pots were enclosed in a plastic bag immediately to prevent dehydration, washed carefully with tap water, and separated into shoots and roots for root growth. After the root was washed, the root was prepared for the determination of the root length by using a measuring tape.

Plant Nutrient Analysis

The dried and ground plant material (0.25 g) was used for digestion. The single wet digestion technique (Cottenie 1980) was conducted to extract the macro elements from the plant tissues. The wet digestion technique is a widely used method for determining plant nutrient concentrations by breaking down organic material using strong acids. To begin, plant samples are first dried at 60-70°C, ground into fine powder, and then weighed (0.25 g) into digestion tubes. A mixture of concentrated sulfuric acid (H_2SO_4) was added to the sample. The sample was then pre-digested overnight. After that, 2 mL 30% H_2O_2 was added and heated on a hotplate or digestion block at 285°C until the solution became clear, indicating a complete breakdown of organic matter. Perchloric acid (HClO_4) may also be used for tougher samples to ensure full digestion. Once digestion is complete, the solution is cooled,

diluted to a 100 mL volume with distilled water, and filtered to remove undissolved particles. The resulting clear solution was analyzed for nutrient concentrations.

N and P concentrations were analyzed using a segmented flow analyzer (AA 500). Additionally, K, Ca, and Mg concentrations were determined using atomic absorption spectroscopy (AAS, PerkinElmer). The plant nutrient uptake was calculated using the following formula (Rabieleh et al. 2015).

$$\text{Uptake (mg.plant}^{-1}\text{)} = \text{Total nutrient concentration (\%)} \times \text{biomass (g)},$$

where the nutrient concentration was found using AAS, and the biomass was the plant's respective dry weight.

Percent Relative Data

The relative data of the values were expressed as percentages, relative to the control for each element recommended by Ashraf & Waheed (1990). The formulas are as follows, where the treatment value was the biochar and microbes amendment, and the control value was without amendment.

$$\text{Relative data (\%)} = (\text{Treatment value} - \text{control value} / \text{control value}) \times 100$$

Statistical Analysis

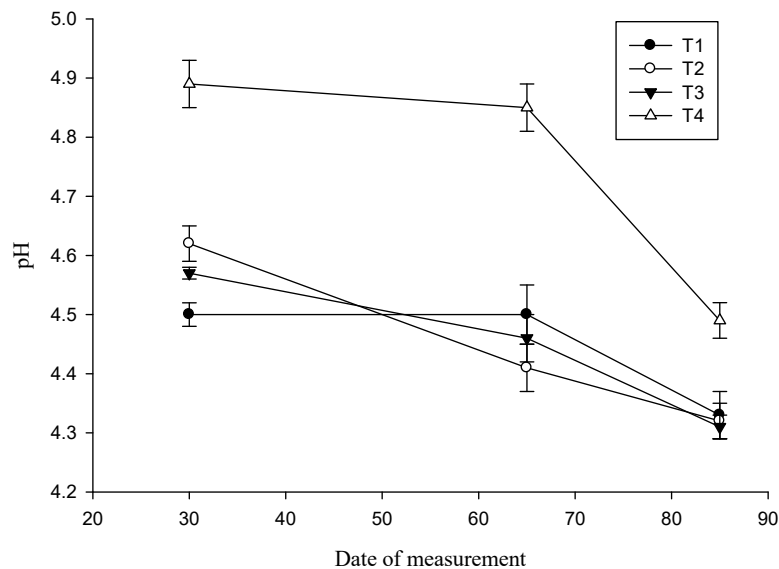
All data were analyzed using the two-way analysis of variance (ANOVA) procedure, and means were separated by Tukey's Honestly Significant Difference (HSD) test at a 5% level of significance using the Statistical Analysis System, JMP software (SAS incorporation).

RESULTS

Soil pH

Rice Husk Biochar (RHB), microbes, and their combination significantly increased soil pH at 30 and 65 days after sowing (DAS) ($P < 0.05$, Fig. 1). At 30 DAS, pH ranges from 4.5 to 4.89. The application of biochar increased soil pH, with 10% oxidized biochar (T2) showing a significant difference ($p < 0.01$) compared to the control (T1), resulting in an increase of pH of 0.12 units. Microbial treatment at T3 showed a significant increase ($p < 0.01$) in soil pH compared to the control. Among all treatments, the combination of 10% oxidized biochar and *Pseudomonas aeruginosa* (T4) demonstrated the highest soil pH (4.89), significantly different ($p = 0.0128$) from the control T1 (4.5).

At 65 DAS, pH ranges from 4.5 to 4.85. The combined effect of 10% oxidized biochar and *Pseudomonas aeruginosa* (T4) resulted in the highest soil pH (4.85), 0.35 units higher



T1 = No microbes and no biochar, T2 = No microbes and 10% oxidized biochar, T3 = *Pseudomonas aeruginosa* and no biochar, T4 = *Pseudomonas aeruginosa* and 10% oxidized biochar. The error bar represents the standard error of the mean.

Fig. 1: pH of different biochar amendments measured at different dates.

than the control and significantly different ($p < 0.01$) from the control T1 (4.5).

At 85 DAS, the lowest pH value (4.33) was found at T1 and the highest value (4.49) at the combined treatment (T4). 10% oxidized biochar and *Pseudomonas aeruginosa* (T4) increased soil pH by 0.16 units, which was significantly different ($p = 0.01$) from the control treatment.

Post-Harvest Soil Nutrient Status

The application of treatments significantly influenced the availability of P in the soil (Table 3). The highest available P was found in the combined treatment T4 (7.92 mg.kg^{-1}), which was significantly greater than the control treatment T1 (1.48 mg.kg^{-1}). Biochar treatment (T2) (4.74 mg.kg^{-1}) and microbes treatment T3 (2.74 mg.kg^{-1}) also showed significant increases. Statistical analysis confirmed that both biochar ($p < 0.0001$), microbial treatments ($p = 0.0002$), and their combination ($p = 0.0233$) significantly increased available P levels by 220%, 85%, and 435%, respectively.

In this study, inorganic nitrogen (N) levels range from 16.97 to 30.54 mg.kg^{-1} and are presented in Table 3. Biochar treatment (T2) significantly increased inorganic N. The combined application of biochar and microbes was not significant.

Table 3 represents the significant effect of biochar, microbes, and their interaction on soil CEC. Soil CEC ranges from 5.25 to $10.19 \text{ mmol.kg}^{-1}$. The Biochar effect was not significantly different, but the microbes effect ($p =$

0.0081) and the biochar microbes combined effect ($p = 0.0142$) were significant. Exchangeable potassium levels ranged from 0.09 to $0.16 \text{ mmol.kg}^{-1}$. Biochar ($p = 0.0001$), microbes ($p = 0.0175$), and their combined effect ($p = 0.0175$) had a significant positive effect on exchangeable K. Combine treatments of biochar and microbes (T4) increased K level by 78% compared to the control. Biochar, microbes, and their combination influenced exchangeable calcium levels. The highest values were observed in the combined treatment T4 ($0.13 \text{ mmol.kg}^{-1}$), and the lowest value ($0.09 \text{ mmol.kg}^{-1}$) was found in the control treatment (T1), and the total increment was 44%.

In this study, Table 3 showed exchangeable magnesium (Mg) values that were highest in 10% oxidized RHB treatments, T2 ($0.15 \text{ mmol.kg}^{-1}$), which indicates significantly increased exchangeable Mg levels ($P = 0.007$). Microbial treatment's effect was not significant, but the combined effect was significant ($p = 0.0200$). In this study, exchangeable aluminum ranged from 0.36 to $0.45 \text{ mmol.kg}^{-1}$. The control treatment ($0.45 \text{ mmol.kg}^{-1}$) demonstrated the highest levels of exchangeable aluminum (Al). Microbes treatment T3 and combined treatment T4 significantly reduced exchangeable Al by 25% and 10%, respectively.

RHB and Microbes' Effect on Transplanted Maize Nutrient Concentration

Tables 4 and 5 demonstrate the effect of oxidized Rice Husk Biochar (RHB), microbes, and their interactions on nutrient concentration and nutrient uptake in maize. The nutrient

Table 3: Effect of oxidized RHB and microbes on changes in nutrients of the post-harvest soil.

Treatments	Available P mg.kg ⁻¹	Inorganic N mg.kg ⁻¹	CEC [mmol. kg ⁻¹]	Exchangeable K [mmol.kg ⁻¹]	Exchangeable ca [mmol.kg ⁻¹]	Exchangeable Mg [mmol.kg ⁻¹]	Exchangeable Al [mmol.kg ⁻¹]
T1	1.48±0.18c	16.97±0.82a	5.25±0.14b	0.09±0.003b	0.09±0.01b	0.06±0.02b	0.45±0.01a
T2	4.75±0.63b	30.87±7.56a	9.82±1.11a	0.12±0.008b	0.08±0.01b	0.15±0.03a	0.39±0.01b
T3	2.74±0.63c	20.16±0.83a	11.79±1.45a	0.09±0.006b	0.10±0.01b	0.12±0.01a	0.36±0.003c
T4	7.92±0.18a	30.54±4.09a	10.19±0.73a	0.16±0.01a	0.13±0.01a	0.14±0.01a	0.41±0.01b
Biochar	<i>P</i> = < 0.0001*	<i>P</i> = 0.0234*	<i>P</i> = 0.1717	<i>P</i> = 0.0001*	<i>P</i> = 0.0606	<i>P</i> = 0.007*	<i>P</i> = 0.4996
Microbes	<i>P</i> = 0.0002*	<i>P</i> = 0.7502	<i>P</i> = 0.0081*	<i>P</i> = 0.0175*	<i>P</i> = 0.0066*	<i>P</i> = 0.0827	<i>P</i> = 0.0006*
BC*M	<i>p</i> = 0.0233*	<i>p</i> = 0.6961	<i>p</i> = 0.0142*	<i>p</i> = 0.0175*	<i>p</i> = 0.0285*	<i>p</i> = 0.0200*	<i>p</i> = < 0.0001*

T1 = No microbes and no biochar, T2 = No microbes and 10% oxidized biochar, T3 = *Pseudomonas aeruginosa* and no biochar, T4 = *Pseudomonas aeruginosa* and 10% oxidized biochar.

concentration of maize plants is presented in Table 4. P levels ranged from 0.13% to 0.51%, with the highest concentration observed in treatment T4 (10% oxidized biochar combined with *P. aeruginosa*), which was significantly higher than the control. This combination increased P concentration by 292% compared to the control. Biochar (*p*=0.0013), microbes (*p*=0.0001), and their combination (*p*=0.0040) all showed significant differences from the control. N concentration ranged from 0.05% to 0.13%, with the highest value in T4, representing a 160% increase. Biochar, microbes, and their combined effect were all statistically significant. K concentration varied from 0.78% to 1.45%, with the lowest value in the control (T1) and the highest in T2 (10% oxidized biochar without microbes). Again, biochar, microbes, and their combination significantly affected K levels. Ca concentrations ranged from 0.01% to 0.11%, with the highest value in T1. Biochar had a significant effect on Ca concentration, but the influence of microbes and their combination was not significant compared to the control. Lastly, magnesium (Mg) concentrations ranged from 0.01% (in the control) to 0.017% (in T3 with *P. aeruginosa* but no biochar). Biochar, microbes, and their combination did not show significant effects on Mg concentration compared to the control.

RHB and Microbes Effect on Transplanted Maize Total Nutrient Uptake

The effect of oxidized Rice Husk Biochar (RHB), microbes, and their combination on phosphorus (P) uptake in maize is represented in Table 5. It was found that P uptake ranges from 189.42 to 460.83 mg.plant⁻¹. P uptake significantly increased with the application of biochar, microbes, and their combination. On average, across microbial treatments, 10% oxidized biochar (T2) increased P uptake by 78%, which was significantly different (*p*=0.0060) from the control (T1). Microbial treatment and combined treatment (10% oxidized biochar and *Pseudomonas aeruginosa*) at T3 and T4 also exhibited a significant increase in P uptake, 133% and 143%, respectively, compared to the control treatment (T1).

Similarly, nitrogen (N) uptake was significantly increased by the application of biochar, and microbes, but not significant on their combination (Table 5). N uptake was ranged from 36.98 to 150.72 mg.plant⁻¹. Biochar treatment at T2 and microbes treatment at T3 represented a 71% and 135% increment of N uptake compared to the control (T1). Among all treatments, the highest N uptake (150.72 mg.plant⁻¹) was observed with the combined application of 10% oxidized biochar and *Pseudomonas aeruginosa* (T4). This study

Table 4: Acidic RHB and microbes' effect on maize plant nutrient concentration.

Treatments	% P concentration	% N concentration	% K concentration	% Ca concentration	% Mg concentration
T1	0.13±0.02c	0.05±0.01b	0.78±0.08a	0.11±0.01a	0.01±0.01a
T2	0.34±0.02b	0.12±0.01a	1.49±0.18	0.07±0.01b	0.014±0.001a
T3	0.47±0.02a	0.13±0.02a	1.27±0.2a	0.01±0.01c	0.017±0.001a
T4	0.51±0.02a	0.13±0.01a	1.45±0.05a	0.01±0.002c	0.016±0.001a
Biochar	<i>p</i> = 0.0013*	0.0284*	0.0341*	0.0046*	0.6605
Microbes	<i>p</i> = 0.0001*	0.0118*	0.1792	<0.0001*	0.2898
BC*M	<i>P</i> = 0.0040*	0.0284*	0.1284	0.0066*	0.5453

Means within the same column followed by different letters are significantly different at *p* ≤ 0.05 (Turkey's HSD test). T1 = No microbes and no biochar, T2 = No microbes and 10% oxidized biochar, T3 = *Pseudomonas aeruginosa* and no biochar, T4 = *Pseudomonas aeruginosa* and 10% oxidized biochar.

Table 5: Oxidized RHB and microbes' effect on transplanted maize plants' total nutrient uptake.

Treatments	Total P mg.plant ⁻¹	Total N mg.plant ⁻¹	K mg.plant ⁻¹	Ca mg.plant ⁻¹	Mg mg.plant ⁻¹
T1	189.42±3.83c	36.98±5.73b	441.42±23.22c	46.46±2.75a	6.17±0.91b
T2	336.26±29.15a	63.18±18.42b	1050.99±82.82b	48.45±9.15a	13.87±0.77a
T3	441.87±18.98ab	86.95±18.60b	1103.97±91.63ab	8.36±1.28b	13.16±2.83ab
T4	460.83±33.33a	150.72±6.17	1401±44.50	7.89±2.41	13.39±0.64a
Biochar	<i>P</i> = 0.0060*	<i>P</i> = 0.0113*	<i>P</i> = 0.0001*	<i>P</i> = 0.8820	<i>P</i> = 0.0352*
Microbes	<i>P</i> = < 0.0001*	<i>P</i> = 0.0011*	<i>P</i> = < 0.0001*	<i>P</i> = < 0.0001*	<i>P</i> = 0.0720*
BC*M	<i>p</i> = 0.0212*	<i>p</i> = 0.2091	<i>p</i> = 0.0471	<i>p</i> = 0.8115	<i>p</i> = 0.0440*

Means within the same column followed by different letters are significantly different at $p \leq 0.05$ (Turkey's HSD test). T1 = No microbes and no biochar, T2 = No microbes and 10% oxidized biochar, T3 = *Pseudomonas aeruginosa* and no biochar, T4 = *Pseudomonas aeruginosa* and 10% oxidized biochar.

represented a significant increase in potassium (K) uptake due to the application of biochar and microbes, as shown in Table 4. Total K ranges from 441.42 to 1401 mg.plant⁻¹. The biochar treatment at T2, the microbes treatment at T3, and the biochar microbes combined treatment at T4 represented K uptake increment 609.57 mg, plant, 662.55 mg.plant⁻¹, and 959.58 mg.plant⁻¹ compared to the control treatment T1. This study exhibited that the biochar and the combined application of biochar and microbes did not show significant differences in calcium (Ca) uptake, as shown in Table 5. The highest Ca uptake (48.45 mg.plant⁻¹) was found at biochar treatment T2, which was not significantly different from the control treatment (T1), and the lowest Ca uptake was found at combined treatment T4 (7.89 mg.plant⁻¹). Table 5 exhibited a significant influence on biochar, microbes, and their combination for Mg uptake. Mg uptake ranges from 6.17 to 13.87. Biochar treatment T2, microbes treatment T3, and combined treatment T4 showed 124%, 113%, and 117% increments of Mg uptake, which are significantly different from the control treatment (T1).

Oxidized RHB and *P. aeruginosa* Combine Effect on SPAD Value

The SPAD value was influenced by the application of biochar, microbe, and the combination of biochar with phosphate-solubilizing bacteria (Table 6). At 30 DAS, SPAD value ranges from 32.13 to 40.8. The combination of biochar and microbes ($p = 0.0031$) showed a significant effect compared to the control T1. At 45 DAS, the highest SPAD value was found at microbes treatment T3 (46.2), and the lowest value was found at biochar treatment T2 (40.63). The application of biochar, microbes, and biochar-biochar-microbes combination effects was not significant at 45 DAS. By 65 DAS highest SPAD value (49.03) was observed at *Pseudomonas aeruginosa* and 10% oxidized biochar treatment T4. The lowest SPAD value (42.26) was found in the control treatment T1. Biochar and the combined effects

Table 6: Effect of RHB and microbes on SPAD value (mean±SE).

Treatments	30 DAS	45 DAS	65 DAS
T1	33.06 ± 0.47b	44.5 ± 1.67a	42.26±0.77b
T2	32.13 ± 0.89b	40.63 ± 0.81a	42.5±1.79b
T3	32.9 ± 1.49b	46.2 ± 2.05a	46.1±1.64ab
T4	40.8±1.09a	45.06±0.64a	49.03±1.06
Biochar (BC)	<i>p</i> =0.0109*	<i>p</i> =0.1165	<i>p</i> =0.2816
Microbes(M)	<i>p</i> =0.0038*	<i>p</i> =0.0629	<i>p</i> =0.0058*
BC×M	<i>p</i> = 0.0031*	<i>p</i> = 0.3642	<i>p</i> = 0.3642

Means within the same column followed by different letters are significantly different at $p \leq 0.05$ (Turkey's HSD test). T1 = No microbes and no biochar, T2 = No microbes and 10% oxidized biochar, T3 = *Pseudomonas aeruginosa* and no biochar, T4 = *Pseudomonas aeruginosa* and 10% oxidized biochar.

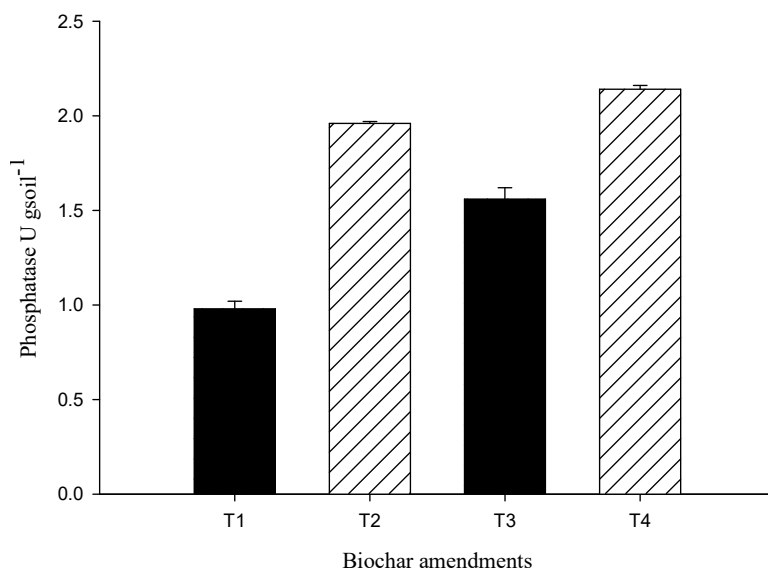
of biochar microbes were not significant at 65 DAS, but the microbes' effect was significant ($p = 0.0058$).

Combined Effect of Oxidized RHB and *P. aeruginosa* on Soil Phosphatase Activity

Application of oxidized RHB and phosphate solubilizing bacteria *Pseudomonas aeruginosa* in soil significantly improved soil phosphatase activity Fig. 2. Among all treatments, the highest enzyme value was found at 10% oxidized RHB and *Pseudomonas aeruginosa* treatment, T4 (2.14 U.g⁻¹ soil) and the lowest value was found at control treatment, T1 (0.98 U.g⁻¹ soil). Biochar, microbes, and their combination showed significant increments of 100%, 59%, and 418% phosphatase activity compared to the control. Inoculation of bacteria *Pseudomonas aeruginosa* on soil phosphatase activity was better than non-inoculated.

Effect of Oxidized RHB and *P. aeruginosa* on Soil Microbial Populations

The influence of phosphate-solubilizing and N₂-fixing bacteria and oxidized RHB biochar on microbial population was presented in Table 7. No significant difference was



T1 = No microbes and no biochar, T2 = No microbes and 10% oxidized biochar, T3 = *Pseudomonas aeruginosa* and no biochar, T4 = *Pseudomonas aeruginosa* and 10% oxidized biochar. The error bar represents the standard error of the mean.

Fig. 2: RHB and microbes' effect on soil phosphatase activity.

found in this study among biochar ($p=0.4092$), microbes ($p=0.8368$), and their combination treatments ($p=0.5760$) compared to the control (T1).

Effect of Oxidized RHB and *P. aeruginosa* on Plant Growth Characters and Yield

In this experiment, Maize plant height, stem diameter, root length, dry biomass, number of grains, and yield were influenced by the addition of biochar, microbes, and their combination, as presented in Table 8. Plant height ranges from 162.33 (microbes treatment, T3) to 183.33 cm (combine treatment, T4). Among all treatments highest plant height (183.33 cm) was found at *Pseudomonas aeruginosa* and 10% oxidized biochar treatment (T4) and was significantly

different from microbial treatment (T3), but not significantly different from other treatments. Stem diameter ranged from 1.36 to 1.49 cm. The highest stem diameter was found at the combined treatment T4 (1.49 cm), and the lowest stem diameter was at the control (1.36 cm). The effect of biochar, microbes, and their combination on stem diameter was not significant in this experiment.

Root length was significantly influenced by biochar ($p=0.0041$), microbes ($p=0.0590$), and the combination of biochar and microbes ($p=0.0463$). The longest roots were observed in treatment T2 (10% RHB biochar and no microbes) with an average length of 63.66 cm. The shortest roots were observed in the control treatment T1 (36.33 cm). Among all treatments, biochar treatments and biochar microbes combined treatment significantly increased root length by 75% and 73%, respectively, compared to the control treatment T1. In this study, dry biomass was significantly influenced by biochar ($p=0.0294$) and microbes ($p=0.0260$), although their interaction was not significant ($p=0.7354$). The highest dry biomass was recorded in treatment T4 (89.93 g.pot⁻¹) and the lowest dry biomass was in the control treatment, T1 (45.42 g.pot⁻¹). The total increment of dry biomass was 98%, which was found by the combination of biochar and microbes treatment (T4).

However, the maize yield exhibited significant differences (Table 8) with the biochar ($p < 0.0001$), microbes ($p < 0.0001$), and combined application of oxidized RHB and *Pseudomonas aeruginosa* ($p = 0.0081$) compared to untreated soil (T1). The highest yield was

Table 7: Oxidized RHB and Microbes' effect on soil microbial populations.

Treatments	Microbial Population [cfu.mL ⁻¹]
T1	$6.3 \times 10^{-5} \pm 1.01 \times 10^{-5}$ a
T2	$3.94 \times 10^{-5} \pm 1.78 \times 10^{-5}$ a
T3	$5.7 \times 10^{-5} \pm 5.29 \times 10^{-6}$ a
T4	$5.2 \times 10^{-5} \pm 2.45 \times 10^{-5}$ a
Biochar (BC)	$p=0.4092$
Microbes(M)	$p=0.8368$
BC×M	$p=0.5760$

Means within the same column followed by different letters are significantly different at $p \leq 0.05$ (Turkey's HSD test). T1= No microbes and no biochar, T2 = No microbes and 10% oxidized biochar, T3 = *Pseudomonas aeruginosa* and no biochar, T4 = *Pseudomonas aeruginosa* and 10% oxidized biochar.

Table 8: Effect of oxidized RHB and *Pseudomonas aeruginosa* on transplanted maize plant growth characters and yield.

Treatments	Plant height [cm]	Stem diameter [cm]	Root length (cm)	Dry biomass [g.pot ⁻¹]	No.of grain/cob	Yield g.pot ⁻¹
T1	168±3.05ab	1.36±0.06a	36.33±2.40b	45.42±1.20b	79±3.5b	10.37±0.73c
T2	165.66±1.85ab	1.37±0.04a	63.66±1.45a	65.10±12.61ab	165±21.5a	21.47±1.32b
T3	162.33±1.85b	1.37±0.03a	56±7.57a	64.45±6.89ab	159±11.5a	21.18±0.99b
T4	183.33±1.85a	1.49±0.09a	63±3.05a	89.93±8.14a	204±12a	42.85±2.42a
Biochar	$p=0.0680$	$p=0.3445$	$p=0.0041^*$	$p=0.0294^*$	$p=0.0087^*$	$p<0.0001^*$
Microbes	$P=0.2122$	$P=0.3217$	$P=0.0590$	$P=0.0260^*$	$P=0.0120^*$	$P<0.0001^*$
BC*M	$P=0.0299^*$	$P=0.4200$	$P=0.0463^*$	$P=0.7354$	$P=0.2135$	$P=0.0081^*$

Means within the same column followed by different letters are significantly different at $p \leq 0.05$ (Turkey's HSD test). T1 = No microbes and no biochar, T2 No microbes and 10% oxidized biochar, T3 *Pseudomonas aeruginosa* and no biochar, T4 *Pseudomonas aeruginosa*, and 10% oxidized biochar.

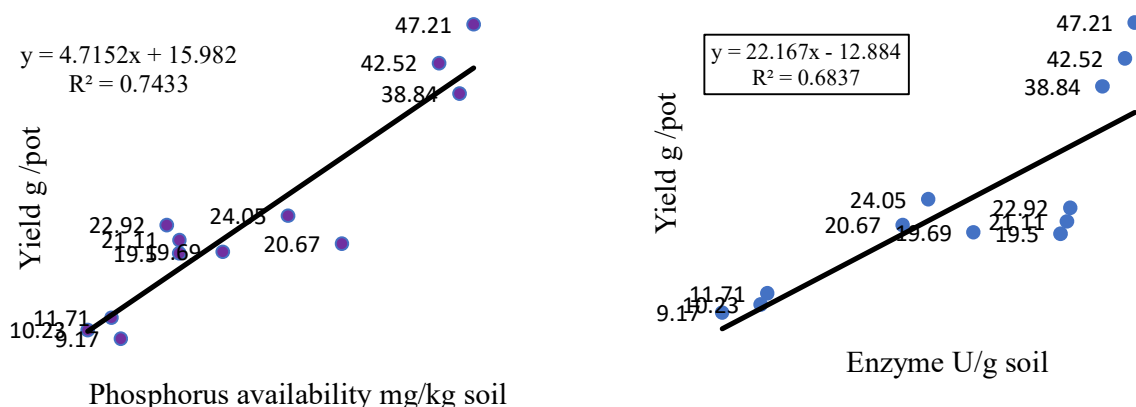


Fig. 3: Regression analysis to show the relationship between the change of soil available P and enzyme against grain yield.

found at T4 (42.85 g.pot⁻¹), and the lowest yield was found at the control treatment (10.37 g.pot⁻¹). 10% Oxidized RHB treatment (T2), microbes treatment (T3), and a combination of biochar and microbes treatment (T4) showed yield increments of 52%, 51%, and 313% respectively.

These improvements in yield might have occurred due to an increase in soil pH, P bioavailability ($r^2=0.74$), and a reduction in Al toxicity ($r^2=0.68$) (Fig. 3).

The biplot (Fig. 4) displayed the first two principal components (PC1 and PC2), which together account for a significant portion of the variability in the data: 54.8% for Component 1 and 16.2% for Component 2. Variables such as Inorganic N mg.plant⁻¹, Enzyme (U.g⁻¹), and CEC have arrows pointing toward the right, indicating a positive association with Component 1. Variables like Ex Al mmol.kg⁻¹ and Microbial population have arrows pointing toward the left, suggesting a negative association with Component 1. Variables such as Average pH and AVP (phosphorus availability) have arrows pointing upward, showing a positive association with Component 2.

Correlation Between Soil Parameters, Plant Parameters, and Nutrient Uptake

Pearson's correlation analysis was conducted to determine the relationship among the soil nutrients, plant parameters, plant nutrient uptake, and yield (Table 9). Grain yield was positively correlated with soil pH ($r=0.89$), Soil available P ($r=0.93$), exchangeable K, Ca, Mg ($r=0.84$, 0.80 and 0.63 respectively), enzyme ($r=0.82$), P uptake ($r=0.73$), N uptake ($r=0.88$), Root length ($r=0.64$) and dry biomass ($r=0.81$). Furthermore, available phosphorus was positively correlated with soil pH ($r=0.84$), Enzyme was positively correlated with available P ($r=0.67$), pH ($r=0.60$), and CEC ($r=0.76$) dry biomass was positively correlated with pH ($r=0.72$), available P ($r=0.77$) P, N, K, Mg uptake ($r=0.70$, 0.86 , 0.82 , and 0.70 respectively).

DISCUSSION

Soil Properties

Biochar and P-solubilizing bacteria can potentially affect soil properties. In our study, rice husk biochar, microbes, and their combination significantly increased soil pH at

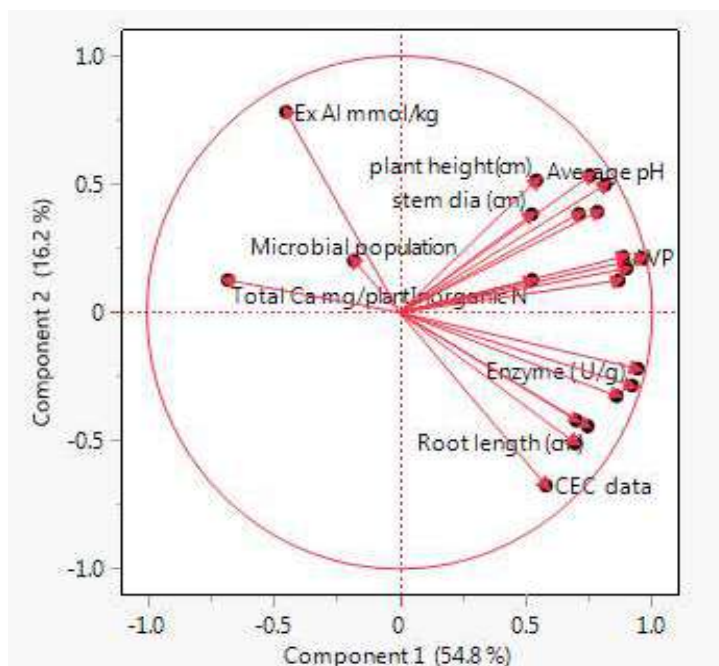


Fig. 4: Analysis of the principal component of different variables.

all three measured dates (30, 65, and 85 days after sowing, DAS). At 30 DAS, 65 DAS, and 85 DAS, the combination of 10% oxidized biochar and *P. aeruginosa* demonstrated an increase in soil pH that ranged from 4.5 to 4.89, 4.5 to 4.85 and 4.33 to 4.49 and the increments were 0.39, 0.35 and 0.16 unit, respectively, compared to the control treatment. An increase in pH contributed to the reduction of Al concentration. Compared to the control treatment, biochar decreased Al^{3+} content by helping to mitigate Al toxicity in acidic soils (Xia et al. 2023). Biochar application is reported to increase soil pH in acidic soil (Tusar et al. 2023). Similarly, individual studies also reported a pH increase. For instance, water hyacinth biochar increased soil pH by 0.72 units at a 1% application rate and by 0.86 units at a 2% application rate, while simultaneously decreasing exchangeable acidity (Lewoyehu et al. 2024).

Oxidized biochars exhibited substantially higher Al^{3+} binding capacity compared to unmodified primary biochars, resulting in a pH increase of 2.5 to 3.5 (Qian & Chen 2014). The addition of biochars significantly enhanced the soil pH buffering capacity (pH buff), with the extent of improvement influenced by the acid buffering capacity of the biochar applied. The primary mechanism driving the increase in pH buff within the pH range of 4.0–7.0 was the release of cations, attributed to the protonation of carboxyl groups on the biochar surfaces and the dissolution of carbonates. This mechanism accounted for over 67% of the observed pH buff increase (Shi et al. 2017).

Our result was in line with the study by Lewoyehu et al. (2024). *Pseudomonas aeruginosa*, an environmentally derived pathogen known for its antibiotic resistance, is capable of producing metabolites such as ammonia, which can raise soil pH by forming ammonium, thereby neutralizing soil acidity (Laborda et al. 2021). Combining oxidized biochar with *Pseudomonas aeruginosa* can boost microbial activity in the soil, support plant growth, enhance soil structure, and facilitate nutrient release, leading to improved soil health and better pH buffering (Ouyang et al. 2023).

The effect of biochar, microbes, and their combination on soil CEC is demonstrated in Table 3. Soil CEC ranges from 5.25 to 10.19 mmol/kg mmol. In this study, the combined effect of biochar and *Pseudomonas aeruginosa* was significantly different from the control treatment T1 on soil CEC. The CEC is one of the most important soil chemical properties that can be changed through biochar application. The functional groups on oxidized biochar can indeed interact with soil organic matter, potentially increasing the effective CEC (Kharel et al. 2019). The aged biochars exhibit an increase in negative surface charge and a reduction in their isoelectric point, attributed to the carboxylation of their surfaces during the aging process. The development of carboxylate groups on biochar surfaces enhances both the negative surface charge and the cation exchange capacity (CEC) (Lawrinenko et al. 2016). The application of biochar enhances CEC by promoting the availability of basic cations like Ca^{2+} and Mg^{2+} , which are crucial for soil fertility. Studies

Table 9: The relationship between transplanted maize soil parameters, plant parameters, and nutrient absorption.

Parameters	Av pH	Av SPAD	AVP	Inorganic N	CEC data	Ex K mmol.kg ⁻¹	Ex Ca mmol.kg ⁻¹	Ex Mg mmol.kg ⁻¹	Ex Al mmol.kg ⁻¹	Microbial population	Enzyme [U.g ⁻¹]	Total P mg.plant ⁻¹	Total N mg.plant ⁻¹	Total K mg.plant ⁻¹	Total Ca mg.plant ⁻¹	Total Mg mg.plant ⁻¹	plant height[m]	stem dia [cm]	Root length [cm]	Dry biomass g.plant ⁻¹	yield gm.pot ⁻¹
Average pH	1.00																				
Average SPAD	0.74	1.00																			
AVP	0.84	0.54	1.00																		
Inorganic N	0.38	0.32	0.47	1.00																	
CEC data	0.18	0.18	0.38	-0.03	1.00																
Ex K mmol.kg ⁻¹	0.85	0.57	0.93	0.24	0.21	1.00															
Ex Ca mmol.kg ⁻¹	0.86	0.78	0.72	0.36	0.12	0.78	1.00														
Ex Mg mmol.kg ⁻¹	0.37	0.17	0.69	0.37	0.66	0.52	0.14	1.00													
Ex Al mmol.kg ⁻¹	0.02	-0.09	-0.14	-0.09	-0.83	0.02	-0.06	-0.49	1.00												
Microbial population	-0.07	-0.02	-0.09	-0.34	-0.14	0.03	0.10	-0.46	0.22	1.00											
Enzyme [U.g ⁻¹]	0.60	0.67	0.67	0.40	0.76	0.53	0.58	0.68	-0.70	-0.19	1.00										
Total P mg.plant ⁻¹	0.50	0.61	0.61	0.27	0.74	0.52	0.55	0.58	-0.70	0.05	0.94	1.00									
Total N mg.plant ⁻¹	0.80	0.70	0.82	0.56	0.37	0.72	0.77	0.43	-0.23	0.14	0.76	0.77	1.00								
Total K mg.plant ⁻¹	0.62	0.55	0.82	0.51	0.64	0.68	0.58	0.75	-0.54	-0.05	0.90	0.91	0.87	1.00							
Total Ca mg.plant ⁻¹	-0.52	-0.76	-0.35	-0.19	-0.60	-0.26	-0.53	-0.27	0.55	0.04	-0.83	-0.78	-0.60	-0.60	1.00						
Total Mg mg.plant ⁻¹	0.26	0.23	0.49	0.34	0.60	0.37	0.21	0.70	-0.61	-0.30	0.71	0.75	0.55	0.79	-0.38	1.00					
plant height [cm]	0.73	0.51	0.60	0.19	0.08	0.61	0.67	0.19	0.15	-0.24	0.34	0.25	0.46	0.30	-0.37	0.16	1.00				
stem dia [cm]	0.58	0.48	0.49	0.55	0.02	0.47	0.55	0.39	0.03	-0.41	0.38	0.18	0.36	0.33	-0.25	0.10	0.42	1.00			
Root length [cm]	0.44	0.12	0.68	0.46	0.67	0.48	0.22	0.86	-0.56	-0.34	0.70	0.62	0.60	0.80	-0.30	0.79	0.16	0.27	1.00		
Dry biomass g.plant ⁻¹	0.72	0.59	0.77	0.53	0.31	0.74	0.76	0.46	-0.28	-0.08	0.69	0.70	0.86	0.82	-0.41	0.70	0.44	0.46	0.65	1.00	
yield g.pot ⁻¹	0.89	0.76	0.93	0.57	0.40	0.84	0.80	0.63	-0.24	-0.18	0.82	0.73	0.88	0.86	-0.61	0.53	0.61	0.58	0.64	0.81	1.00

Av. P: available P; Ex. K: exchangeable K; Ex. Ca: exchangeable Ca; Ex. Mg: exchangeable Mg; Ex. Al: ex-changeable Al;

have shown that applying biochar to soil improved soil pH, reaching 1.49 units and larger increases in CEC (Haile *et al.* 2024). Microbially induced soils amended with biochar have been demonstrated to enhance soil properties, including cation exchange capacity (CEC), through the activity of microorganisms (Shukla & Sharma 2024). The combined application of biochar and *Pseudomonas aeruginosa* substantially increases microbial populations. It enhances microbial activity, and results in the release of byproducts that improve soil structure and increase nutrient availability, thereby further boosting soil CEC (Olajumoke *et al.* 2024).

The study investigated the impact of the phosphate-solubilizing bacterium *P. aeruginosa*, in combination with oxidized rice husk biochar (RHB), on the soil microbial population. The findings, as presented in Table 7, offer insights into how these amendments interact and their combined effects on soil microbial dynamics. When applied separately or combined, neither the biochar nor the microbial treatment resulted in a significant difference in the microbial population compared to the unamended control. At pH levels below 5.0, *P. aeruginosa* shows a significant reduction in cell count and metabolic activity, suggesting a critical point where its growth is greatly hindered (Wang *et al.* 2024). Our experimental pH ranges between 4 to 4.5. It is crucial to recognize that strong acids and bases can influence the functional activity of microorganisms (Teng *et al.* 2020).

Oxidized RHB and phosphate-solubilizing bacteria *Pseudomonas aeruginosa* in soil significantly influenced soil phosphatase activity (Fig. 2). Biochar, microbes, and their combination showed significant increments of 100%, 59%, and 418% phosphatase activity compared to the control. *Pseudomonas* species, including *P. aeruginosa*, are known to produce phosphatases that catalyze the hydrolysis of organic phosphorus compounds (Chauhan & Dhaked 2022). The combined use of biochar and *P. aeruginosa* has been demonstrated to substantially enhance microbial populations and enzyme activities, such as phosphatase (Olajumoke *et al.* 2024). The porous nature of biochar allows for better microbial colonization, which can protect enzymes from degradation, thus sustaining their activity over time (Sun *et al.* 2024). Phosphatase enzymes generally show greater activity in alkaline pH conditions; therefore, the rise in pH caused by the biochar can boost the activity of these enzymes (Guan & Wang 2023, Schalk & Perraud 2023).

The application of treatments significantly influenced the availability of phosphorus (P) in the soil (Table 3). Statistical analysis confirmed that biochar, microbial treatments, and their combination significantly influenced available P levels at 220%, 85%, and 435%, respectively. The possible mechanisms are – a) Enhance soil pH and liming effect. b)

Improved P desorption and c) other activities such as surface functionalization of biochar, reduction of iron and aluminum binding of phosphorus, stimulation of microbial activity, increased CEC, enhanced organic matter mineralization, Mitigation of soil compaction, etc. Soil amendment can affect the availability and adsorption ability of phosphorus in the soil, and biochar has the best effect on the availability of soil phosphorus (Hong *et al.* 2018)

Biochar can enhance phosphorus (P) availability in soil by offering a habitat and carbon source for phosphate-solubilizing bacteria (PSB), which are capable of dissolving low-solubility P compounds. Thus, the combined use of biochar and PSB presents an eco-friendly approach to boost PSB activity in soil, thereby increasing P mobilization and improving plant productivity (Siddiqui *et al.* 2016).

Acidic oxidized biochar can reduce phosphorus fixation and increase plant phosphorus availability in acidic soils (Zhao *et al.* 2023). Phosphate-solubilizing bacteria can dissolve insoluble phosphorus compounds in the soil, making the phosphorus available for plant uptake (Tao GuangCan *et al.* 2008).

Biochar can facilitate the formation of *Pseudomonas aeruginosa* biofilms, improving soil colonization and nutrient solubilization, which may enhance phosphorus availability and microbial activity in specific areas of the soil (Egamberdieva *et al.* 2018). The interaction of oxidized biochar with soil and microorganisms may lead to the release of phosphorus, thereby increasing the amount of available phosphorus in the soil solution and affecting nutrient dynamics (Liu *et al.* 2023). The combination of organic material and phosphate-solubilizing bacteria can increase phosphorus availability more effectively than individual components (Timofeeva *et al.* 2023).

RHB and Microbial Interactions on Transplanted Maize Growth and Yield

In this experiment, maize plant height, stem diameter, root length, dry biomass, and yield were influenced by the addition of biochar, microbes, and their combination, as presented in Table 8. Among all treatments highest plant height (183.33 cm) was found at *Pseudomonas aeruginosa* and 10% oxidized biochar treatment (T4) and was significantly different from microbial treatment (T3), but not significantly different from other treatments. The effect of biochar, microbes, and their combination on stem diameter was not significant in this experiment. The longest roots were observed in treatment T2 (10% RHB biochar and no microbes) with an average length of 63.66 cm. The shortest roots were observed in the control treatment T1 (36.33 cm). Among all treatments, biochar treatments and

biochar microbes combined treatment significantly increased root length by 75% and 73%, respectively, compared to the control treatment T1. In this study, dry biomass was significantly influenced by biochar ($p=0.0294$) and microbes ($p=0.0260$), although their interaction was not significant ($p=0.7354$). The highest dry biomass was recorded in treatment T4 (89.93 g.pot⁻¹), and the lowest dry biomass was in the control treatment, T1 (45.42 g.pot⁻¹). The total increment of dry biomass was 98%, which was found by the combination of biochar and microbes treatment (T4).

However, the maize yield exhibited significant differences (refer to Table 8) with the biochar ($p < 0.0001$), microbes ($p < 0.0001$), and combined application of oxidized RHB and *Pseudomonas aeruginosa* ($p 0.0081$) compared to untreated soil (T1). The highest yield was found at T4 (42.85 g.pot⁻¹), and the lowest yield was found at the control treatment (10.37 g.pot⁻¹). 10% Oxidized RHB treatment (T2), microbes treatment (T3), and a combination of biochar and microbes treatment (T4) showed yield increments of 52%, 51%, and 313% respectively compared to the control.

The PCA (Table 9, Fig. 4) biplot suggests that certain soil properties and nutrient uptakes (such as soil pH, available P, and enzyme activity) have a strong influence on maize yield and growth parameters. Biochar alone increased root length and volume, while microbial inoculation improved stem diameter and dry biomass. Its porosity and adsorption capacity make biochar an effective carrier for plant growth-promoting rhizobacteria (PGPR), enhancing crop growth (Ajeng et al. 2020). The combination of biochar (5 t.ha⁻¹) and bacteria (Sb16) was found to enhance soil quality and promote the growth of maize (Abdulrahman et al. 2017). In a greenhouse experiment, applying biochar at 5 tons per hectare along with bacterial inoculation significantly ($P < 0.05$) boosted the growth of maize, improving shoot and root biomass, root length, root volume, plant height, and leaf chlorophyll content. Oxidized rice husk biochar likely helped neutralize harmful substances, promoting healthier plants with increased biomass (Rizwan et al. 2016).

Ashry & Hassan (2019) studied the combined effects of biochar and plant growth-promoting endophytic bacteria (PGPE) on pepper plants. The combination significantly improved all growth metrics and yield compared to individual applications. (El-Naggar et al. 2019) discussed various studies where biochar combined with microbial inoculants, such as *Pseudomonas* species, resulted in yield increases ranging from 20-30% due to enhanced nutrient cycling and reduced soil acidity.

The combination of biochar and *P. aeruginosa* led to a notable increase in maize yield, ranging from 15-25% compared to untreated control groups (Rafique et al. 2017).

The combined effect of biochar and beneficial microbes likely promoted the growth of a well-developed, branched root system in maize plants. This enhanced the efficiency of nutrient uptake and assimilation, ultimately resulting in a higher grain yield (Sun et al. 2022, Ain & Noraini 2023). This is a short-term study, and the results may vary for long-term experiments under field conditions. A long-term field study should be carried out to validate the results.

CONCLUSIONS

This study concludes that the combined application of oxidized rice husk biochar (RHB) and *Pseudomonas aeruginosa* effectively enhances soil properties and boosts transplanted maize yield in acidic soils. The use of acidic oxidized RHB and phosphate-solubilizing bacteria significantly improved phosphorus availability by 435% and phosphatase enzyme activity by 418%. Additionally, a combination of biochar and microbes treatment showed a yield increment of 413.21%. These findings highlight the potential of this combined treatment for improving soil fertility and crop productivity in challenging soil conditions.

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Tuning the Traits of Nickel Oxide Nanoparticles Employing Green Synthesis Using *Brassica oleracea* L. Var. *capitata* for Exploring the Antimicrobial, Anticancer and Supercapacitor Applications

Ruquaiya Shaikh¹, Arif Pathan², Mohd. Anis³, Satish P. Mardikar⁴, M. I. Baig⁵, Mazahar Farooqui⁶ and R. D. Isankar^{1†}

¹Department of Chemistry, Government Vidharbha Institute of Science and Humanities, Amravati, Maharashtra, India

²Department of Chemistry, Maulana Azad College of Arts, Science and Commerce, Chhatrapati Sambhajinagar, Formerly Aurangabad, 431001, Maharashtra, India

³Department of Physics & Electronics, Maulana Azad College of Arts, Science and Commerce, Chhatrapati Sambhajinagar (Aurangabad) 431001, Maharashtra, India

⁴Smt. R.S. College, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India

⁵Physics Department, Ram Meghe College of Engineering and Management, Badnera, Maharashtra, India

⁶Maulana Azad College of Arts, Science and Commerce, Chhatrapati Sambhajinagar (Aurangabad) 431001, Maharashtra, India

†Corresponding author: R.D.Isankar; ramisankarrd@gmail.com

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ABSTRACT

The Nickel Oxide (NiO) nanoparticles are synthesized using the green synthesis method utilizing *Brassica oleracea* L. var. *capitata* leaf extracts. The X-ray diffraction studies revealed that synthesized NiO-NPs exhibit face-centered cubic structure. SEM images of NiO-NPs exhibited agglomerated structures. TEM images of NiO-NPs revealed spherical morphology with variable sizes ranging from 5-50 nm. FT-IR spectrum was used to confirm the various functional groups. The absorbance spectral studies reveal the existence of three important peaks at wavelength 545 nm, 589 nm, and 636 nm. The antibacterial activity of NiO-NPs against Gram +ve *Staphylococcus aureus* (NCIM2079), *Bacillus subtilis* (NCIM2250), and Gram -ve *Pseudomonas aeruginosa* (MTCC3541), *Escherichia coli* (NCIM2065) was further studied. The anticancer activity of NiO-NPs against lung cancer cells (NRU-A549) was examined. The IC₅₀ value was found to be 326.4 µg/mL. The synthesized NiO-NPs were further studied for electrochemical and supercapacitor applications.

INTRODUCTION

Metal oxide nanoparticles have garnered significant attention due to their distinct optical, electronic, and physiochemical characteristics (Balgude & Mardikar 2021, Kumar et al. 2020, Mardikar et al. 2021, 2020, Mone et al. 2020). Recently, there has been a growing interest in producing nanoparticles of Fe, Co, and Ni owing to their exceptional properties and wide-ranging applications in fields such as sensors, memory storage devices, photocatalytic processes, drug delivery systems, catalysis, magnetic resonance imaging and even cancer cell treatment (Baek & Cho, 2017, Ewaiss et al. 2017, Farzin et al. 2020, Lokesh et al. 2016, Magdalane et al. 2016, Manikandan et al. 2015, Prabhakar, 2023, Sreekanth et al. 2020, Tahir et al. 2022, Zhang et al. 2017). Metal oxides find utility in diverse sectors, with biomedicine being a particularly promising area (Iqbal et al. 2021). Researchers are actively exploring eco-friendly and non-hazardous methods to synthesize materials for treating tumors and cancer cells, leveraging the enhanced adsorption capabilities of these nanoparticles to induce cytotoxic effects through medium starvation. Their unique properties like surface area, metal ion releasing, and adsorbing ability. It is also utilized as a competent material in the elimination of pathogens from wastewater, owing to its primary characteristics such as stability,

gradual release of metal ions from the nanoparticle, and effective biological properties (Ahmad & Rawat 2023). Nanoparticles exhibit various properties that distinguish them from bulk materials, including surface area to volume ratio, electro-optical, magneto-optical, chemical, and mechanical properties. These properties serve as a powerful tool in combating bacteria (Binns 2021, McNamara & Tofail 2017). Numerous antimicrobial nanoparticles have demonstrated their effectiveness against infectious diseases in vitro and animal cells (Kalashgarani & Babapoor 2022). The antibacterial activity is not only dependent on the metal oxide used but also on the nature of the bacterial species. Bacteria can be classified into Gram-positive (+) and Gram-negative (-) based on their cell wall structure. Gram-positive cells have thick layered walls (20-50 nm), while Gram-negative bacteria have thin layered walls. In this study, an attempt was made to synthesize NiO nanoparticles with a small particle size and large surface area, possessing good antibacterial and anti-inflammatory properties. NiO nanoparticles have garnered significant interest in recent research due to their high chemical stability, electrocatalytic properties, super conductance characteristics, and electron transfer capability. Nickel oxide is a p-type semiconductor metal oxide with a band gap ranging from 3.6 to 4.0 eV (Goel et al. 2020). It is an environmentally active material, finding applications in the adsorption of hazardous dyes and inorganic pollutants (Pandian et al. 2015). Additionally, due to their anti-inflammatory properties, they are employed in the field of biomedicine (Uddin et al. 2021). NiO nanoparticles have been found to exhibit toxic effects on bacteria and microalgae (Isaacoff & Brown 2017, Liu et al. 2022), attributed to their potential to induce oxidative stress and release nickel ions (Ni^{2+}) inside the cell (Berhe & Gebreslassie 2023, Waseem et al. 2022). NiO nanoparticles have been associated with cytotoxic effects due to their unique properties, such as surface area, metal ion release, and adsorption ability.

Many techniques, like co-precipitation, electro-deposition, sol-gel method, and solvothermal method, have been created for producing nanoparticles with increased reaction times and high energy consumption (Shang et al. 2017, Sharma et al. 2018). The conventional method of combustion is a self-sustaining exothermic reaction that produces various metal oxides in large amounts (Ahmad et al. 2022, Samrot et al. 2021). There are several environmentally eco-friendly methods for the synthesis of metal nanoparticles. The use of less hazardous chemicals or natural materials in place of toxic chemicals is one of the green approaches. The nanomaterials are synthesized by using micro-organisms plant extract. The green synthesis of nanoparticles, mainly plant extract, is used, and the extensive use of chemicals is avoided (Ahmed et al. 2016, Akarca et al.

2020, Gupta et al. 2020, Habtemariam & Oumer 2020, Kar & Ray 2014, Sheikholeslami 2022). Plant leaves and petals have a hardness that acts as a bio template, controlling the size of the nanoparticles and preventing their aggregation. The plant consists of many fatty acids, vitamins, amino acids and nutrients, glucosinolates (Dai et al. 2019), and phenolics (flavonoids, anthocyanins, proanthocyanides, and cinnamates), which are responsible for capturing the metal ions.

The bottom-up approach for the synthesis of nanoparticles mainly deals with the creation of NPs from the assembly of miniature matter, viz. Atoms and molecules into novel nuclei, which further develop into a particle having nanoscopic sizes using different chemical and biological techniques (Pandit et al. 2022). The manipulation of the biological synthesis route is considered an innovative supernumerary for the physicochemical synthesis routes. This technique utilizes natural biomass sources like micro and macro-organisms (fungi, algae, bacteria, and selected viruses), biomolecules (lipids, pigments, proteins, polysaccharides, etc.), and plants as biologic-reducing and stabilizing agents for the green production of NPs with minimum contamination (Hamida et al. 2021, Jeevanandam et al. 2022). The clear expansion of NPs synthesis options and green methods that make use of various biological sources is quite breathtaking. Various remarkable features of the green (bio-synthesis) technique are the nonexistence of noxious chemical compounds being employed as stabilizing or reducing agents resulting in biocompatibility, no noxious yield being created from this procedure using low energy consumption at a sensible cost, and being highly scalable (Hamida et al. 2021). The applicability of bio-NPs facilitated by the green route is more analogous to metal oxide nanoparticles with unconventional properties such as quantum size, miniature size, quantum tunneling, and surface effects (Ali et al. 2023, Berta et al. 2021). Additionally, features such as enhanced morphologies, high yield, and enduring stability in metal oxides are achieved by employing a greener method (Nadeem et al. 2020).

Brassica oleracea L. var. *capitata* belongs to the Brassicaceae family, genus *Brassica*, and species *oleracea*. *Brassica oleracea* L.var.*capitata* contains alkaloids, amino acids, flavonoids, glucoside, phenol, tannin, steroids, and carbohydrates. The phenolic compounds are the most important groups of plant metabolites compound (Arun et al. 2021).

Supercapacitors have been considered one of the most promising options owing to their simple design and capacity to supply the electricity demand (Uke et al. 2021a, Yu et al. 2015). Transition metal oxides such as NiO are the most promising electrode materials in energy storage devices.

They have a large surface area. A variety of substances can be found easily and are eco-friendly to the environment (Lu et al. 2017, Uke et al. 2021b). These metals are very significant because they provide a clear increase in capacitance through the tuning and control of different imperfections at interfaces and surfaces (Hashem et al. 2022, Uke et al. 2020).

Here in the present study green synthesis of NiO nanoparticles from *Brassica oleracea* L.var. *capitata* extract as a reducing agent has been reported and their antimicrobial and anticancer activity has been evaluated. Synthesized NiO nanoparticles also showed good supercapacitor application.

MATERIALS AND METHODS

Chemicals and Materials

Nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), distilled water, and *Brassica oleracea* L. var. *capitata* extract were used for synthesis. Nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was procured from Loba Chemie and was used as received.

Preparation of extract of *Brassica oleracea* L. var. *capitata*

10 g of dehydrated leaf powder of *Brassica oleracea* L.var. *capitata* was taken in soxhlet, and it was attached to a 250 mL round bottom flask. During extraction, 100 mL of distilled water was used as a solvent. The round bottom flask was heated at a constant temperature of 60°C during extraction. After completion of all cycles of the soxhlet extractor, the extract was collected in a 250 mL beaker & this aqueous extract was utilized further for green synthesis of NiO NPs.

Green synthesis of NiO-NPs

100 mL 0.1 M nickel nitrate solution was taken in a 250 mL round bottom flask and kept on the magnetic stirrer. 50 mL of aqueous leaf extract of *Brassica oleracea* L. var. *capitata* was added dropwise under constant magnetic stirring. After complete addition, the mixture was stirred for 90 min. Further, the reaction mass was isolated by centrifugation at 1500 rpm for 15 min. The supernatant liquid was discarded, and Ni-NPs were collected. The obtained precipitate was washed several times with ethanol and distilled water.

After washing, the precipitate was dried and calcinated at 700°C for 2 h. The calcined product was kept in a sealed container bottle and was utilized for further characterization. Fig. 1 shows the schematic representation of the preparation process of NiO-NPs.

Fabrication of NiO Electrode

The preparation of NiO electrodes involved the use of grade 304 stainless steel (SS) as the substrate. Prior to its application, the SS was subjected to a cleaning process with detergent and subsequently immersed in 4N HNO_3 . This was followed by a series of washings with acetone and deionized distilled water (DDW). The cleaned substrate was then dried in an oven. A doctor blade was employed to apply a uniform layer of a mixture consisting of the active material (the prepared electrode material), poly vinylidene fluoride (PVDF), and carbon black (acetylene black) in weight ratios of 75%, 15%, and 10%, respectively, in dimethyl formamide (DMF) onto the stainless-steel substrate. The electrodes were then dried overnight at a temperature of 60°C (Ezhilarasi et al. 2016, Karpagavinayagam et al. 2022). After this drying process, the weight of the electrode, measuring $1 \times 1 \text{ cm}^2$, was found to be approximately 2 mg.

Electrochemical analysis was performed using the EmStat4S. LR electrochemical workstations and the Potentiostat-Galvanostat model PG12110 (Techno Science, Instrument). For the electrochemical characterization, an electrochemical cell was constructed with the fabricated electrode serving as the working electrode, a platinum electrode as the counter electrode, and an Ag/AgCl electrode as the reference electrode. A $1 \text{ mol.L}^{-1} \text{ Na}_2\text{SO}_4$ aqueous solution was utilized as the electrolyte. The electrochemical assessments of the prepared electrodes were conducted using various techniques, including cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS). The specific capacitance (C_s , F.g^{-1}) was determined using the CV and GCD methods, as outlined in equations (1) and (2), respectively. Additionally, the energy density (ED, Wh.kg^{-1}), power density (PD, W.kg^{-1}), and Coulombic efficiency were calculated using equations (3), (4), and (5), respectively.

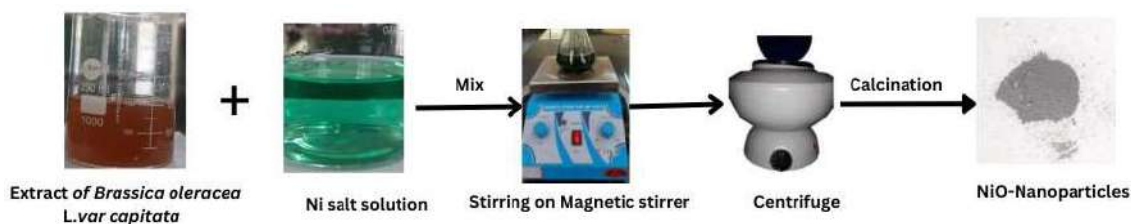


Fig. 1: Schematic representation of the preparation process of NiO-NPs.

$$Cs = \frac{1}{mv(V_{max} - V_{min})} \int_{V_a}^{V_c} I(v) dv \quad \dots(1)$$

$$Cs = \frac{Id \times td}{\Delta vm} \quad \dots(2)$$

$$E = \frac{0.5 \times Cs(V_{max}^2 - V_{min}^2)}{3.6} \quad \dots(3)$$

$$P = \frac{E \times 3600}{td} \quad \dots(4)$$

$$\eta = \frac{td}{tc} \times 100 \quad \dots(5)$$

Where 'V' refers to the scan rate, and '(V_{max} - V_{min})' denotes the operational potential window in volts. 'V_{max}' represents the cathodic current, while 'V_{min}' represents the anodic current. The variable 'm' indicates the mass deposited in grams, and 'I(v)' represents the current in milliamperes (mA). The Id represents the current density (mA.cm⁻²), and td and tc represent the discharge and charge time of the electrode in the GCD curve, respectively.

Characterization Techniques

The crystal structure of green synthesized NiO-NPs was confirmed by powder X-ray diffraction having Rigaku Mini Flex 600 X-Ray diffractometer. It operated at a voltage of 40 kV and a current of 50 mA with Cu-K radiation over a 2θ range of 20° to 90°. The UV-visible spectrum was recorded by JASCO V-750 of Serial No. D084261799 has accessory USE-753 ranging in 200-900 nm. The functional group of synthesized NiO-NPs was identified by SHIMADZU FT-IR, having a frequency range of 400-4000 cm⁻¹. The surface morphology of green synthesized NiO-NPs was identified by scanning electron microscopy (SEM, model JSM-IT500LA, Jeol Japan), elemental detection spectroscopy (EDS), high-resolution scanning electron microscopy (HR-TEM, JEOL ASIA PTE-LTD, model JEM 2100 PLUS).

Antimicrobial Activity

The green synthesized NiO-NPs were examined for their antibacterial activity against Gram +ve *Staphylococcus aureus* (NCIM2079), *Bacillus subtilis* (NCIM2250) and Gram -ve *Pseudomonas aeruginosa* (MTCC3541), *Escherichia coli* (NCIM2065) by using agar diffusion method (Disc diffusion method). Overnight grown culture was prepared in the nutrient agar, the composition of nutrient agar is peptone, yeast extract, and NaCl. A loop full of culture was spread on the nutrient agar plate. The zone of inhibition in mm was then calculated by Vernier Calliper.

Anticancer Activity

Cytotoxicity of the NiO-NPs on the A-549 cell line was determined by NRU Assay. The cells (5000-8000 cells/well)

were cultured in 96 well plates for 24 h in DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO₂. The next day, the medium was removed, and fresh culture medium was added to each well of the plate. 5 µl of treatment dilutions of different concentrations (0-1000 µg.mL⁻¹ final concentration) were added to the defined wells, and treated plates were incubated for 24 h. 10 µL of NRU (40 µg.mL⁻¹ in PBS) per 100 µL medium was added and incubated for 1 h. After that medium was removed then, NRU was dissolved in 100 µL of NRU Destain solution. Finally, plates were read at 490/660 nm.

RESULTS AND DISCUSSION

XRD Study of NiO-NPs

The powdered XRD pattern of green synthesized NiO-NPs from *Brassica oleracea* L. var. *capitata* are as shown in Fig. 2. All the peaks appearing at 37.24, 43.29, 62.85, 75.38, 79.37 can be indexed to (111), (200), (220), (311) and (222) planes respectively which are very well matching with JCPDS Card No. 04-0835. FCC (Face-centered cubic) crystalline Bunsenite structure of NiO-NPs was observed. The crystallite size (D) was found to be 16.68 nm according to the Scherer equation.

$$D = \frac{0.89\lambda}{\beta \cos \theta}$$

Where λ is the wavelength of the x-ray, θ is the Bragg diffraction angle, and β is the FWHM (Kaviyarasu et al. 2016). Lattice strain and interplanar distance (d) were found to be 0.00586 and 1.094, respectively. Besides these, no impurities were observed, which suggested the high purity of monophasic NiO nanoparticles. The reported literature studies indicate that the crystal size of green synthesized NiO nanoparticles was between 8-43.9 nm (Kuchekar et al. 2018, Nouneh et al. 2011).

UV-Visible Analysis of NiO-NPs

NiO-NPs synthesized using *Brassica oleracea* L. var. *capitata* were analyzed using the UV spectroscopic technique, and the results obtained are shown in Fig. 3. The synthesis of NiO-NPs using the green route was confirmed by observing the color change from green to dark brown, which was further confirmed by UV-visible analysis. The results reveal the emergence of three important peaks obtained at wavelength 545 nm, 589 nm, and 636 nm. Kuchekar et al. (2018) reported the absorption band at 656 nm owing to surface plasmon resonance. It was noticeable that the broadening of the peak was evident at the 374 nm to 422 nm range, corresponding to the SPR of nickel (Khalil et al. 2017).

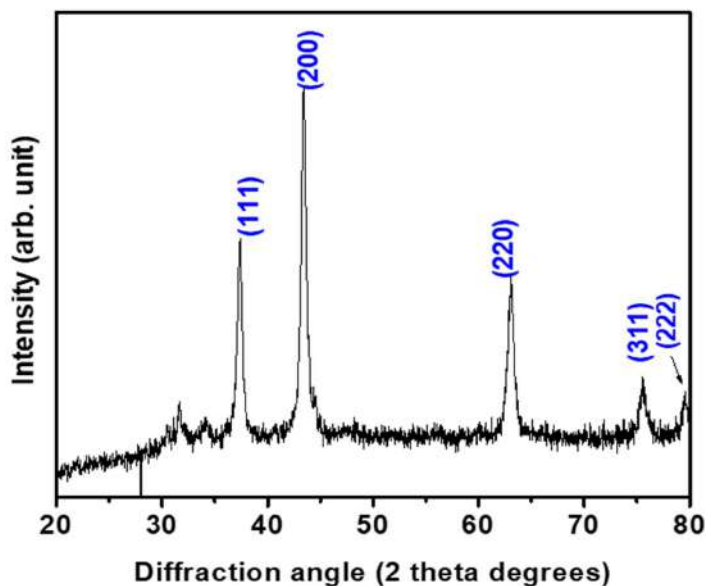


Fig. 2: XRD pattern of green synthesized NiO-NPs.

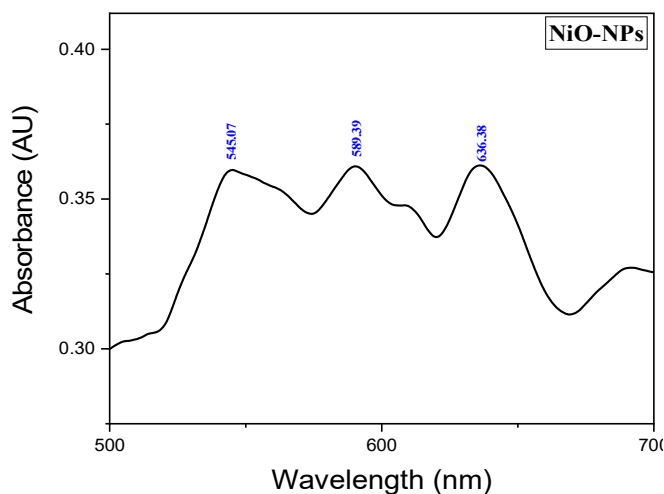


Fig. 3: UV-visible Absorption Spectra of NiO-NPs.

FT-IR Study of NiO-NPs

Fourier transform infrared (FT-IR) analysis was carried out to identify the different functional groups present in NiO-NPs synthesized by *Brassica oleracea* L. var. *capitata* extract. The FTIR spectrum of synthesized NiO-NPs is shown in Fig. 4. The bands at 3026 cm^{-1} correspond to the -OH stretching. The band at 2950 cm^{-1} is due to the stretching of C-H or N-H. The band at 1321 cm^{-1} corresponds to C-O stretching. The band at 1207 cm^{-1} and 1100 cm^{-1} corresponds to C-O and C=C stretching of aromatic ring and polyphenol. The band at 880 cm^{-1} can be ascribed to M-O bond stretching. The presence of the -OH group indicates the presence of

water molecules on NiO-NPs. (Huang et al. 2021, Iqbal et al. 2020, Uddin et al. 2021).

Elemental Analysis of Green Synthesized NiO-NPs

The quantitative and qualitative analysis of elemental composition was determined by the energy dispersive spectroscopy (EDS) technique. Fig. 5 shows the EDS elemental mapping of NiO-NPs. The NiO-NPs exhibit stronger absorption peaks because of the surface plasmon resonance. In the EDS pattern, peaks of nickel (Ni) and oxygen (O) elements were predominantly observed, suggesting the successful formation of NiO-NPs. The

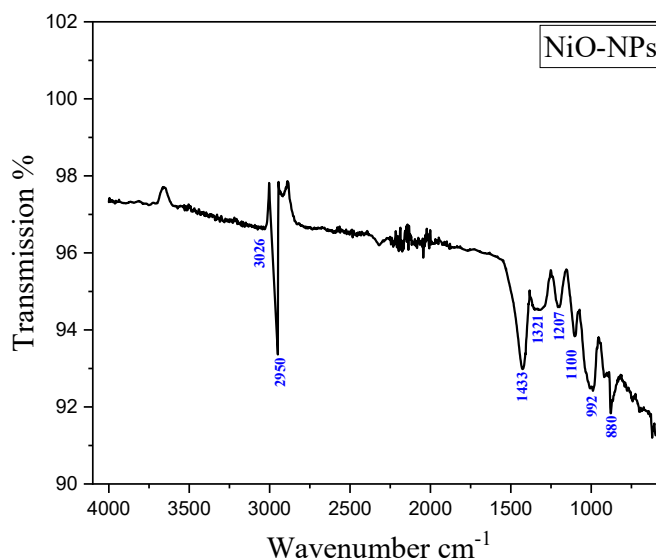


Fig. 4: FT-IR Spectrum of NiO-NPs from *Brassica oleracea* L. var. *capitata*.

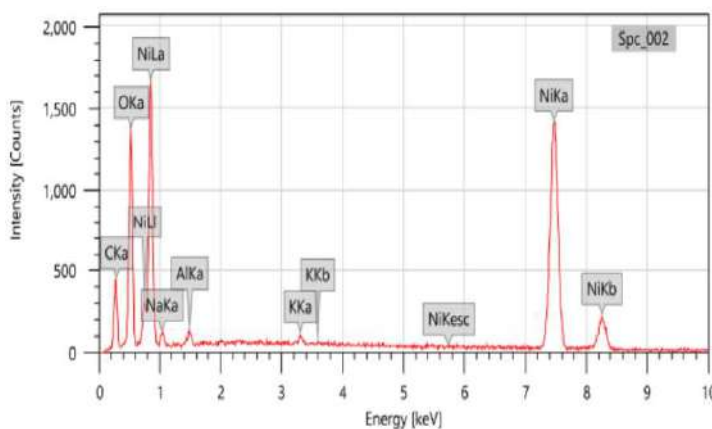


Fig. 5: EDS of NiO-NPs.

presence of carbon and oxygen can be ascribed to the organic compounds present in the plant extract (Alamgeer et al. 2018). The elemental composition of green synthesized NiO-NPs by using *Brassica oleracea* L. var. *capitata* is shown in Table 1, which is in close agreement

Table 1: Elemental Composition of Synthesised NiO-NPs.

Element	Line	% Mass	Atom %
C	K	15.87±0.13	34.20±0.29
O	K	22.88±0.20	37.02±0.32
Na	K	1.90±0.09	2.14±0.10
Al	K	0.74±0.04	0.71±0.04
K	K	0.42±0.03	0.28±0.02
Ni	K	58.19±0.41	25.66±0.18
Total		100.00	100.00

with the elemental composition of NiO reported by (Pandian et al. 2015)

Particle Size Study of NiO-NPs

The dynamic light scattering was used to determine the particle size of green synthesized NiO-NPs using *Brassica oleracea* L. var. *capitata*. The average particle size was found to be 429.2 nm, as shown in Fig.6. (Sudhasree et al. 2014). Uddin et al. (2022) reported that the average particle size of Ni-NPs by green and chemical methods was found to be 2695 nm with a PDI of 1.31 and 2453 nm with a PDI of 0.401 respectively.

SEM Study of NiO-NPs

Low- and high-resolution SEM images of as-synthesized NiO-NPs are depicted in Fig. 7 (a-d). Low-resolution images

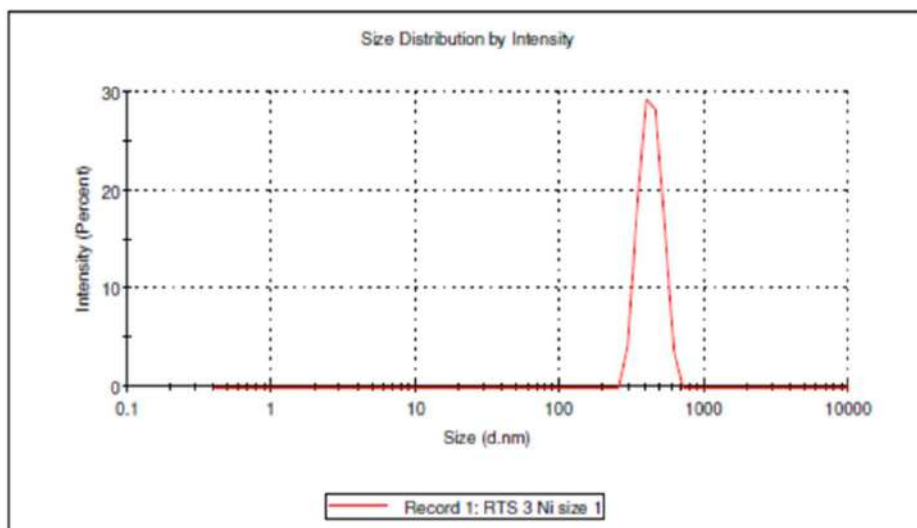


Fig. 6: Particle size distribution of green synthesized NiO-NPs.

reveal that the NiO nanoparticles exhibit agglomerated structures. More insights into these agglomerated structures can be found by close observation of high-resolution SEM images, as shown in Fig. 7 (c-d). It can be seen that the agglomerated structures are formed due to the clustering of small nanoparticles. In the literature, it was reported that agglomerated structure may be due to the magnetic interaction and polymeric adherence of NPs (Uddin et al. 2022).

TEM Study of NiO-NPs

The morphological investigations were further confirmed by studying the synthesized NiO-NPs by TEM. Fig. 8-(a-b) shows low- and high-resolution TEM images, in which it is observed that the synthesized NiO nanoparticles exhibit spherical morphology with variable sizes ranging from 5-50 nm. Here, in the present case, the particle sizes obtained using PSD and TEM vary significantly from 5-50 nm for

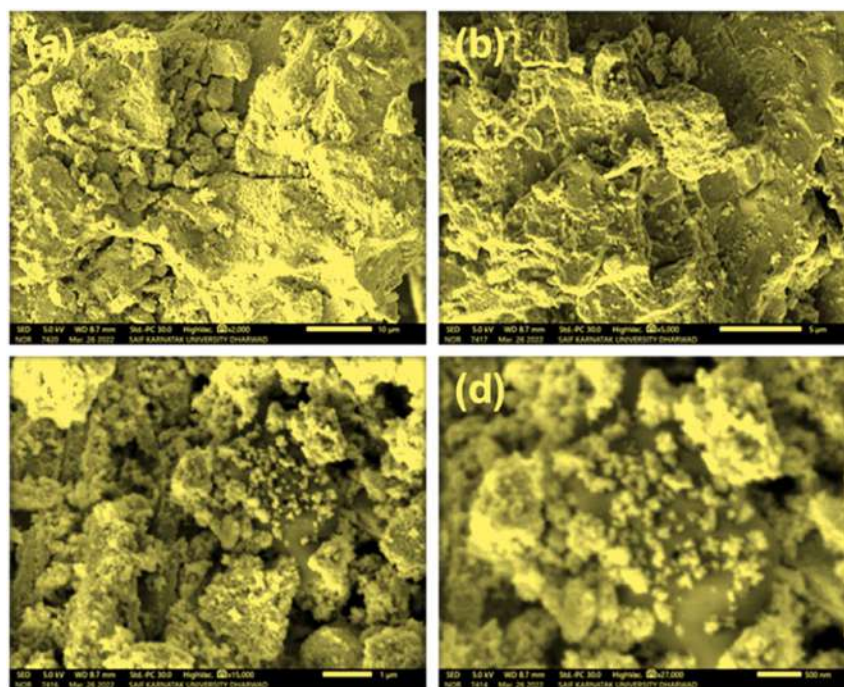


Fig. 7: (a-d) Low- and high-resolution SEM images of synthesized NiO-NPs.

TEM to 490 nm for PSD. These results can be attributed to the green synthetic architecture employed for the synthesis of NiO nanoparticles. It has been reported that the extract of medicinal plants is a rich source of phytochemical compounds (Iqbal et al. 2020). These phytochemicals act as fuel to synthesize smaller dimensions of nanoparticles (Al-Zaqri et al. 2022, Moradnia et al. 2024).

Although several techniques have been used to measure the size of the particles, each technique has its advantages and limitations (Domingos et al. 2009). TEM expresses the primary particle size of the particles under study. However, DLS measurements are often done to determine the true state of particles in media, especially if they will be applied/studied in media. Herein present, since the NiO particles have been

synthesized using the green method, it might be the tendency of these particles to agglomerate in an aqueous solution and preserve their monodispersed owing to the surface charge and functional groups present on them. Another reason for the significant difference between the particle sizes observed by DLS and TEM can be attributed to DLS being an intensity-based technique, and this puts higher emphasis on the larger particle sizes, whereas TEM is a number-based technique and will thus show stronger emphasis on the smallest components in the size distribution.

Antimicrobial Assay

Antibacterial activity: Green synthesized NiO-NPs from the extract of *Brassica oleracea* L. var. *capitata* were evaluated

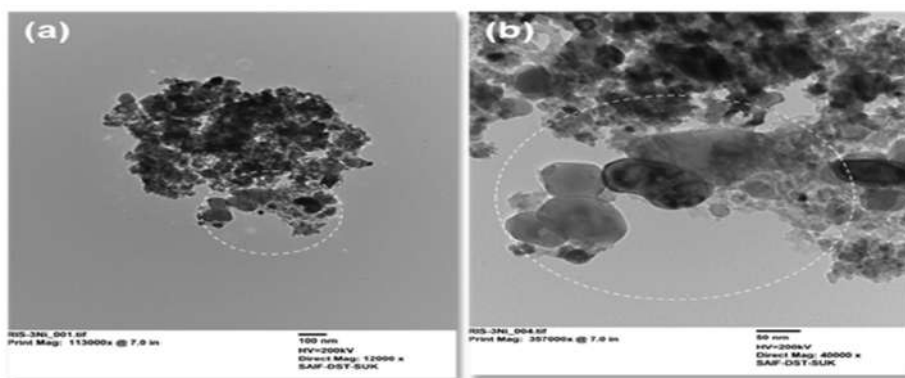


Fig. 8: (a-b) Low and high-resolution TEM images of synthesized Ni-NPs.

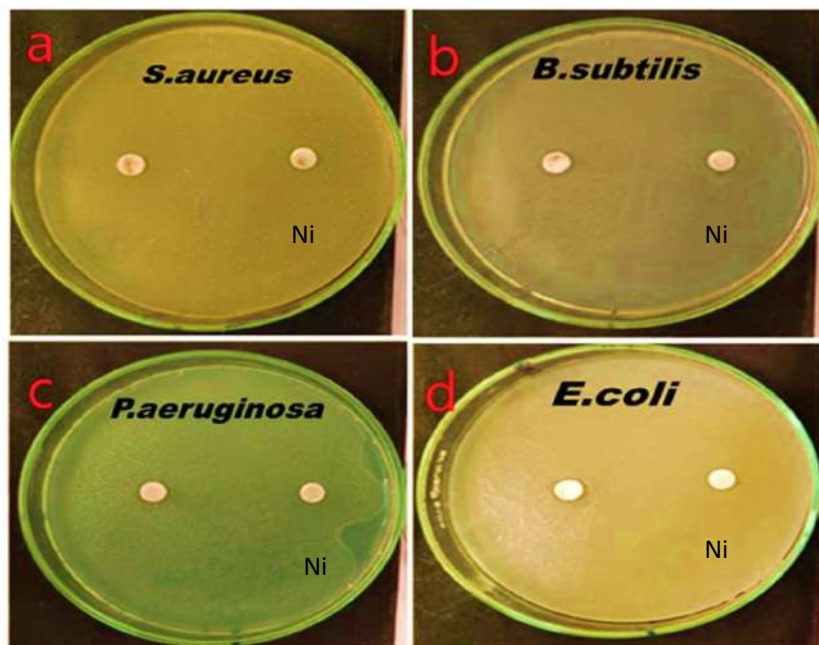


Fig. 9: (a-d) Antibacterial activity of green synthesized NiO-NPs against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*.

for their potential killing efficiency of both Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative (*P. aeruginosa*, *E. coli*) bacteria by performing disc diffusion method (Fig. 9). The chloramphenicol is used standard antibiotic as a positive control. The zone of inhibition resulting from green synthetic nanoparticles was used to prove the bactericidal efficiency. The synthesized NiO-NPs from an extract of *Brassica oleracea* L. var. *capitata* showed good bacterial activity of Gram-positive *B. subtilis*, whereas Gram-negative bacteria, i.e., *P. aeruginosa*, *E. coli*, showed good bacterial activity as compared to Gram-positive bacteria. NiO-NPs show good antibacterial activity against Gram-ve bacteria *P. aeruginosa*. The precise mechanism underlying the bactericidal activity of nanoparticles (NPs) has not yet been elucidated. However, it is hypothesized that these nanoparticles interact with bacterial cells, leading to alterations in the morphology of the cell membrane and an increase in permeability. This disruption interferes with the normal transport processes across the plasma membrane, ultimately resulting in cell death. Evidence suggests that biosynthesized nickel oxide nanoparticles (NiO-NPs), characterized by their small size and high stability, possess a large surface area-to-volume ratio. This property enhances their interaction with bacterial cells, thereby increasing their bactericidal efficacy through the release of Ni^{2+} ions. These ions can easily penetrate the cell wall, subsequently disrupting the electron transport system and affecting cellular components such as proteins and DNA while also inducing oxidative stress, which ultimately leads to cell damage. Consequently, the bactericidal activity and stability of NiO nanoparticles are highly effective, indicating their potential application in antimicrobial coatings for various environmental and biomedical uses (Nagajyothi et al.

Table 2: Antibacterial activity zone diameter of NiO-NPs.

Sr. No.	Name of NPs	Zone of inhibition in millimeters [mm]			
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
1.	NiO-NPs	-	9.44	9.53	9.31
2.	Chloramphenicol Standard sample	19.45	24.55	21.30	27.1

2014). The overall mechanism is schematically represented in Fig. 10.

The anti-bacterial zone of inhibition in millimeters of NiO-NPs is shown in Table 2. The data shows that NiO-NPs contribute to the antimicrobial activity of the studied microorganisms. The efficacy of the NiO-NPS is compared with previous studies in Table 3. It can be seen from the data that the green synthesized pristine NiO-NPs exhibit comparable biological activity.

The anti-bacterial zone of inhibition in millimeters of NiO-NPs is shown in Table 2.

Anticancer Activity

The cytotoxic potential of NiO nanoparticles was evaluated using the Neutral Red Uptake (NRU) assay on A549 cancer cells. Exposure to NiO nanoparticles at concentrations ranging from 1 to 1000 $\mu\text{g.mL}^{-1}$ for 24 h resulted in a concentration-dependent decrease in cell viability. The results of NiO showed that the cell viability is slightly decreased at a concentration of 10 mg/mL. A significant reduction of approximately 60% in cell viability was observed at the highest concentration (1000 $\mu\text{g.mL}^{-1}$). These findings indicate that NiO nanoparticles exhibit cytotoxic

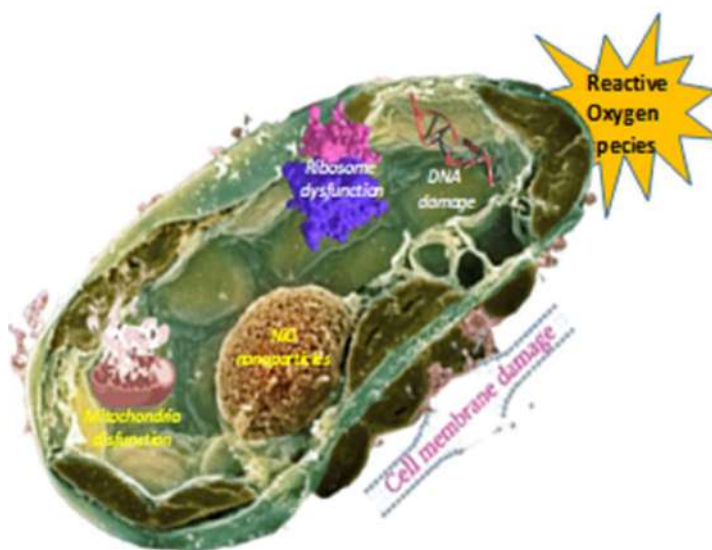


Fig. 10: The schematic diagram representing the proposed antibacterial mechanism of NiO-NPs.

Table 3: Antimicrobial activity of NiO NPs against gram-positive (*S. aureus*) and gram-negative (*S. typhimurium*) compared with previous studies.

Sr. No.	Nanomaterial employed	Synthesis method used	Bacteria used for study	Observed Amoxicillin Zone of Inhibition [mm]	References
1.	Chitosan-based nanocomposite films incorporated with NiO nanoparticles	Solution combustion synthesis method	gram-positive (<i>S. aureus</i>) gram-negative (<i>S. typhimurium</i>)	17.61 ± 0.39 11.47 ± 0.04	(Marand et al. 2021)
2.	Cobalt-doped NiO nanoparticles	<i>Murraya koenigii</i> mediated green synthesis	gram-negative (<i>Klebsiella pneumoniae</i> , <i>Shigella dysenteriae</i>) gram positive (<i>Staphylococcus aureus</i> , and <i>Bacillus</i>)	15.5 & 17 7 & 14	(Elamin et al. 2022)
3.	Nanostructured Ag/NiO composite	Using <i>W. somnifera</i> leaf extract (WSLE)	human pathogen <i>Klebsiella pneumoniae</i>	15.4	(Chinnaiah et al. 2024)
4.	Nanostructured CdO-NiO composite	microwave-assisted precipitation method	Gram-negative bacterium <i>Bacillus subtilis</i>	27	(Karthik et al. 2018)
5.	NiO nanoparticles	Using <i>Brassica oleracea</i> L. var. <i>capitata</i> extract	Gram positive <i>B. subtilis</i> Gram negative <i>P. aeruginosa</i> , <i>E. coli</i>	9.44 9.53 & 9.31	Present study

Table 4: Cytotoxicity effect of NiO-NPs against cancer cells.

Sr. No.	Name of Nanoparticles	IC50 value [$\mu\text{g.mL}^{-1}$]
1.	NiO-NPs	326.4

effects by compromising lysosomal integrity, as assessed by the NRU assay. The observed cytotoxicity suggests that biosynthesized NiO nanoparticles endorse further investigation as potential anticancer agents.

Fig. 11 showed that more than 50% of cell death at $1000\mu\text{g.mL}^{-1}$ of nanoparticles. The cytotoxic effects induced by nanoparticles at lower concentrations are due to the plant components attached to the nanoparticles. The reduction

in cell viability associated with elevated concentrations of NiO nanoparticles indicates a notable cytotoxic effect, resulting in the accumulation of internal cellular stress and ultimately culminating in apoptosis (Ahmad et al. 2015, Vani et al. 2018). The results obtained from this research work are also very well supported with various pieces of evidence for the cytotoxic effect of green synthesized metal nanoparticles by using *Annona squamosa* leaf extract against the breast cancer mcf-7 cell line (Vivek et al. 2012). *Piper longum* leaf extracts against hep-2 cancer cell line (Jacob et al. 2012) and *morinda citrifolia* against hela cell lines in-vitro (Meli et al. 2019). Table 4 shows the

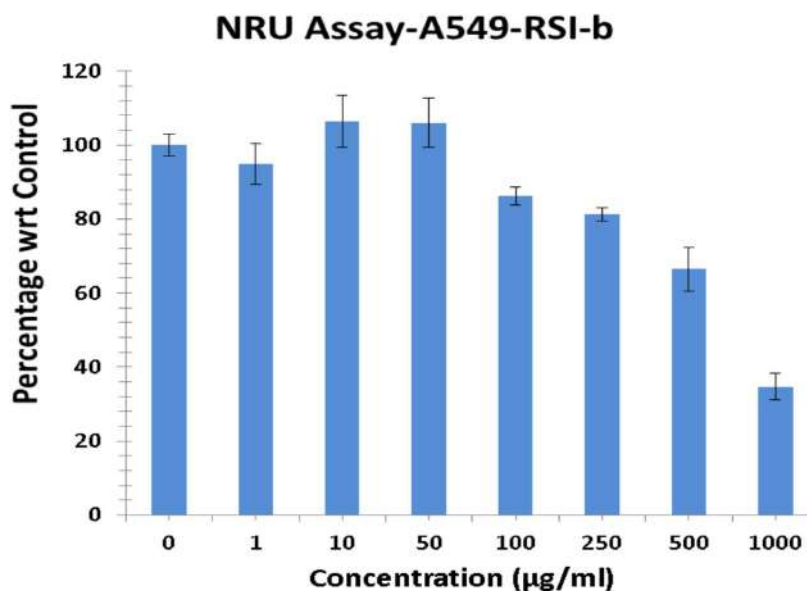


Fig. 11: Anticancer Potential of NiO-NPs.

cytotoxicity effect of NiO-NPs on cancer cells by NRU assay A549.

Supercapacitor Study of Synthesized NiO-NPs

Utilizing 1 M Na_2SO_4 as the electrolyte, cyclic voltammetry tests have been carried out. The cyclic voltammetry tests have been carried out on NiO electrodes in the potential range of 0 to 0.5 V. At the electrode/electrolyte interface (electric double layer) and electrode surface (via redox reactions), electrodes in an alkaline electrolyte store charge. The CV curve of NiO electrodes is shown in Fig. 12 (a) for various scan rates. The Faradic nature of the NiO is confirmed by the clearly marked redox peaks on CV curves as opposed to the perfect rectangular shape of EDLC. Their corresponding anodic and cathodic scans do not have symmetric oxidation and reduction peaks. This is because the redox reaction is kinetically irreversible. The following redox process produces the charge storage function of NiO electrodes: electrodes in the potential range of 0 to 0.5 V. Galvanostatic charge-discharge curves at different current densities are shown in Fig.12 (b). The specific capacitance obtained from CV at 5, 10, 25, 50

and 100 mV.s^{-1} are 226 F.g^{-1} , 201.3 F.g^{-1} , 159.0 F.g^{-1} , 115.66 F.g^{-1} and 78.82 F.g^{-1} respectively.

Moreover, the variation of specific capacitance with scan rate is demonstrated in Fig.12 (a-d). The specific capacitance, energy density, and power density of NiO Supercapacitor electrodes calculated from the Galvanostatic discharge curve are shown in Table 5. Fig.13 shows the Nyquist plot for the NiO electrode. From this graph, the electrolyte resistance and charge transfer resistance for NiO electrodes in 1 Molar Na_2SO_4 electrolyte obtained 0.8 Ohm.cm^{-2} and 3.2 Ohm.cm^{-2} , respectively. The low electrolyte resistance and charge transfer resistance of NiO electrodes are highly desirable for the higher electrochemical performance of the electrode material. The obtained low values of electrolyte resistance, charge transfer resistance, high specific capacitance, and energy density for NiO electrodes demonstrated the patentability of NiO in terms of its applicability as an electrode for supercapacitor applications. Table 5 gives the specific capacitance, energy density, and power density of NiO. The supercapacitor electrode was calculated from the Galvanostatic discharge curve.

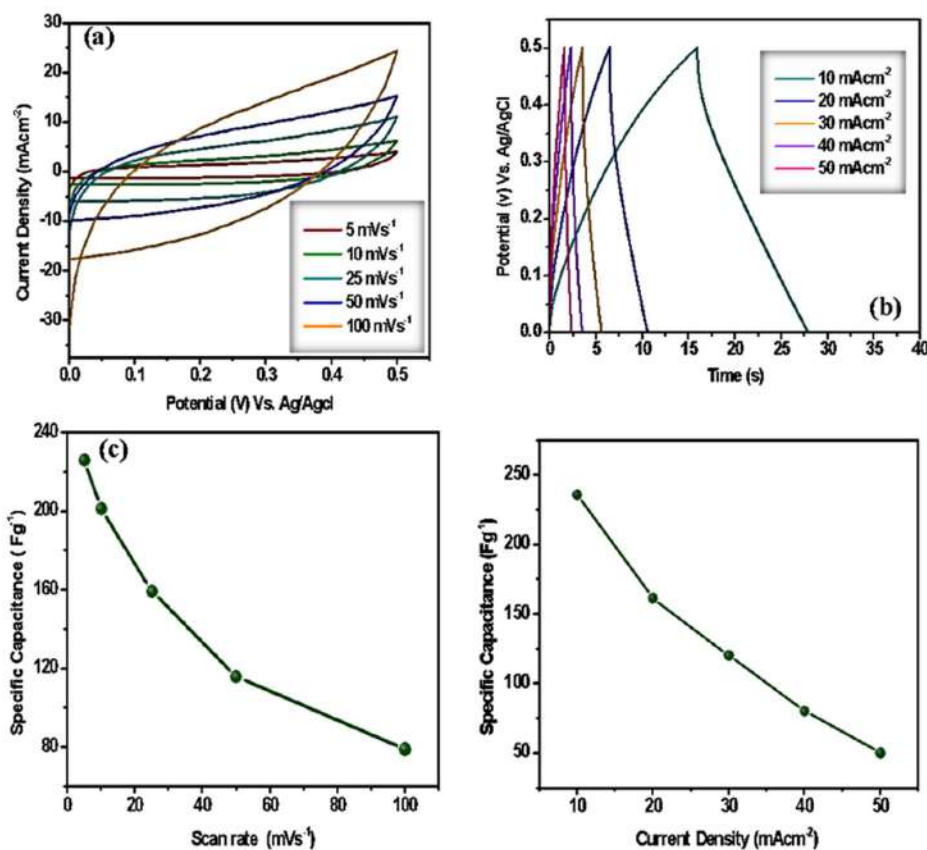


Fig. 12: (a) Cyclic voltammetry curves at different scan rates, (b) Galvanostatic charge-discharge curves at different current densities. (c) Specific capacitance Vs. Scan rate (d) Specific capacitance Vs. Current density.

Table 5: Specific capacitance, energy density, and power density of NiO-NPs.

Current density [mA.cm ⁻²]	Specific capacitance [F.g ⁻¹]	Energy density [Wh K.g ⁻¹]	Power Density [W.kg ⁻¹]
10	235.4	5.23	1600
20	161.2	3.58	3200
30	120.2	2.66	4800
40	80.1	1.78	6400
50	50.6	1.1	8000

A comparative summary of calculated specific capacitance with previous literature is provided in Table 6. At the different current densities, the potential window for as-prepared NiO electrodes linearly increases during the charging process and linearly decreases during the discharge process, revealing good electrochemical properties. The as-prepared NiO NPs have spherical morphology, and these special features provide more electroactive surface area for ion transportation.

CONCLUSIONS

In this research work, we have successfully synthesized

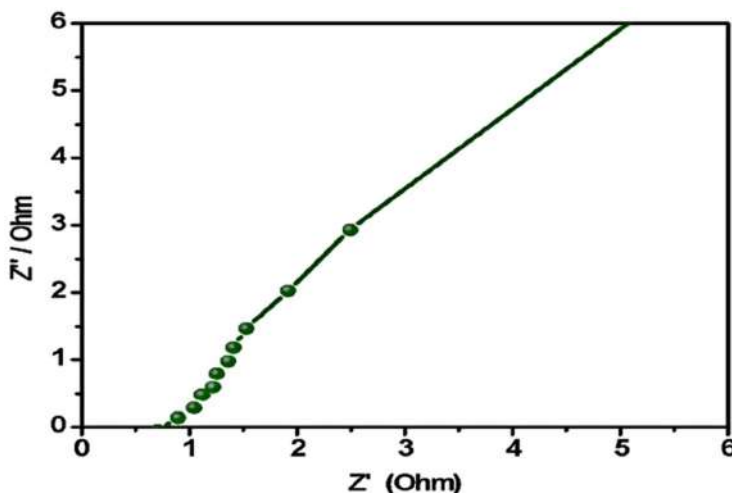


Fig. 13: Nyquist Plot of NiO-NPs Electrode.

Table 6: Comparative study of the capacitive performance of as-synthesized NiO NPs electrode with literature reports.

Sr. No.	Electrode Material	Method of synthesis	Electrolyte	Sp. capacitance (F.g ⁻¹), scan rate (mV. s ⁻¹)/ current density (A.g ⁻¹)	Ed [Wh.kg ⁻¹]	Coulombic efficiency (%), cycles,	Reference
1.	Cu ₂ O@MnO ₂ @NiO	Green synthesis using <i>Sarcophrynium Brachystachys</i> leaf extract	1.0 M Na ₂ SO ₄	1.00 mV/s scan rate, 1092 F/g	12.74 W/kg at 0.5 A/g	-	(Obodo et al. 2024)
2.	NiO@MnO ₂ nanoparticles	Hydrothermal	0.5 M Na ₂ SO ₄	343 (F.g ⁻¹)	energy density of 7.62 Wh/kg with a power density of 249 W/kg	-	(Islam et al. 2024)
3.	NiO bio-composite materials	Co-precipitation method	6.0 M KOH	348 F.g ⁻¹	-	-	(Avinash et al. 2021)
4.	NiO/ZnO Nano-composite	Hydrothermal and subsequent calcination process	3 mol L ⁻¹ KOH	723 F.g ⁻¹	-	75, 3000	(Zhu & Shao, 2018)
5.	NiO NPs	Green synthesis using <i>Brassica oleracea</i> L. var. <i>capitata</i>	1 M Na ₂ SO ₄	235 F.g ⁻¹	5.23WhKg ⁻¹ with a power density of 1600 W/kg	-	Present study

NiO-NPs from an extract of *Brassica oleracea* L.var.capitata. The formation of NiO-NPs was confirmed by a change in the color of the solution by UV-visible spectroscopy. The FT-IR spectrum shows different functional groups in synthesized NiO-NPs. The morphological study utilizing SEM and TEM revealed the formation of spherical morphology. XRD results revealed the successful formation of NiO nanoparticles utilizing the green method. Particle sizes were determined using PSD, which was found to be 490 nm. The as-synthesized NiO-NPs exhibited competing antibacterial and anticancer activities. Further, the as-synthesized NiO-NPs were also employed for supercapacitor applications. The results demonstrated that NiO electrodes exhibit superior electrochemical characteristics, including high specific capacitance, favorable energy, power densities, and low resistive losses. These findings highlight the potential of NiO as an efficient and robust electrode material for supercapacitor applications. The demonstrated high energy, power density performance, and low internal resistances position NiO as a promising candidate for future energy storage technologies. Further research and development could optimize these properties, making NiO-based supercapacitors viable for a wide range of practical applications in energy storage and management.

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Single-Step Green Method of Synthesis of Activated Carbon from Lignocellulosic Biomass Waste of *Jacaranda mimosifolia* for Sustainable Water Purification

Vishal Haribhai Patel^{1,2}, Abdul Gani^{1†} and Anamika Paul³

¹School of Engineering, Galgotias University, Plot No.2, Sector 17-A, Yamuna Expressway, Greater Noida-201310, Uttar Pradesh, India

²Amity University, Room No. G05, J3 Block, Amity University, Noida-201313, Uttar Pradesh, India

³WSP Global Inc., 1st Floor, FC24, Sector 16A, Noida-201306, Uttar Pradesh, India

†Corresponding author: Abdul Gani; abdul.gani@galgotiasuniversity.edu.in

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ABSTRACT

Abundant lignocellulosic biomass components have been a source of inspiration for designing complex materials with high surface area and potent applications in a wide variety of commercial products, including water purification, biosensors, catalysis, and others. Billion tons of lignocellulosic biomass waste are produced in a year. This lignocellulosic biomass waste could be a good source of precursor for activated carbon and other carbon-based nanomaterials. Activated carbon was prepared from Seed pods of lignocellulosic biomass of *Jacaranda mimosifolia*, which was treated as waste using a single-step green method of synthesis. Synthesized activated carbon was characterized using high-resolution scanning electron microscopy (HRSEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), N₂ adsorption/desorption, and Zeta potential. It was evident that the synthesis method was free from chemical use and thus eco-friendly. We have reported maximum removal of heavy metal, lead ion (Pb²⁺), and dye Eriochrome Black T (EBT) using prepared activated carbon was 58.77 and 286.56 mg·g⁻¹, respectively. The adsorption was rapid, with 97% of Pb²⁺ and 90% of EBT adsorption accomplished within 60 min. The synthesized material could be used in the design of a filter for sustainable water purification.

INTRODUCTION

Many organic and inorganic water-soluble pollutants are discharged into water bodies, giving rise to serious environmental problems. The wastewater produced from various industries such as manufacturing, printing, textile (Yao et al. 2020), chemicals, electronics, and pharmaceutical industries (Özdemir et al. 2019) carries a noticeable quantity of pollutants, including dyes and heavy metals. This has remained a major environmental challenge. They are responsible for the seepage of different pollutants, such as dyes (Tonato et al. 2019), heavy metals, and phenols (Mohammed et al. 2018), into the aquatic environment without proper removal. Both heavy metals and dyes are envisaged to be some of the most dangerous pollutants in water systems as they exhibit mutagenic, immunogenic, carcinogenic, and teratogenic characteristics (Azari et al. 2019) and also cause serious environmental and health problems for aquatic flora, fauna, and finally human health (Yin et al. 2019).

Dyes are used in varied sectors, including textile, paper, leather, dyestuff, printing, plastic, cosmetics, and coatings, because of their low cost, brightness, and high resistance against environmental conditions (Reck et al. 2018). Moreover, dyes were very stable to light, temperature, microbial attack, and even at low concentrations optically active and detectable (Bello et al. 2018),

causing a very acute impact on water bodies (dos Santos et al. 2018).

Industries generating a massive quantity of heavy metals-laden wastewater contain arsenic, cadmium, lead, cobalt, copper, chromium, mercury, zinc, iron, manganese, nickel, and others. These effluents are released into water bodies without proper treatment (Liu et al. 2019, Dobrowolski et al. 2019), and poisonous at low concentrations (Ali et al. 2019), could be bio-accumulated/magnified along with food chains, and pose hazards to human health (Que et al. 2019) and found within the different environmental compartments (Alonso-Magdalena et al. 2019). Owing to the mentioned impacts on different biota, a strong demand for water purification from heavy metals and dyes becomes crucial.

The key techniques that are used to remove pollutants from the water phase include physical and chemical processes including coagulation/flocculation (Hou et al. 2019), precipitation (Chen et al. 2018), ultrafiltration (Kavitha et al. 2019), photo-oxidation (Du & Chen 2018), electrodialysis (Nemati et al. 2017), electrocoagulation (Doggaz et al. 2019), membrane separation (Hosseini et al. 2018), ion exchange (Feng et al. 2019), forward osmosis (Qiu & He 2019), electrochemical oxidation of Wastewater (Rai & Sinha 2022), irradiation (Ghobashy & Elhady 2017), and adsorption (Wei et al. 2021). The biological processes include phytoextraction (Napoli et al. 2019) and biological degradation (Jacob et al. 2018).

In the past few years, bio templates from natural sources like extracts of various plant components have been a source of inspiration for designing complex materials with high surface area, which have potent applications in a wide variety of commercial products, including filters for water purification, biosensors, catalysis, and others.

Biosorption, using bio-wastes for the elimination of different water pollutants, is an eco-friendly, economical, and efficient technique (Gupta et al. 2018) that limits the concentration of different water pollutants to the acceptable limits recommended by different federal regulations (Krstić et al. 2018). Numerous benefits are attained due to the recycling of these biomasses, including waste minimization, and could rectify different ecological as well as environmental problems (Gupta et al. 2019). Adsorption using a low-cost and sustainable biosorbent derived from biomass provides an alternative to a circular economy (Madelá & Skuza 2021).

Jacaranda mimosifolia used in the study belongs to the botanical family Bignoniaceae reported to be native of South America. The jacaranda has been cultivated in almost every part of the world and used in numerous metropolises in tropical and sub-tropical countries as an ornamental tree (Mostafa et al. 2014) (Ragsac et al. 2019). *Jacaranda*

mimosifolia can be categorized as a medium-sized tree which is usually less than 10 m tall and can reach 12-15 m under ideal conditions (*Agroforestry database*). Seed pods, drying dark brown, have a compressed-elliptical shape and are 3.5–8.5 cm long. Seeds are surrounded by a thin membrane, usually 0.8–1.4 cm long. Its seed pods were not edible and attractive to birds as they lacked a pleasing color or scent. Therefore, upon ripening, they dry and fall, generating a large amount of organic biomass over the soil. Very limited studies were found in the literature related to the use of *Jacaranda mimosifolia* seed pods (JMSP) for the removal of pollutants from water or wastewater.

In the present study, activated carbons (biosorbent) for water treatment have been synthesized using biomass of JMSP to investigate the adsorption performance of Pb^{+2} and Eriochrome Black T (EBT) in aqueous solution. The effect of various operational parameters, kinetics, thermodynamics, adsorption isotherms and adsorption mechanism were investigated by combining different methods, including high-resolution scanning electron microscopy (HRSEM), X-ray diffraction (XRD), Fourier transforms infrared spectroscopy (FTIR), N_2 adsorption/desorption and Zeta potential.

MATERIALS AND METHODS

Chemicals and Reagents Used

The JMSP was collected from local city gardens of greater Noida, India. Reagents used in experiments were analytical grade, and no further processing was carried out before use. Unless specified, the deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}$) was used during all experiments. The standard stock solutions of $1000 \text{ mg}\cdot\text{L}^{-1}$ were prepared for metal ion Pb^{+2} and EBT. Table 1 describes the specification and chemical structure of EBT used for the study. The pH of the solution was adjusted using 0.01-1 M HCl and NaOH.

Preparation of Activated Carbon

The seeds were removed from the collected JMSP before processing them. First, collected JMSP were washed with tap water to remove the residual dirt, sand, and other soluble impurities, then washed with deionized water 4 times. Washed JMSP were dried in a laboratory oven at 70°C for 24 h until constant weight. The dried JMSP was ground using an electronic blender and sieved into the particles $< 177 \mu\text{m}$ and stored in plastic bottles. The sieved JMSP powder was placed into the specially designed reactor under the inert atmosphere with an N_2 flow rate of $0.001 \text{ m}^3\cdot\text{min}^{-1}$ with a heating rate maintained at $300^\circ\text{C}\cdot\text{min}^{-1}$ to the final temperature of 1950°C , which was maintained for 05 min (carbonization) followed by activation at 1950°C

Table 1: Specification and Chemical Structure of Eriochrome Black T (EBT).

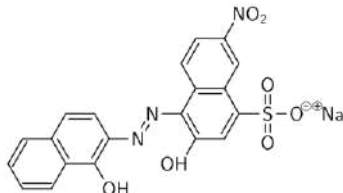
Dye Name	IUPAC Name	Chemical Formula & Molecular Weight	Chemical Structure	$\lambda_{\max}$ (nm)
Eriochrome Black T (EBT)	sodium;4-[(1-hydroxy naphthalene-2-yl)hydrazinylidene]-7-nitro-3-oxonaphthalene-1-sulfonate CAS Number 1787-61-7	$C_{20}H_{12}N_3O_7SNa$ 461.381 g·mol ⁻¹		526

Table 2: Proximate analysis of synthesized JMSP activated carbon.

Particular	Moisture % on a dry basis	Volatile matter % on a dry basis	Ash % on a dry basis	Fixed carbon % on a dry basis
JMSP AC	6.67	22.26	3.03	74.71

under CO₂ with flow rate of 0.001 m³·min⁻¹ for 05 min. The temperature of 1950°C was maintained for a total time of 10 min, 05 min for carbonization, and 05 min for activation. The prepared activated carbon was left to cool to room temperature and washed with deionized water; the activated carbon was separated using a 0.45 µm nylon membrane filter. The activated carbon obtained was dried at 110°C for 24 h, cooled to room temperature in a desiccator, and kept in airtight bottles for further analysis. The prepared activated carbon was labeled as JMSP AC. The single-step synthesis method for the production of activated carbon (biosorbent) was efficient and free from the use of chemical, thus eco-friendly. Additionally, the synthesis process operated with flexibility and scalability. The quantitative analysis (Table 2) confirms the higher conversion of available elemental carbon from lignocellulosic biomass to activated carbon using the proposed synthesis method.

The percent yield of JMSP AC was determined from the relation:

$$Yield(\%) = \frac{w_{ac}}{w_0} \times 100 \quad \dots(1)$$

where w_{ac} and w_0 were the final JMSP AC dry weight (g) and the precursor dry weight (g), respectively.

Adsorption Studies

The efficacy of the synthesized JMSP AC was tested as an adsorbent for the Pb⁺² and EBT sorption from aqueous solutions in the batch experiments. All experiments were triplicate, and the mean of these were reported with the control experiment in the same operating conditions without adding adsorbent. Different standard solutions (10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm) of Pb⁺² metal ion and (10 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm) for EBT dye were prepared and kept in the refrigerator to maintain the constant volume

and concentration. The adsorption experiments were carried out in a 250 ml flask containing 100 ml of metal and dye solution of various concentrations, dosage of JMSP AC, and contact time at natural solution pH. Samples were shaken in a thermostat incubator at 200 rpm for 2 h. The residual metal ion concentration was determined using ICP-OES (Agilent Technologies 700 series), and that of the dye was determined using a UV-VIS spectrophotometer (Agilent Carry 100 UV-Vis). The amount of metal ion and dye adsorbed per unit mass of adsorbent was calculated according to the equation:

$$Q_e = \frac{(C_0 - C_e)V}{W} \quad \dots (2)$$

Where Q_e was the amount of metal ion/dye adsorbed on biosorbent (mg·g⁻¹), and C_0 and C_e were the initial and equilibrium concentrations of metal ion/dye solution (mg·L⁻¹). V - volume of metal ion/dye solution (L), and W - amount of JMSP AC biosorbent (g).

The following equation determined the % removal:

$$\text{Removal } (\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \quad \dots(3)$$

The adsorption kinetics were done at 25 °C and natural pH with concentrations at 100 and 250 mg·L⁻¹ for metal ion and dye, respectively. The samples were analyzed at predefined time intervals, and metal ion/dye uptake on biosorbent at time t was determined using Eq. 4

$$Q_t = \frac{(C_0 - C_t)V}{W} \quad \dots(4)$$

Where C_t (mg·L⁻¹) - concentration of metal ion/dye at any time.

The adsorption isotherm data were analyzed using four different types of adsorption isotherm models, including the Langmuir, Freundlich, Temkin, and Redlich-Peterson (R-P); the kinetic models used were pseudo-first-order, pseudo-second-order, and intraparticle diffusion (Table 3).

Furthermore, to elucidate the adsorption thermodynamics, Pb⁺², and EBT adsorption was conducted at different

Table 3: Adsorption isotherms and kinetic models.

Models	Equation	Parameters
Langmuir	$Q_e = \frac{Q_m K_L C_e}{1 + K_L C_e}$	K_L = constant related to the affinity of binding sites ($L \cdot mg^{-1}$)
Freundlich	$Q_e = K_F C_e^{\frac{1}{n}}$	K_F = constant related to adsorption capacity ($L \cdot g^{-1}$), n = constant related to the intensity of adsorption
Temkin	$Q_e = \frac{RT}{B_T} \ln(A_T C_e)$	A_T = equilibrium binding constant ($L \cdot g^{-1}$), R = universal gas constant ($8.314 J \cdot mol^{-1} K^{-1}$), T = absolute temperature (K), B_T = constant related to adsorption heat ($J \cdot mol^{-1}$)
Redlich-Peterson (R-P)	$Q_e = \frac{K_R C_e}{1 + a_R C_e^g}$	K_R = R-P constant ($L \cdot g^{-1}$) a_R = R-P constants ($L \cdot mg^{-1}$) g = exponent related to surface heterogeneity
Pseudo-first order	$Q_t = Q_e (1 - e^{-k_1 t})$	k_1 = equilibrium rate constant (min^{-1})
Pseudo-second order	$Q_t = \frac{Q_e^2 k_2 t}{1 + Q_e k_2 t}$	k_2 = equilibrium rate constant ($g \cdot mg^{-1} \cdot min^{-1}$)
Intraparticle Diffusion model	$Q_t = k_i t^{1/2} + C$	k_i = rate constant $mg \cdot g^{-1} \cdot min^{-0.5}$, C = boundary layer thickness

temperatures in the range of 25 to 40°C with 0.8 g·L⁻¹ of adsorbent suspended in 50 mL 50 ppm Pb⁺²/EBT solution agitated at 200 rpm for 2 h. The various thermodynamic parameters at equilibrium, including Gibb's free energy change (ΔG^0), enthalpy change (ΔH^0), and entropy change (ΔS^0), were calculated based on the adsorbate concentration C_e (mg·L⁻¹) and adsorption capacity Q_e (mg·g⁻¹) in solution in equilibrium at different temperatures.

Biosorbent Regeneration

Regeneration of the used JMSP biosorbent was carried with 0.2 M HNO₃ to desorb the sequestered Pb⁺² and EBT, using a solid/liquid ratio of 3 g·L⁻¹ for 2 h. Subsequently, the biosorbent was separated using a 0.45 µm nylon filter. The filtrate was analyzed for desorbed Pb⁺² and EBT, while the separated biosorbent was washed with milli-Q water 3 times and then used for resorption study after drying in the oven at 110°C for 12 h.

Characterization of JMSP AC Biosorbent

To elucidate the underlying adsorption mechanisms and active binding sites, JMSP biosorbent were characterized using a variety of physicochemical and spectroscopic approaches before and after Pb⁺²/EBT adsorption (i.e., pristine JMSP biosorbent and Pb⁺²/EBT loaded JMSP AC). The surface electric property of JMSP biosorbent was examined by a Malvern Zetasizer (Nano Series) and pH drift method (Faria et al. 2004). Textural characterization of the JMSP AC was carried out by N₂ adsorption at 77 K using Quantachrome Autosorb IQ Surface area analyzer. The XRD diffractogram of the JMSP was recorded using CuKα radiation ($\lambda = 1.54184 \text{ \AA}$) at 30 kV and 10 mA in the range of 2θ from 5° to 90° (Bruker, Germany). The

surface morphological and elemental properties of JMSP were investigated by a high-resolution scanning electron microscopy (Thermo scientific Apreo S) coupled with an EDX. Furthermore, the functional groups on the JMSP surface were identified by FTIR spectroscopy in the range of 500–4000 cm⁻¹ wavenumber (PerkinElmer Frontier).

RESULTS AND DISCUSSION

Yield and Surface Electric Property

The activated carbon synthesized in this study had an approximate yield of 26.80 %, which was in agreement with the previous work related to the processing of JMSP for activated carbon (Treviño-Cordero et al. 2013) (Elizalde-González & Hernández-Montoya 2009). The zeta potential measurement revealed (Table 4) that the surface charge for prepared activated carbon (JMSP AC), activated carbon loaded with Pb⁺² (JMSP AC-Pb⁺²) and EBT (JMSP AC-EBT) were -16.2 mV, -12.8 mV and -26.9 mV respectively. The result showed that the stability of the JMSP AC-EBT complex was more stable than the JMSP AC-Pb⁺² complex compared to JMSP AC. The increase in zeta potential after adsorption with Pb⁺² ion indicates the binding of metal ions on the surface of the biosorbent, while the attachment of negatively charged functional groups on the JMSP AC surface resulted in a decrease of zeta potential after EBT adsorption. The point of zero charge (pH_{PZC}) for the synthesized activated carbon was 7.42. With increasing pH, the surface becomes more negatively charged, leading to a stronger electrostatic attraction, while a decrease in the pH results in a more positive charge, leading to stronger repulsion forces (Rambabu et al. 2020).

Table 4: The Zeta potential of JMSP AC before and after Pb^{+2} and EBT adsorption.

Material	Zeta Potential ξ [mV]
JMSP AC	-16.2
JMSP AC- Pb^{+2}	-12.8
JMSP AC-EBT	-26.9

Characterization of Activated Carbons

The N_2 adsorption/desorption isotherms of the prepared JMSP AC are shown in Fig.1. According to isotherm, a progressive increase of relative pressure resulted in increased N_2 volume even at very low relative pressure (inset Fig.1). The adsorption isotherm for prepared JMSP biosorbent belong to type IV isotherm according to the IUPAC classification (Sing et al. 1985), which indicates the mesoporous structure.

The predominant hysteresis loop indicates the adsorption with capillary condensation (Donohue & Aranovich 1998, Thommes et al. 2015). The development of mesoporous distribution of JMSP AC and isotherm at low relative pressure (0.010).

The structure was affirmed by the pore size distribution (Fig.1), as shown most of the pores had sizes between 2.9 to 38 nm, which according to IUPAC classification associated

with mesoporous materials. (Sing et al. 1985, Thommes et al. 2015).

Different BET study parameters shown in Table 5 revealed that the mesopore volume was 92% of the total pore volume with a surface area of $136 \text{ m}^2 \cdot \text{g}^{-1}$. The average pore size of the JMSP AC was 3.10 nm. In the literature, similar structure characteristics were reported with activated carbon prepared from plant-based materials (Danish et al. 2018, Lima et al. 2019, Cunha et al. 2020, Kasperiski et al. 2018, Salomón et al. 2021).

The surface morphological aspects of the synthesized biosorbent by SEM are represented in Fig. 2. The prepared JMSP AC biosorbent (Fig. 2b) observed greater surface irregularity compared to the raw Jacaranda mimosifolia seed pods (Fig. 2a) with even distribution of the cavities over the surface. These pores were favorable to adsorption, as they could increase the adsorption capacity of the adsorbate molecules of Pb^{+2} and EBT. After the Pb^{+2} adsorption on the biosorbent, some metal particles precipitate was observed. The elemental mapping exhibits the intense signal for Pb and O, confirming the microprecipitation of Pb^{+2} on the surface of the biosorbent.

The XRD diffraction study reveals the crystalline property of JMSP (Fig. 3a). The broad peak at $2\theta^0$ of 8-29 characterized various amorphous phases such as

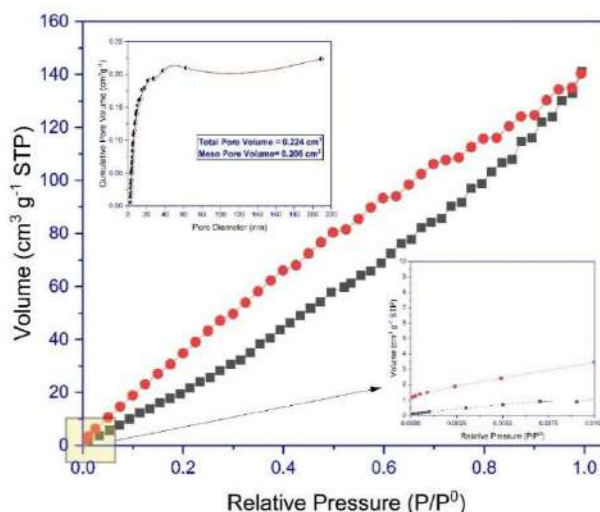
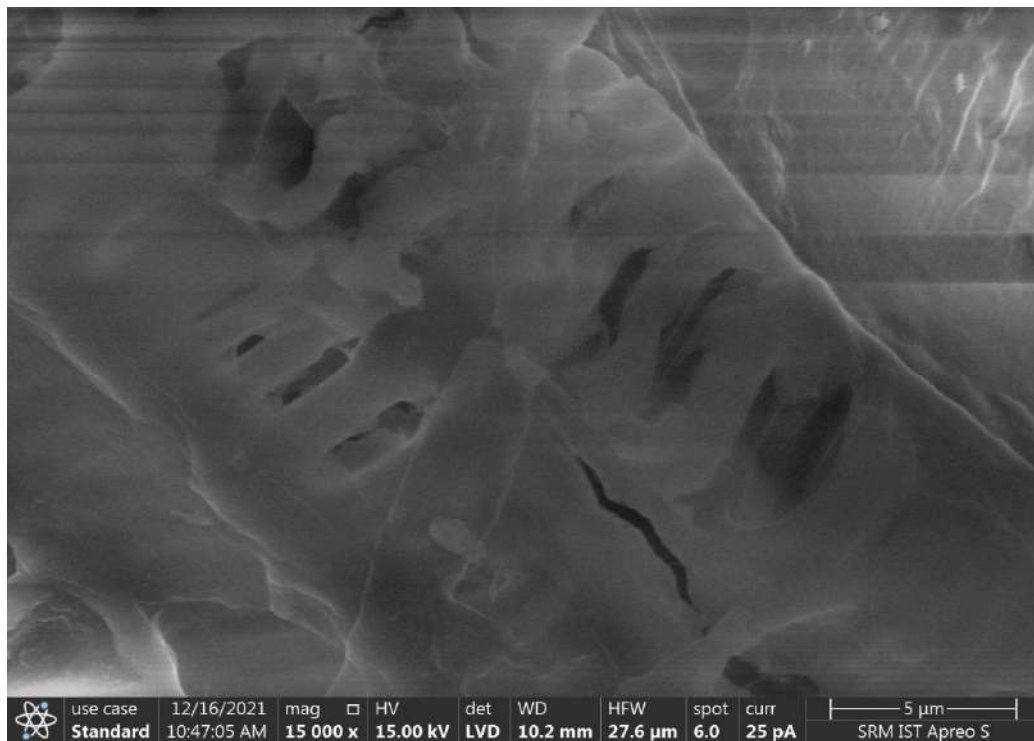


Fig. 1: N_2 adsorption/desorption isotherms of the prepared JMSP AC inset show the pore size.

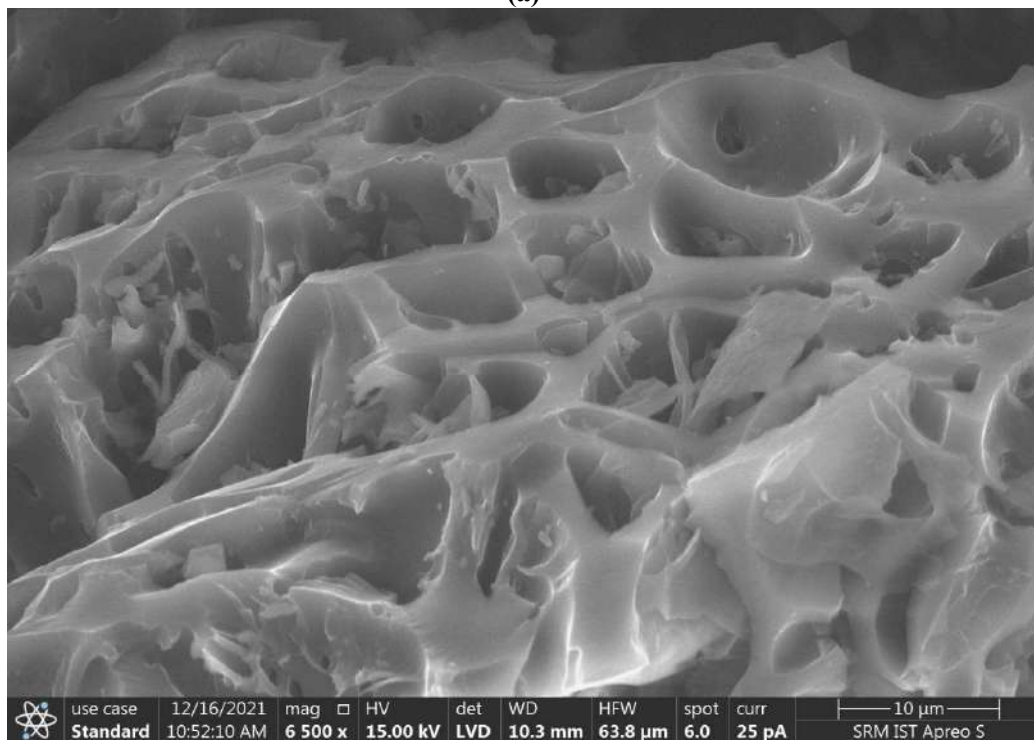
Table 5: BET study parameters of prepared JMSP AC.

	D_p (nm)	S_{BET} [$\text{m}^2 \cdot \text{g}^{-1}$]	V_T [$\text{cm}^3 \cdot \text{g}^{-1}$]	V_μ [$\text{cm}^3 \cdot \text{g}^{-1}$]	V_m [$\text{cm}^3 \cdot \text{g}^{-1}$]	V_m/V_T [%]	V_μ/V_T [%]
JMSP AC	3.10	136	0.224	0	0.206	92	0

D_p = average pore diameter, S_{BET} = BET surface area, V_T = total pore volume, V_μ = micropore volume, V_m = mesopore volume, V_m/V_T = percentage of mesopore, V_μ/V_T = percentage of micropore



(a)



(b)

Fig. 2: SEM images of (a) Raw Jacaranda mimosifolia seed pods (JMSP) (b) prepared JMSP AC biosorbent.

hemicellulose, lignin, and amorphous cellulose of the prepared biosorbent. The distinct reflection at $2\theta^0$ of 16 and 26, along with unresolved doublet at 22 and 23, corresponded to starch of *Jacaranda mimosifolia* seed pod biosorbent (Sandhu & Lim 2008) (Patiño-Rodríguez et al. 2020). Cellulose from the JMSP has a well-defined crystal structure with absorption peaks in XRD diffractogram at 2θ angles of 16° corresponding to crystal plane (101) and 23° (002), respectively. The presence of all the peaks in the diffractogram pattern of the JMSP AC indicated the successful formation of the desired porous carbon. It was elucidated that after the adsorption of Pb^{+2} and EBT on JMSP AC, the diffractogram showed broader and weaker reflections, confirming the reduced crystallinity of JMSP AC. This should be due to the binding of JMSP AC with Pb^{+2} and EBT, resulting in the disorientation of the crystalline regions of JMSP AC because of hydrogen bond breakage to prevent the disorientation of crystalline regions (Moyo et al. 2017). These amorphous regions were responsible for the adsorption of Pb^{+2} and EBT on biosorbent.

The changes in various functional groups on the JMSP AC biosorbent before and after adsorption of Pb^{+2} and EBT were confirmed from FTIR spectra (Fig. 3b). The complex composition of JMSP AC biosorbent could be realized from the various adsorption bands observed. The FTIR spectra of JMSP AC biosorbent exhibited broad weak adsorption bands for stretching vibration of O–H with free hydroxyl at 3660 cm^{-1} , twin sharp bands at 2981 cm^{-1} and 2973 cm^{-1} at 2924 cm^{-1} for C–H in alkanes, C=O stretch in saturated carbonyl at 1715 cm^{-1} , bending vibrations of N–H in primary amines at 1591 cm^{-1} , stretching of acyl C–O at 1286 cm^{-1} and 1206 cm^{-1} , stretching of amines C–N at 1109 cm^{-1} ,

C–O in primary alcohol at 1028 cm^{-1} and bending of alkene C–H at 839 cm^{-1} . This confirmed the existence of various functional groups in JMSP AC, e.g., hydroxyl, amine, carboxyl and alkene. After the adsorption with Pb^{+2} and EBT on biosorbents, all such bands were removed for Pb^{+2} adsorption and weakened for EBT suggesting the binding of Pb^{+2} and EBT to the functional groups (Hufton et al. 2021) (Ren et al. 2022) which strongly favor the adsorption of pollutants on the biosorbent. Furthermore, following Pb^{+2} and EBT adsorption, the prominent peaks at 2981 cm^{-1} and 2973 cm^{-1} were decreased and displaced, suggesting that hydrogen bonding may be associated with the Pb^{+2} and EBT sorption process by JMSP AC. The peak at 1591 cm^{-1} may have been caused by the JMSP AC stretching vibration, which shifted following the adsorption of Pb^{+2} and EBT, suggesting that π - π type interaction may have been involved in the adsorption process by JMSP AC.

Adsorption performance of JMSP AC for Pb^{+2} and EBT

Effect of pH on adsorption: It was evident that the pH of the solution plays a pivotal role in adsorption studies due to its effect on the electrostatic interaction between adsorbates and adsorbents. In the present study, the effect of pH on the removal of heavy metal Pb^{+2} and dye EBT was determined in the pH range from 2-9. For $pH > 9$, under basic conditions the formation of the precipitation of salts occurred as their hydroxide can influence the adsorption process, therefore adsorption study was conducted for pH up to 9. Lead ions (Pb^{+2}) showed the maximum adsorption of about $59.24\text{ mg}\cdot\text{L}^{-1}$ at pH 5 (Fig. 4a) and for EBT, pH 6 was found to be optimum with maximum adsorption of $289.32\text{ mg}\cdot\text{L}^{-1}$ (Fig. 4b).

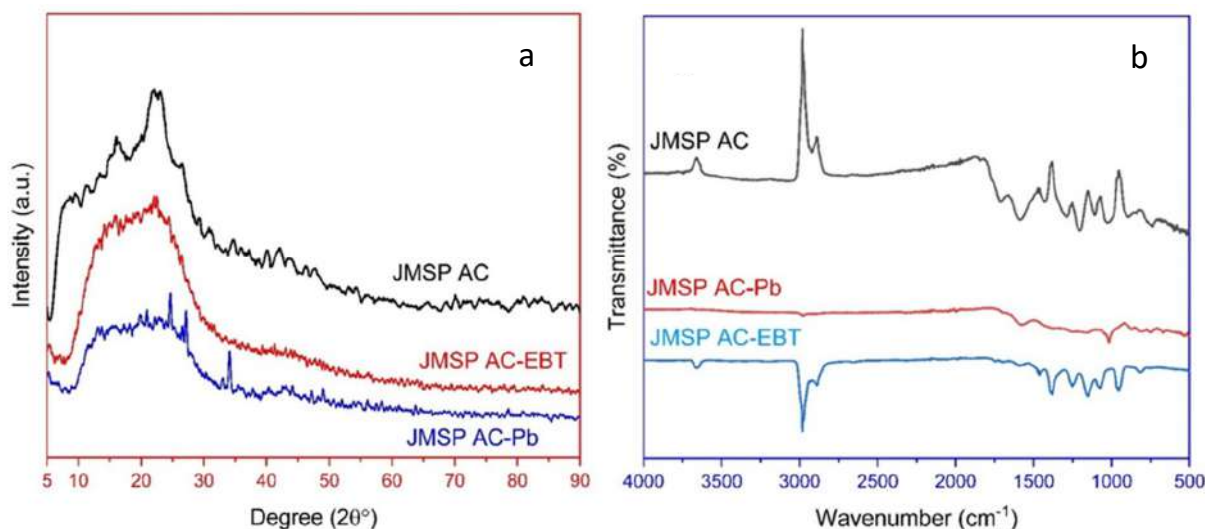


Fig. 3: (a) XRD diffractogram of JMSP AC before and after Pb^{+2} and EBT adsorption. (b) FTIR Spectra of JMSP AC before and after Pb^{+2} and EBT.

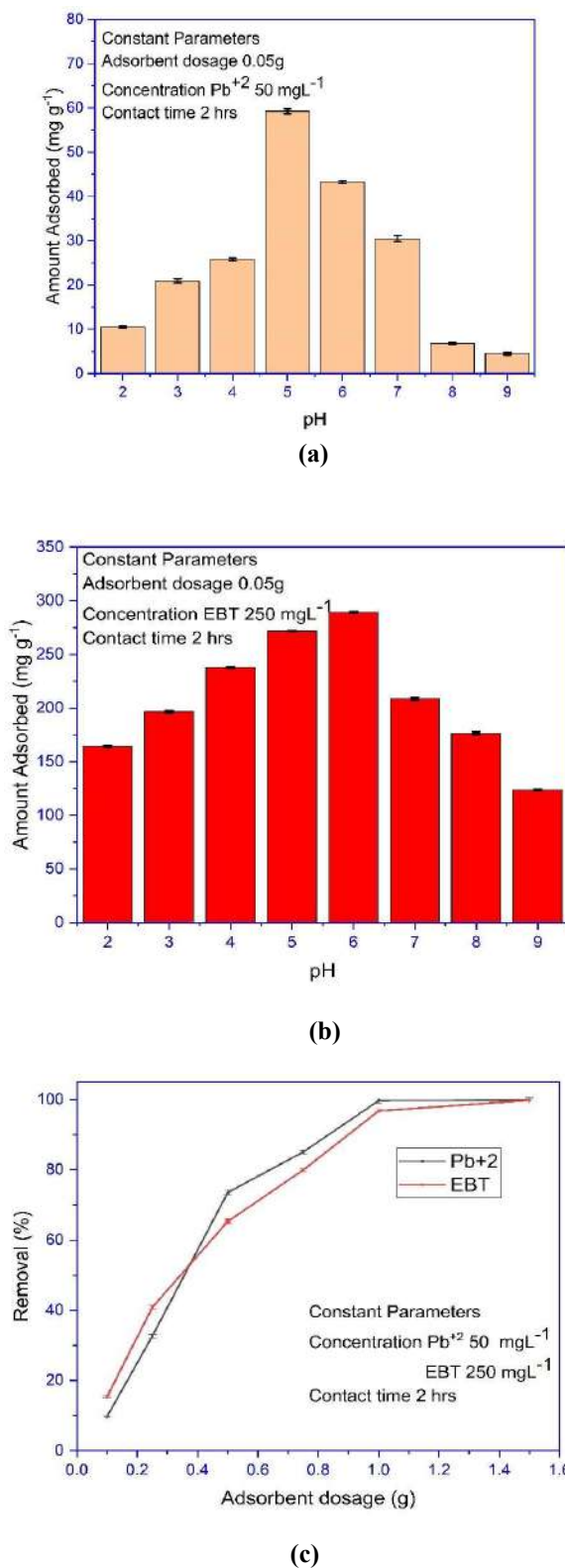
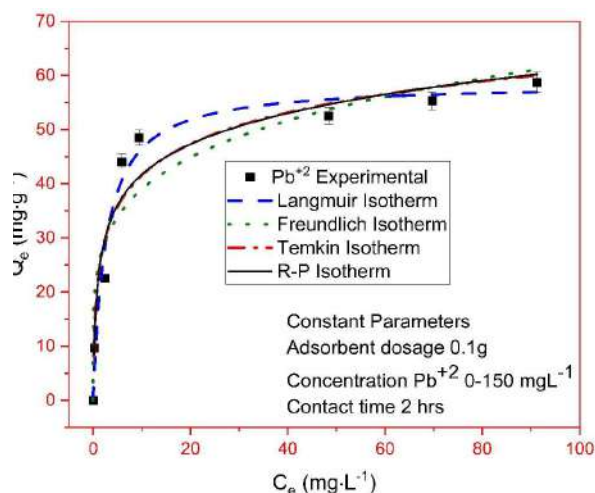
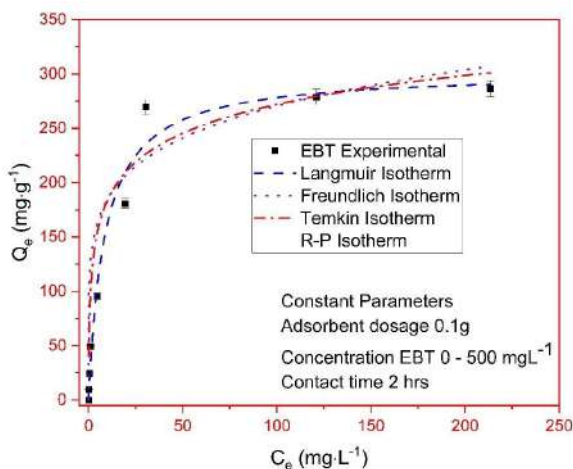


Fig. 4: Effect of pH (a & b) and dosage (c) for the adsorption of Pb⁺² and EBT on JMSP AC.

Effect of dosage on adsorption: The minimum possible dosage for the optimum adsorption of Pb^{+2} and EBT was determined, and the amount of biosorbent dosage was varied 0.1 g, 0.25 g, 0.50 g, 0.75 g, 1.0 g, and 1.5 g in 100 ml of $50 \text{ mg}\cdot\text{L}^{-1}$ Pb^{+2} and $250 \text{ mg}\cdot\text{L}^{-1}$ EBT solution. The adsorption was increased with the amount of biosorbent dosage for the Pb^{+2} ions and EBT, which was found to be 32.62% and 40.83% at a dosage of 0.25 g and reached 85.10% and 79.96% at a dosage of 0.50 g (Fig. 4c). The adsorption reached to about 100% removal of metal ion Pb^{+2} and dye EBT at 1.5 g of JMSP AC. However, the overall adsorption of Pb^{+2} is lower than the EBT on the biosorbent.



(a)



(b)

Fig. 5: Adsorption isotherm of Pb^{+2} (a) and EBT (b) adsorption on JMSP AC with nonlinear fitting to langmuir, freundlich, temkin and R-P models.

As can be observed trend of adsorption with the adsorbent dosage, with increase in dosage, the adsorption increases and reaches to maximum for 1.5 g. These observations evince that adsorption is directly proportional to the amount of the adsorbent dosage.

Adsorption Isotherms: In the equilibrium study, the nonlinear isotherm models of Langmuir, Freundlich, Temkin, and Redlich-Peterson (R-P) were realized to determine the mode of adsorption and interaction between the metal ion Pb^{+2} and dye EBT. Fig. 5 depicted the nonlinear fitting for the above models for Pb^{+2} (Fig. 5a) and EBT (Fig. 5b), and Table 6 illustrated the values of constants and correlation coefficient for all four isotherm models. The maximum Pb^{+2} adsorption capacity of JMSP AC was determined to be $58.77 \text{ mg}\cdot\text{g}^{-1}$, and the R-P adsorption model provided the best description (Fig. 5a, Table 6) with a correlation coefficient (R^2) of 0.9998. For EBT adsorption, the maximum adsorption capacity was $286.56 \text{ mg}\cdot\text{g}^{-1}$, and a similar nonlinear R-P adsorption model provided the best description (Fig. 5b, Table 6) with a correlation coefficient (R^2) of 0.9996. The features of both, Langmuir and Freundlich isotherms were incorporated into the R-P model, and it reduces to the Freundlich model if the value of a_{rcin}^g was much higher than 1.0 and approaches the Langmuir model when g equals 1.0. This confirmed the multilayer adsorption of Pb^{+2} and EBT on the heterogeneous surface of JMSP AC.

Furthermore, a dimensionless constant, the separation factor (R_L) defined by the following relationship predicting the adsorption nature of the process.

$$R_L = \frac{1}{1 + K_L C_0} \quad \dots(5)$$

where K_L refers to the Langmuir constant and C_0 was the initial concentration of adsorbate in ($\text{mg}\cdot\text{L}^{-1}$). The separation factor indicates the nature of adsorption to either be irreversible ($R_L = 0$), unfavorable ($R_L > 1$), linear ($R_L = 1$), or favorable ($0 < R_L < 1$).

In the present study, the experimentally calculated R_L values for the adsorption of Pb^{+2} and EBT on synthesized JMSP AC were 0.0172 - 0.2083 and 0.0164 - 0.4545, respectively. These R_L values confirmed that biosorbent JMSP AC was advantageous for adsorption of Pb^{+2} and EBT from water.

Moreover, values of Freundlich constant n were found to be 4.92 ($1/n = 0.203$) and 6.01 ($1/n = 0.166$) for the adsorption of Pb^{+2} and EBT, respectively, suggesting favorable adsorption ($0 < 1/n < 1$) on the surface of synthesized biosorbent. This encourages the utilization of low-cost JMSP AC for the removal/recovery of heavy metals and dyes from wastewater.

Table 6: Parameters for adsorption isotherm and kinetic models of Pb⁺² and EBT adsorption by JMSP AC.

Models	Parameter	Pb ⁺²	EBT
Isotherms			
Langmuir	Q _m	58.56	302.1
	K _L	0.38	0.12
	R ²	0.9762	0.9779
Freundlich	K _F	24.41	125.8
	n	4.92	6.01
	R ²	0.9106	0.8617
Temkin	A _T	14.49	12.02
	B _T	8.35	38.36
	R ²	0.9524	0.9859
Redlich-Peterson	K _R	69.65	232.66
	a _R	2.18	1.58
	g	0.86	0.87
	R ²	0.9998	0.9996
Kinetics			
Pseudo-first order	Q _e	59.51	234.28
	k ₁	0.05	0.1
	R ²	0.9945	0.9736
Pseudo-second order	Q _e	68.26	253.39
	k ₂	9.72 x 10 ⁻⁴	6.13 x 10 ⁻⁴
	R ²	0.9813	0.9967
Intraparticle Diffusion	k _i	4.17	7.95
	C	17.28	153.57
	R ²	0.7462	0.8799

(Note: Q_e - Equilibrium adsorption capacity, Q_m - Maximum adsorption capacity. Details of other parameters are referred to in Table 3.

Adsorption kinetics: To determine the kinetics of Pb⁺² and EBT adsorption on JMSP AC, different models, including pseudo-first order, pseudo-second order, and Intraparticle diffusion, were applied. Fig. 6 shows the kinetic fitting for different models and Table 6 represents the various constants for the fitted models along with the correlation coefficient. It was found that the adsorption follows the pseudo-first order and pseudo-second order for the adsorption of Pb⁺² (Fig 6a, Table 6) and EBT (Fig 6b, Table 6) on the synthesized JMSP AC, respectively, with the correlation coefficient (R²) 0.9945 and 0.9967. The intercept C for the intraparticle diffusion (Fig 6c, Table 6) for Pb⁺² and EBT adsorption was 17.28 and 153.57, respectively, evident that the effect of the boundary layer is higher for EBT adsorption than that of Pb⁺² on the surface of biosorbent. Further, analysis of the kinetic data for the intraparticle diffusion model observed that the plot does not pass through the origin, suggesting that

intraparticle diffusion was not the only rate-limiting step for the adsorption of Pb⁺² and EBT on biosorbent.

The equilibria of the Pb⁺² and EBT adsorption processes on JMSP AC were readily reached within 180 min, with 97.04% of Pb⁺² and 90.28% of EBT adsorption reached in the first 60 min (Fig. 6).

The Pb⁺² and EBT adsorption processes had three phases: in first phase, during first 20 minutes, Pb⁺² and EBT quickly bound to JMSP AC due to the high availability of the free surface binding sites; during second phase from 20 to 60 minutes, it was gradual decrease in adsorption rate and final third stage start after 60 minutes, which characterize as equilibrium stage with slow uptake of Pb⁺² and EBT on the biosorbent due to the less free binding sites available as a result of surface saturation of the adsorbent and decreased concentration of Pb⁺² and EBT in the liquid phase.

Adsorption thermodynamics: The temperature (25 to 40°C) effect on the adsorption of Pb⁺² and EBT was investigated. Thermodynamic parameters such as free energy change (ΔG⁰), enthalpy (ΔH⁰), and entropy (ΔS⁰) were calculated according to equations.

$$\Delta G^0 = -RT \ln K_d \quad \dots(6)$$

$$K_d = \frac{Q_e}{C_e} \quad \dots(7)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad \dots(8)$$

The parameters ΔH⁰ and ΔS⁰ were obtained from the slope and intercept of the plot lnK_d versus 1/T. The thermodynamic parameters are given in Table 7. The ΔG⁰ of Pb⁺² adsorption by JMSP AC biosorbent was -7.39, -5.93, -4.17, and -2.78 kJ.mol⁻¹ at 298, 303, 308, and 313 K, respectively. This confirmed that the Pb⁺² adsorption process became less spontaneous with increased temperature. The ΔH⁰ and ΔS⁰ were estimated to be -100.22 kJ.mol⁻¹ and -0.311 kJ.K.mol⁻¹, respectively implied that the Pb⁺² adsorption by JMSP AC was exothermic and reduced randomness at the solid-liquid interface of the adsorption system.

For EBT adsorption, the ΔG⁰ was -9.11, -7.10, -5.39, and -3.67 kJ.mol⁻¹ at 298, 303, 308, and 313 K, respectively, indicating the reduced adsorption spontaneity at higher temperatures. The ΔH⁰ and ΔS⁰ were estimated to be -116.66 kJ.mol⁻¹ and -0.361 kJ.K⁻¹.mol⁻¹, respectively. The decreased adsorption of Pb⁺² and EBT by biosorbent at higher temperatures could reduce the thickness of thermal boundary layer for the adsorbed Pb⁺²/EBT leads to the escape from the JMSP AC surface back into the bulk solution (Rambabu et al. 2020). This probably indicates a poor interplay between adsorbate and various surface functionalities of JMSP AC.

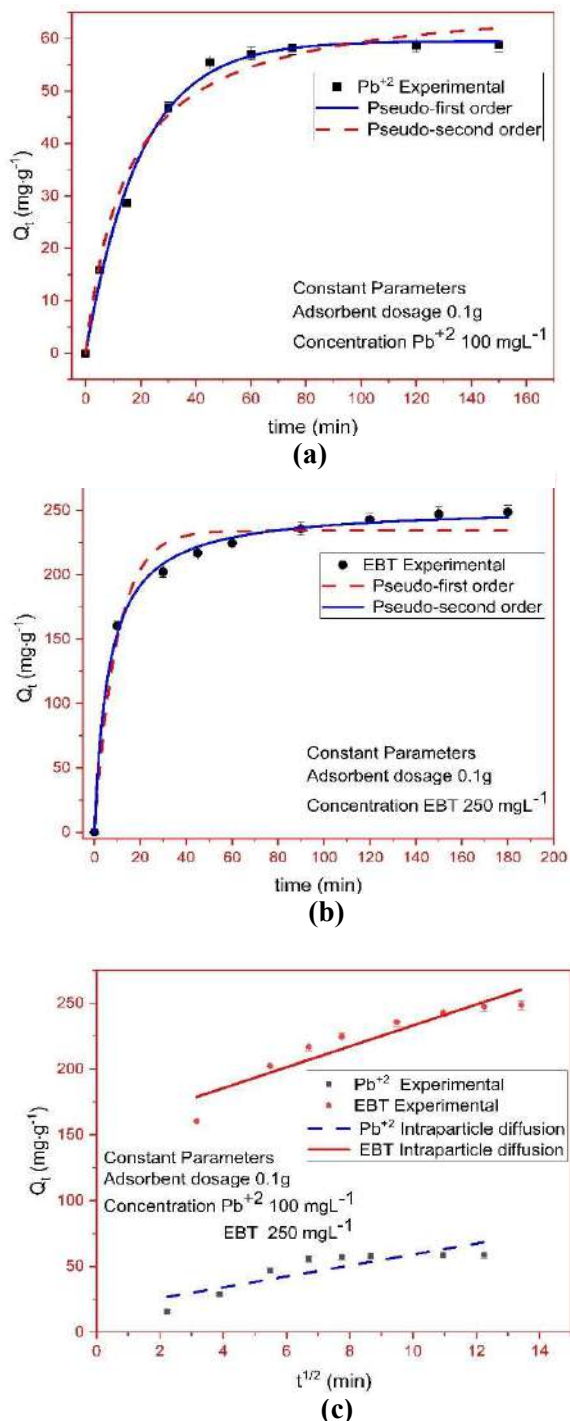


Fig. 6: Adsorption kinetics of Pb²⁺ (a) and EBT (b) adsorption on JMSP AC with nonlinear fitting to pseudo-first order, pseudo-second order and Intraparticle diffusion (c) kinetic models.

The adsorption mechanism of Pb²⁺ and EBT on JMSP AC biosorbents was explored by examining experimental data through various kinetic models, including pseudo-first

Table 7: The thermodynamic parameters of Pb²⁺ and EBT adsorption by JMSP AC biosorbent.

	ΔG^0 [kJ. mol ⁻¹]	ΔH^0 [kJ. mol ⁻¹]	ΔS^0 [kJ.K ⁻¹ . mol ⁻¹]
Pb ²⁺ adsorption			
298 K	-7.39	-100.22	-0.311
303 K	-5.93		
308 K	-4.17		
313 K	-2.78		
EBT adsorption			
298 K	-9.11	-116.66	-0.361
303 K	-7.10		
308 K	-5.39		
313 K	-3.67		

order, pseudo-second order, and intraparticle diffusion. The proton quantity could influence the adsorption for Pb²⁺ and EBT, suggesting the surface-active regions on JMSP AC. The various interactions between the surface of JMSP AC and Pb²⁺/EBT resulted in adsorption, including surface complexation, microprecipitation, and electrostatic attraction (Fig. 7).

Regeneration of biosorbent: Stability and the biosorbent's capacity for regeneration were typically crucial elements in practical applications, which was investigated through six consecutive adsorption-desorption cycles (Fig. 8) using 0.2 M HNO₃ as effective eluting agents for regeneration of the JMSP AC biosorbents. However, plant biomass based biosorbents were inexpensive, widely accessible, and recyclable. It was observed that as the number of regeneration cycles increased, the adsorption efficiency dropped, for Pb²⁺ efficiency was reduced from 83% to 68 % during the first three cycles and further reduced to 39% after the sixth regeneration cycle, whereas for EBT, efficiency was reduced from 92% to 85 % and further reduced to 12% at the end of the sixth regeneration cycle. This suggested that, with the right procedures, the biosorbent may be recycled. To optimize the regeneration process, the eluting agents' solution was stirred at 50-100 rpm for 120 min at 298.15 K, and this helped to minimize pickling of the surface of JMSP AC, which facilitated the reduction of the decline in adsorption efficiency over the cycles.

Comparative studies: Different biosorbents synthesized from lignocellulosic biomass were applied for the removal of heavy metal ion Pb²⁺ and dye EBT from water. Table 8 illustrated the maximum adsorption capacity (Q_m) of different adsorbents for the removal of Pb²⁺ and EBT confirmed that the synthesized biosorbent JMSP AC was much more efficient compared to other adsorbents. The previous study had various limitations in terms of cost-effectiveness, scale-up, energy consumption, regeneration potential, reproducibility, implementation in resource-constrained regions for large-scale water treatment, and



Fig. 7: Schematics of Adsorption mechanism of Pb^{+2} and EBT on JMSP AC.

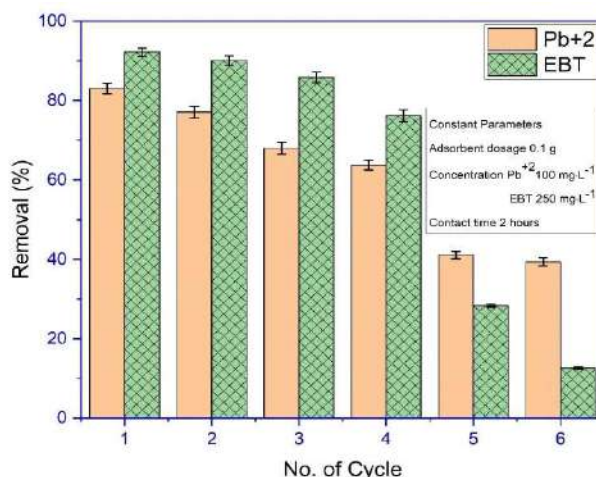


Fig. 8: Sequential adsorption- desorption cycles of the prepared JMSP AC for regeneration.

environmental sustainability. As major synthesis processes used toxic chemicals in their production cycle (Melliti et al. 2023, Eletta et al. 2023, Bumajdad & Hasila 2023, Beyan et al. 2024, Isam et al. 2024, Al-Ma'abreh et al. 2024, Aziz et al. 2019, Abiza et al. 2024, Ali et al. 2022, Ayuba & Sani 2022, El Mansouri et al. 2022, Rath et al. 2024, Sadeghi et al. 2021)) for activated carbon including chemicals used in pre and post-treatment steps. The scalability and reproducibility of disclosed processes has limited scope as insufficient disclosure of entire production process (Mandal et al. 2021, Melliti et al. 2023, Eletta et al. 2023, Bumajdad & Hasila 2023, Isam et al. 2024, Mkilima et al. 2024, Ali et al. 2022, El Mansouri et al. 2022), this prone to the hurdle in adaptation of production processes at commercial scale. Major constraints for implementation of large-scale production of activated carbon include the availability of

energy and emission of huge amounts of greenhouse gases (GHG) as major production processes were very energy intensive, these processes had very high specific energy consumption in terms of kilowatt hours (KWH) per kilogram of activated carbon produces which leads to high GHG footprint. In contrast to the above limitations, our single-step green synthesis process addresses major constrain for its adaptability at industrial scale production. First, our process did not require any environmentally hazardous chemicals; secondly, required little energy compared to previously reported processes; and finally, the disclosed production process could easily scale up and reproduce as our study has sufficient disclosure of the entire production process. The cost-effectiveness of our single-step green synthesis process made it easy to adopt for large-scale water treatment facilities for environmental sustainability. Thus, our study

Table 8: The adsorbent JMSP AC comparison of adsorption capacities for Pb+2 and EBT with different adsorbents.

Sr.No.	Pollutants	Adsorbents	Q_m (mg·g ⁻¹)	Ref.
	Pb ⁺²	Kenaf-based activated carbon (KAC)	11.236	(Mandal et al. 2021)
		activated carbon (date palm fiber)	29.859	(Melliti et al. 2023)
		Activated carbon (<i>Theobroma tithonia</i>)	46.95	(Eletta et al. 2023)
		f-activated carbon (palm tree)	4.112	(Bumajdad & Hasila 2023)
		activated carbon (red algae- <i>Gracilaria changii</i>)	36.231	(Isam et al. 2024)
		Activated carbon (Teff Straw)	42.97	(Beyan et al. 2024)
		Activated carbon-sodium alginate-oxalate gel particles	53.04	(Li et al. 2024)
		activated carbon (banana peel)	6	(Mkilima et al. 2024)
		Activated Carbon (<i>Cypress</i> Fruit)	56.18	(Al-Ma'abreh et al. 2024)
		kenaf-core activated carbon	12.7	(Mandal et al. 2021)
		Chemically activated Jute	25	(Aziz et al. 2019)
		Jacaranda mimosifolia Seed pod Activated Carbon	58.77	present work
	EBT	Activated carbon (<i>Spartium junceum</i>)	261.36	(Abiza et al. 2024)
		Chitosan (Shrimp shell)	162.3	(Khelifira et al. 2021)
		Activated carbon (Datura metal seeds)	154.5	(Ali et al. 2022)
		biochar (giant reed)	9.06	(Abdu et al. 2024)
		Activated Carbon (Waste Hemp <i>Cannabis sativa</i> L.)	14.025	(El Mansouri et al. 2022)
		graphene (wheat straw)	146.2	(Sadeghi et al. 2021)
		Activated carbon (<i>Typha</i> grass)	47.619	(Ayuba & Sani 2022)
		graphene oxide/activated carbon (<i>Carica papaya</i> seeds)	51.84	(Rath et al. 2024)
		Nano Carbon (<i>Morus Nigra</i> (Mulberry) Stem)	192.308	(Ahmed et al. 2024)
		Activated Carbon (Pepper Stalks)	55.56	(DOLAŞ 2023)
		Jacaranda mimosifolia Seed pod Activated Carbon	286.56	present work

linked with availability and sustainable management of water (SDG6) and sustainable consumption of natural resources (SDG12) for the production of Activated carbon with a smaller environmental footprint leads to reduced GHG Emissions (SDG13).

Based on the current study, we learned distinct reasons for the high adsorption capacities of the synthesized JMSP AC.

- 1) The single-step synthesis method produces several types of active sites.
- 2) Surface hydroxyl groups produce the surface complexation between contaminants.
- 3) Electrostatic attraction, co-exchanges, microprecipitation, and surface diffusion occur between Pb⁺² and EBT and prepared biosorbent.
- 4) The porous architecture of the synthesized biosorbent allowed the adsorption of various contaminants.
- 5) The adopted single-step synthesis method produces abundant transformation sites responsible for introducing physiochemical partition and stacking of sites to adsorb contaminant molecules.

CONCLUSIONS

In summary, we investigated novel and scalable synthesis of JMSP AC and its application for the removal of contaminants from water and wastewater. The synthesized biosorbent, a waste-to-resource product, manifested efficient adsorption of Pb⁺² and EBT (Q_m of 58.77 mg-Pb⁺²/g and 286.56 mg-EBT/g). The adopted single-step synthesis method was more energy efficient and eco-friendly compared to expensive conventional energy-intensive production and treatment processes. The binding sites for Pb⁺² and EBT were identified as hydroxyl, amine, ether, and carboxyl groups. The underlying adsorption mechanism comprises electrostatic attraction, microprecipitation, diffusion, and complexation. The mechanism provides an understanding of interactions between water pollutants and biosorbents. The study revealed the single-step, eco-friendly, and scalable synthesis method for producing activated carbon (biosorbent) for the removal of heavy metal and dye from water.

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The Effect of Plastoquinone Derivatives on Barley (*Hordeum vulgare* L.) Tolerance to Zinc Oxide Particles of Different Dispersion

N. G. Duplii¹, A. V. Usatov¹, K. V. Azarin¹, A. Ranjan^{1,2†} and E. V. Prazdnova¹

¹Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don, Russia

²Amity Institute of Environmental Toxicology, Safety and Management, Amity University, Noida, Uttar Pradesh, India

†Corresponding author: A. Ranjan; aranjan@amity.edu

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ABSTRACT

The plastoquinone derivatives 10-(6'-plastoquinonyl) decyl triphenylphosphonium (SkQ1) and 10-(6'-methyl-plastoquinonyl) decyl triphenylphosphonium (SkQ3) are lipophilic cationic antioxidants which can effectively penetrate cell membranes, targeting mitochondria to provide antioxidant defense in cells. In this study, barley (*Hordeum vulgare* L.) grown with zinc oxide (ZnO) nanoparticle (NPs) stress treated with SkQ1 and SkQ3 to observe the growth rate and level of expression of oxidative stress-related genes in barley seedlings. The study reported that a concentration of 300 mg.L⁻¹ of ZnO NPs inhibits the growth and development of the root system, while the addition of larger ZnO NPs in a similar concentration did not affect the development of the root. With the addition of SkQ1 and SkQ3, the length and weight of roots of the plants grown treated with 300 mg.L⁻¹ of ZnO NPs remain at the control level, while without the antioxidant, root length, and weight decrease by 14% and 12%, respectively, compared to the control indicating an increase in plant resistance under NPs induced stress conditions. In the case of 2000 mg.L⁻¹ of ZnO concentration, size-dependent effects were observed on the length of the roots, which decreased by 41.5% and 53.8%, and the weight of the roots by 23.3% and 38.8%, respectively, compared to the control. When plants are grown with 2000 mg.L⁻¹ of ZnO NPs along with SkQ1 and SkQ3 treatment, an increase in root length is observed by 14.6% and 17.4%, and dry weight increases by 12.2% and 13.2%, respectively, compared to the control. When a similar concentration of ZnO in the form of NPs is added, similar indicators increase by 15.5% and 14.3% in root length and 16.0% and 17.1% in root dry weight, respectively. The level of expression of antioxidant system genes in roots decreases by 10-18 times, and in leaves, it changes only slightly, which proves the effect of plastoquinone derivatives on mitochondria, which in the roots are the main suppliers of ROS.

INTRODUCTION

The growing need for nanotechnology may lead to the accumulation of NPs in the environment. Concerns are being raised about the impact of NPs on the ecological state of agrocenoses (Rastogi et al. 2017). The widespread use of metal-based NPs has revealed both the beneficial and harmful effects they have on plants (Javed et al. 2024). Stress tolerance can be controlled by the application of NPs. Due to their small size, NPs are highly reactive, making them suitable for altering various biological functions of plants (Javed et al. 2023) and promoting plant growth in a species-specific manner, even under biotic and abiotic stress conditions (Giraldo et al. 2014 Hanif et al. 2023).

However, in addition to the positive effects of MNPs on plants, these particles also have negative effects on plants (Liu et al. 2020). The induction of abiotic stress resistance by metal NPs is considered to be the main cause of nano-pollution or nanotoxicity. MNPs lead to the production of excessive reactive oxygen species (ROS) and, as a result, cause oxidative stress in plants, which leads to phyto, cyto and genotoxicity.



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Zinc oxide NPs are among the most widely used in the nano-industry (Meena et al. 2021). Zinc (Zn) in the divalent state (Zn^{2+}) is the most common form found in soil and absorbed by plants (Gupta et al. 2016). However, Zn^{2+} at high concentrations is highly toxic to plants (Balafrej et al. 2020). Excess Zn^{2+} in cells can cause oxidative stress and disrupt pro-oxidant and antioxidant homeostasis in plant cells (Sachdev et al. 2021). It has also been found that excess Zn has a genotoxic effect on plants, leading to genetic disorders and diminished plant health (Oladele et al. 2013, Kaur & Garg 2021). A study has shown that a high concentration of Zn (100 mg.L^{-1}) in cells causes chromosomal aberrations, including disruption of metaphase and uneven chromosome segregation. Other studies have also shown the negative effect of increased Zn concentration on plant growth parameters and crop yield (Ranjan et al. 2021, Todeschini et al. 2011). For example, under the influence of Zn^{2+} , the morphology and ultrastructure of the leaf sharply changed, and the formation of calcium oxalate crystals also increased. In addition, Zn^{2+} treatment has been shown to reduce root and shoot length, as well as leaf area (Rani et al. 2024).

One of the most frequently developed nanomaterials is ZnO NPs, which have gained popularity in industrial applications and are also largely studied in agricultural applications, inevitably ending up in the environment due to their production in large quantities and their widespread use. Agricultural application of Zn NPs is also associated with direct environmental release and exposure, and their repeated application in different cropping cycles is also allowing its accumulation and biomagnification in plants exhibiting its deleterious effects (Rajput et al. 2021). Zn NPs primarily exhibit a negative impact on the plants by triggering ROS production, which ultimately affects the plants multifacetedly (Rajput et al. 2018). Studies have also confirmed the role of ZnO NPs in reducing root cell viability and genomic matrix stability, as well as upregulating and downregulating microRNAs in barley seedlings (Plaksenkova et al. 2020). Further studies on exposure to bulk- and ZnO NPs at 300 and 2000 mg.L^{-1} confirmed differential gene expression in barley roots, with upregulation of Mn-SOD and Fe-SOD genes associated with chloroplast protection. Cat 1 isoform predominated, contributing to increased catalase activity, suggesting activation of antioxidant defense mechanisms against oxidative stress, potentially mitigating early ontogenetic damage but leading to energy resource depletion at higher concentrations (Azarin et al. 2022).

In this regard, one of the effective methods of combating the negative impact of stress factors on plants is the regulation of ROS production (Sharma et al. 2012) through the action of natural or artificially synthesized antioxidants (Flieger

et al. 2021). The pursuit and introduction of antioxidants-based treatment to stressed plants for modulating ROS-induced stress is a promising and contemporary approach. Low-molecular-weight antioxidants are best suited for such purposes as they are capable, due to their structure, of targeted penetration into cell mitochondria (Smith et al. 2011). Skulachev and his team in the year 2009 developed many such compounds, such as SkQ1 (10-6'-plastoquinonyl decyl triphenyl-phosphonium) and SkQ3 (10-6'-methyl-plastoquinonyl decyl triphenyl-phosphonium) related to mitochondria-targeted antioxidants were synthesized (Skulachev et al. 2009). Due to plastoquinone antioxidants, as the base of this class of chemical compounds, they are potentially able to be reduced in the mitochondrial respiratory chain and can exhibit unique properties, acting as multi-acting antioxidants (Antonenko et al. 2008). During the studies, substances such as SkQ1 and SkQ3 showed the greatest antioxidant activity (Samuilov et al. 2019).

To understand the effect of oxidative stress caused by pollutants is to analyze the dynamics of gene expression of the pro- and antioxidant systems of plants. In this study, we have evaluated the role of SkQ1 and SkQ3 in alleviating ZnO (bulk and nano-forms)-induced ROS stress at different concentrations and sizes as per our previous studies (Azarin et al. 2022). It can be concluded that mitochondria-targeted antioxidants SkQ1 and SkQ3 are promising protectors capable of compensating for the insufficiency of protective mechanisms under stress due to the ability of these substances to reduce the excessive amount of ROS. This hypothesis can be confirmed both by direct measurement of the ROS level in the leaves and roots of barley sprouts and by determining the transcriptional activity of the antioxidant system genes, which correlate with the rate of plant growth, expressed with the length and weight of leaves and roots.

The objective of the study was to measure the ROS level and assess the expression of antioxidant-related genes to validate the role of SkQ1 and SkQ3 in stress modulation in barley seedlings. It would help assess the insights into how plants respond to NPs' polluted environmental conditions in the presence of plastoquinone derivatives and ensure the development of application methods for enhancing agricultural crop productivity.

Barley (*H. vulgare* L.) is a convenient model plant for studying the influence of various factors on resistance. Barley sprouts were used as a study's object of the impact of NPs cerium and titanium dioxide (Marchiol et al. 2016), silver (Fayez et al. 2017), selenium (Nagdalian et al. 2023), copper (Rajput et al. 2018) and other metals. Due to its short growth period and unpretentiousness, barley is easy to grow both in hydroponics (Wang et al. 2017) or artificial and natural

substrates (Ali et al. 2004), as well as in greenhouse or phytotron conditions. Early work on enzyme isolation and characterization also meant that barley grain enzymes were one of the first plant proteins to be crystallized and with solved structures (Langridge 2018). Currently, genes of the antioxidant system associated with enzymes that inhibit ROS are widely known for barley. That is why we chose barley as the object of our study.

It should be noted that plants, unlike other organisms, have developed a specific antioxidant defense mechanism to maintain the dynamic balance of ROS (Wang et al. 2018). The antioxidant defense system consists of two components: its own metabolites and enzymes that are coordinated in inactivating ROS and maintaining the balance between reduced and oxidized molecules in the body. Intracellular and extracellular antioxidants form complex networks that protect against oxidation and “form” stress signals (Peng et al. 2022). Thus, studying the effect of antioxidants using SkQ1 and SkQ3 as an example at the molecular level can complement the modern understanding of the mechanisms for maintaining plant redox homeostasis. Studying the reaction of the plant antioxidant system to contamination with zinc oxide nanoparticles can serve as a model to help assess the toxicity of heavy metal particles of varying degrees of dispersion. A toxicogenomic approach based on the study of the expression of genes of defense systems in various organs and tissues when exposed to heavy metal compounds is necessary for predicting possible damage to agriculture from soil pollution.

MATERIALS AND METHODS

Seed Treatment

The study included *H. vulgare* L. variety Medicum 157 OS seeds soaked in solutions of SkQ1 and SkQ3 at a

concentration of 2.5 nM for 6 hs and then germinated in Petri dishes on wet filter paper for 36 hs at a temperature of 25 °C and relative humidity of 60 ± 2.8 %. Then, equally germinated seeds were transferred to water (control) and ZnO NPs solution at concentrations of 300 and 2000 mg.L⁻¹. (Azarin et al. 2022, 2024). The bulk form for ZnO was also selected at the same concentration. These concentrations of ZnO (bulk and nano-forms) NPs zinc oxide were not chosen randomly also but were selected based on our previous studies. (Azarin et al. 2022). Our previous research shows that high doses of ZnO NP (2000 mg.L⁻¹ and 10000 mg.L⁻¹) reduce the respiration efficiency and ATP content in roots. NP ZnO also seriously changes the structure of mitochondria, reducing their number and efficiency (Azarin et al. 2024). The bulk form for ZnO was also selected at the same concentration. These concentrations of ZnO (bulk and nano-forms) were chosen based on our previous studies. (Azarin et al. 2022). Since ZnO NPs are insoluble in water, barley seeds were germinated on a rotary shaker (Helicon, Russia) at a speed of 115 rpm at a temperature of 25°C and illumination of 4 ± 0.5 kLux. After 9 days, the length of roots and sprouts was measured, then the plants were placed in a drying oven for 48 hs at a temperature of 105°C, and their weight was measured.

Characterization of Nanoparticles

Zinc oxide nanoparticles were commercially obtained from Aldrich, USA. The highest fraction of particles had a minimum Feret diameter (minFeret) of 60 ± 4 nm and a maximum Feret diameter (maxFeret) of 132 ± 8 nm.

The results of X-ray structural analysis showed the presence of standard peaks and high purity of the studied compound (Fig. 1). Previous measurements of the ζ -potential (-22 mV to -6.5 mV) demonstrated the stability of the

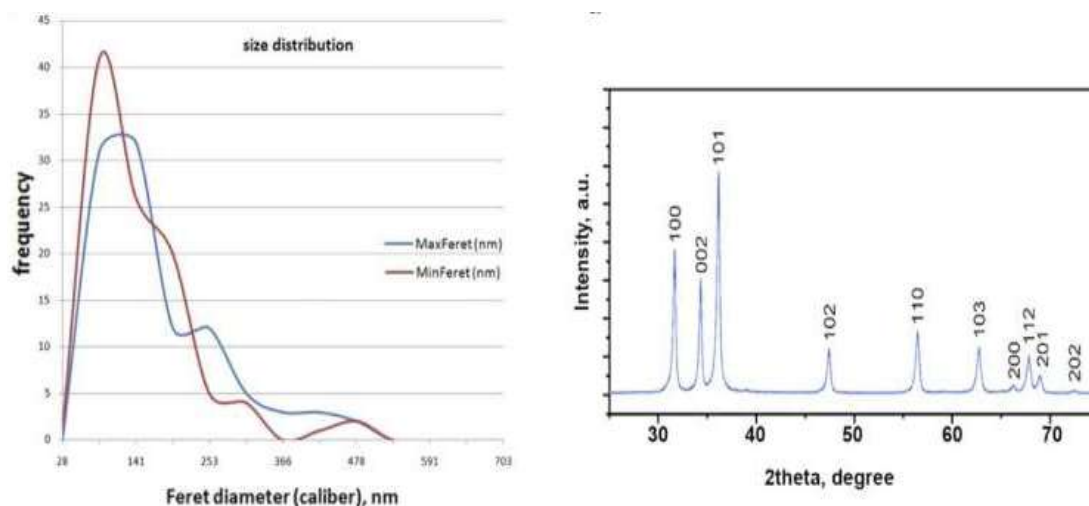


Fig. 1: Characteristics of ZnO nanoparticles: size distribution and X-ray diffraction pattern.

colloidal system of ZnO nanoparticles (Voloshina et al. 2022). Fourier transform infrared spectroscopy confirmed the presence of the ZnO functional group in the samples.

Measurement of ROS Level

The total ROS pool was determined using a fluorescence assay based on the formation of dichlorofluorescein from non-fluorescent dichlorofluorescein diacetate. A 0.5 g sample of fresh plant material was ground in liquid nitrogen with the addition of 2 ml of 0.2 N HClO₄ and subsequent neutralization with 37 μ L of 4 M KOH. Then 950 μ L of 0.15 M Tris-HCl buffer (pH 7.5), 25 μ L of supernatant, and 25 μ L of 0.5 mM dichlorofluorescein diacetate solution were added successively. The samples were stored in a thermostat at 37°C for 20 min, after which the fluorescence spectra were recorded (λ_{ex} = 496 nm/ λ_{em} = 524 nm) on an RF-5301 spectrofluorimeter (Shimadzu). The ROS content was calculated in mkg.g^{-1} FW.

RNA Extraction

RNA from leaf and root tissues was isolated according to the Chomczynski method (Chomczynski & Sacchi 1987) using a commercial ExtractRNA kit (Evrogen, Russia). RNA isolation was carried out in several steps. First, plant tissues were homogenized in a mortar with the addition of liquid nitrogen, and then lysis was carried out with ExtractRNA buffer. The liquid lysate was sedimented in a centrifuge, after which chloroform was added to the supernatant and mixed in a microcentrifuge. Then, the RNA was precipitated using isopropanol, incubating the mixture for 30 min at 4°C to precipitate the RNA. The resulting RNA precipitate was washed with 80% ethanol and dried in the open air at 37°C, which was further dissolved in 60 μ L of RNase-free water, stirring with a pipette tip. The RNA isolated was again treated with DNase from the commercial kit DNase I, RNase-free (ThermoFisherScientific, USA). The reaction mixture contained 6 μ L of RNA, 1 μ L of 10 $\times$ DNase buffer, and 1.2 μ L of DNase. The mixture was kept for 30 min at 37°C. 1 μ L of 50 mM EDTA was added and kept for 10 min at 65°C. This

process was performed on RNA to remove contaminating DNA, followed by inactivation of DNase enzyme.

Reverse Transcription Using PCR

The reverse transcription reaction was carried out using a commercial kit MMLVRTkit (Evrogen, Russia). To 4 μ L of RNA, 0.5 μ L (20 μ M) of primer was added and then heated for 2 min at 70°C. The samples were then placed in the cold. A pre-prepared mixture of the following composition was added to each sample: 5 $\times$ buffer for first-strand synthesis 5 μ L, dNTP mixture (10 mM of each nucleotide) 2 μ L, dithiothreitol (20 mM) 2 μ L, MMLV reversease 1 μ L. The final volume of the reaction mixture was adjusted to 25 μ L with sterile RNase-free water. The content was mixed in a microcentrifuge and incubated for 1 h at 39°C. Further heating of the mixture at 70°C for 10 min stopped the reaction. The synthesized cDNA was used as a matrix for real-time PCR. The polymerase chain reaction was carried out using a commercial kit in the presence of SYBR Green I (Evrogen, Russia). To carry out RT-PCR, 25 μ L of the reaction mixture of the following composition was used: 5 μ L of 5 $\times$ buffer from the kit, 3 μ L of cDNA, 1 μ L of forward and reverse 10 pM primers, after which the final volume of the reaction mixture was adjusted to 25 μ L with sterile water. Amplification was carried out in a CFX96 thermal cycler (Bio-Rad, USA). A thermal regime with initial denaturation at 94°C for 3 min, then 36 cycles subject to the temperature-time regime: primer annealing at 58°C for 20 sec, elongation - 30 sec at 70°C, denaturation at 95°C – 10 sec., final elongation – 2 min at 70°C.

qRT-PCR analysis of oxidative stress-related genes in the roots and leaves in *H. vulgare*. SODA - superoxide dismutase [Mn], SODB - superoxide dismutase [Fe], CAT1 - catalase-1; CAT2 - catalase-2; GR - glutathione reductase; APX- ascorbate peroxidase. Primers used for RT-PCR (Table 1) were tested for specificity using the Primer3 program and the GenBank database of NCBI.

To calculate quantitative changes in gene transcription, the $2^{-\Delta\Delta C_t}$ method was used, where C_t is the threshold value

Table 1: Primers used for RT-PCR.

Gene	NCBI Gene ID:	Sequence (5'-3') forward primer	Sequence (5'-3') reverse primer
<i>SodA1</i>	123428270	TGGATGGGTGTGGCTAGCTTT	AGTATGCATGCTCCAGACAT
<i>SodB</i>	123412483	TACTACGGCCTCACGACTCC	CGGGTTGCCGTTGTTGTAGG
<i>GR</i>	123447388	GAGCTACGACTACGACCTCTTC	CACGTATCACGCACGTCCC
<i>Cat1</i>	123407150	AACTCCGCCTACTGGAAAAC	ACGTTCAGGTATGCGTTCCC
<i>Cat2</i>	123402406	ACCGCAACGTCGACAACTTC	TTGACGGGGAGCATCAGGTA
<i>Apx1</i>	123448947	GAGGTCTGGCTTTGAGGGAC	TCAGCAGAGTTTGTCACTTGA
β -tubulin	123444643	GGAGGCTGAGAACTGTGACTG	TCAGGGTACTCCTCCCTGATT

of the cycle during which fluorescence is first detected reliably above the threshold level, and 2 is the maximum PCR efficiency coefficient. Beta-tubulin was used as a reference gene. The resulting $\Delta\Delta C_t$ value was used to determine changes in the expression level of the gene of interest.

Plants not treated with zinc oxide served as controls. When assessing the effect of SkQ, the expression level was calculated relative to plants treated with a similar dose of zinc oxide but without the addition of SkQ.

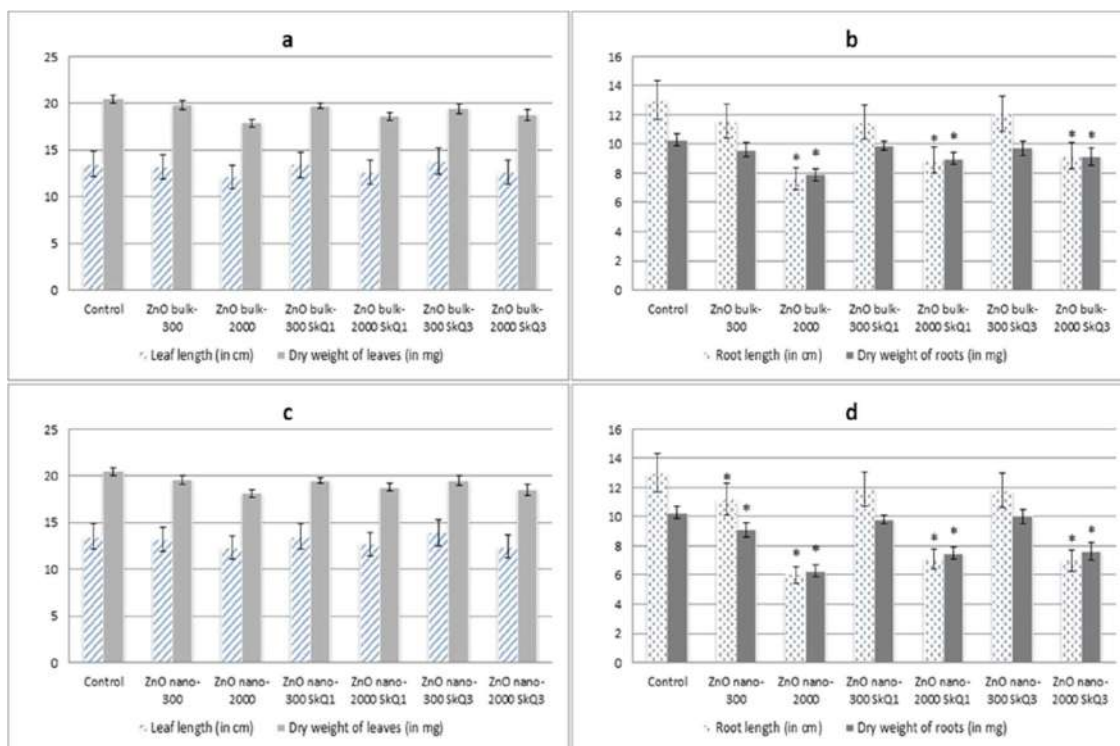
Statistical Validation

All experimental variants were carried out in three independent replicates. Data are presented as arithmetic means and their standard deviations. The reliability of the differences in the data obtained in the experimental and control groups was assessed using Student's t-test, $p < 0.05$. Statistical data processing was carried out using the Statistica 10.0 program. Statistical processing of PCR results was carried out using the statistical program R-Studio. Each PCR was carried out at least 2 times in three replicates for each gene; the nonparametric Mann-Whitney U test was used to assess the significance of differences. Data were considered reliable at $p < 0.05$, where p is an indicator of statistical reliability of the data.

RESULTS AND DISCUSSION

Effect on Plant Morphology

The plants under ZnO NPs exposure and further treatment with SkQ1 and SkQ3 affected the length and dry weight of leaves and roots of 14-day-old barley seedlings. The findings show variations in leaf and root parameters in response to different treatments and NPs dispersions. Across treatments, the leaf length and dry weight of leaves generally remained relatively consistent compared to the control group, with leaf lengths ranging from approximately 12.1 to 13.9 cm and dry weights of leaves ranging from around 17.9 to 20.5 mg. These observations indicate the limited impact on above-ground growth parameters. However, significant changes were observed in root length and dry weight of roots, particularly in seedlings exposed to ZnO NPs with smaller dispersions (2000 nm). In these cases, root lengths ranged from approximately 6.0 to 9.2 cm, and dry weights of roots ranged from approximately 6.3 to 10.3 mg. The addition of ZnO NPs with a concentration of 300 mg.L⁻¹ led to a decrease in root length by 14% and weight by 12%, respectively. Similarly, when the ZnO concentration increases to 2000 mg.L⁻¹, depending on the size of ZnO NPs, the length of



*Differences are significant ($p < 0.05$)

Fig. 2: Effect of treatment with SkQ1 and SKQ3 on the length and dry weight of leaves and roots of 14-day-old barley seedlings under conditions of contamination with zinc oxide particles of different dispersion. Experiments were carried out in three independent replicates.

the roots decreases by 41.5% and 53.8%, and the weight of the roots by 23.3% and 38.8%, respectively. These values represent significant reductions compared to both the control and bulk ZnO treatments, suggesting a detrimental effect of NPs size on root development.

Interestingly, the addition of SkQ1 and SkQ3 appeared to mitigate some of the negative effects of ZnO NPs exposure, as evidenced by fewer reductions in root parameters compared to NP-only treatments. For example, root lengths ranged from approximately 7.0 to 12.1 cm, and dry weights of roots ranged from around 7.5 to 10.0 mg in seedlings treated with SkQ1 and SkQ3. When treating plants grown with SkQ1 and SkQ3 with the addition of bulk ZnO at a concentration of 2000 mg.L⁻¹, an increase in root length is observed by 14.6% and 17.4%, respectively, and dry weight increases by 12.2% and 13.2%, respectively compared to untreated plants. When adding a similar concentration of ZnO NPs was used, an increase of 15.5% and 14.3% in root length and 16.0% and 17.1% in root dry weight was

observed, respectively. Such findings indicate a potential protective role of these compounds against NP-induced stress in barley seedlings. Fig.2 presents the length and dry weight of leaves and roots of barley seedlings with the addition of ZnO in various forms and concentrations, treated with SKQ1 and SKQ1. The findings suggest an increase in growth rate under conditions of high concentrations of ZnO upon treatment with plastoquinone derivatives SkQ1 and SkQ3 may indirectly indicate the restoration of the balance between the formation and elimination of ROS under the action of the antioxidant when exposed to heavy metals.

Our finding is also supported by other studies, which have shown that seed priming with a 2.5nM aqueous solution of SkQ3 significantly enhanced emergence and seedling growth performance, particularly under drought stress conditions (Sadoyan et al. 2021). The study has also demonstrated the mechanism by which SkQ1 improved chloroplast function by exerting a dramatic quenching effect on chlorophyll fluorescence in photosynthetic cells, likely mediated by

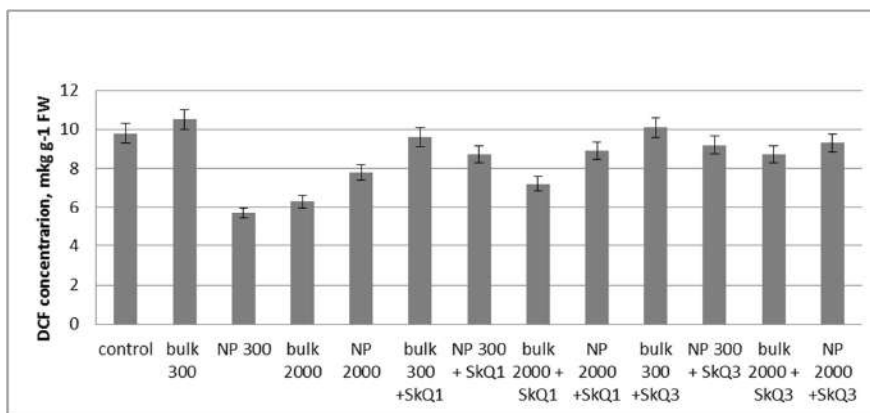


Fig. 3: Total ROS level in the leaves of barley under bulk ZnO and NP ZnO treatments. Different letters on the columns refer to statistically significant differences at $P \leq 0.05$.

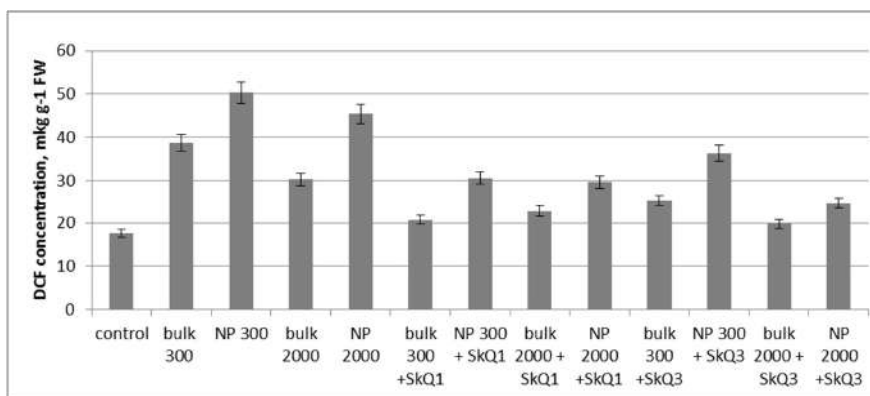


Fig. 4: Total ROS level in the root of barley under bulk ZnO and NP ZnO treatments. Different letters on the columns refer to statistically significant differences at $P \leq 0.05$.

its interaction with the manganese cluster in Photosystem II (PSII) of chloroplasts. This interaction disables the oxygen-evolving complex (OEC), halting water splitting and generating potent quenchers, providing detailed insights into plant physiology and stress response mechanisms (Ptushenko et al. 2019).

Total ROS Level

Figs. 3 and 4 show the total level of ROS in leaf and root cells in the control and under SkQ1 and SKQ3 treatment under bulk ZnO and NP ZnO at concentrations of 300 mg.L⁻¹ and 2000 mg.L⁻¹.

In leaf cells, the total amount of ROS when treated with 300 mg.L⁻¹ ZnO in macroform remained almost unchanged compared to the control. With an increase in the ZnO concentration to 2000 mg.L⁻¹, as well as with treatment with 300 mg.L⁻¹ ZnO nanoparticles, a decrease

in the ROS concentration was recorded. The introduction of SkQ1 and SKQ3 brought all values closer to the control values (Fig. 3). An increase in the total amount of ROS in root cells was recorded when exposed to bulk-ZnO and ZnO NPs at all concentrations studied while at the same dosage, the nanodispersed form had a greater effect than the macrodispersed form of ZnO (Fig. 4). The introduction of SkQ1 and SKQ3 leads to a decrease in the ROS concentration by 2 times compared to the samples not treated with the antioxidant.

Effect on Antioxidant-Related Gene Expression

The levels of relative expression of ROS-related genes in the leaves and roots of barley plants varied heterogeneously. As can be seen in Fig. 1 and Fig. 5, the expression level of all the studied genes at a ZnO concentration of 300 mg.L⁻¹ decreases by 0.5-6 times in the leaves except for the *Cat1* and

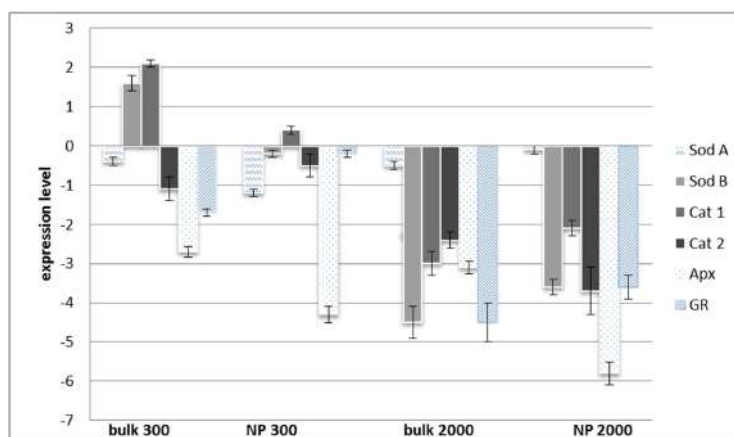


Fig. 5: Effect of bulk-ZnO and ZnO NPs on the level of expression of genes of the antioxidant system of leaf *Hordeum vulgare* L. (relative to control). Experiments were carried out in three independent replicates.

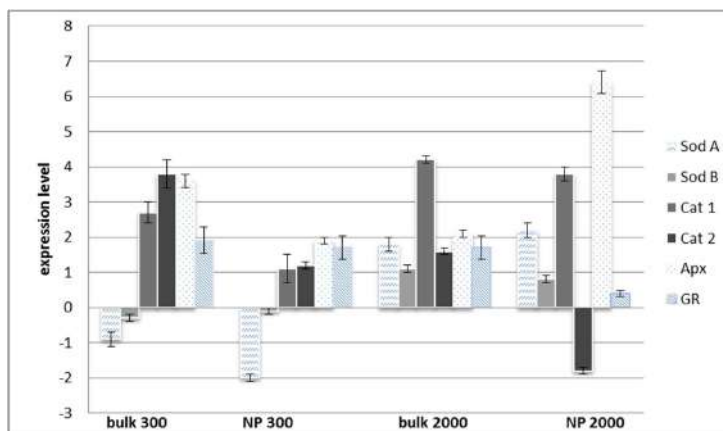


Fig. 6: Effect of bulk-ZnO and ZnO NPs on the level of expression of genes of the antioxidant system of roots *Hordeum vulgare* L. (relative to control). Experiments were carried out in three independent replicates.

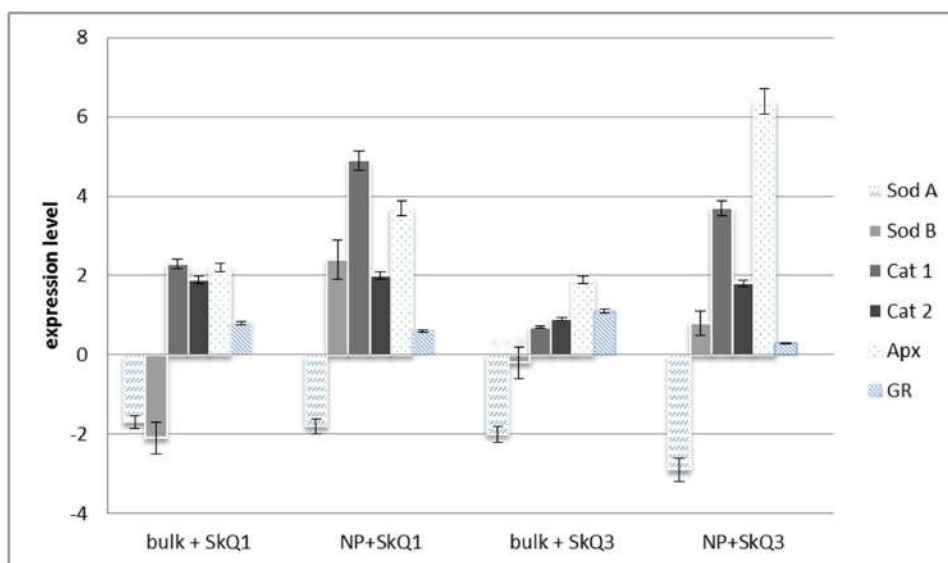


Fig 7: Effect of SkQ1 and SkQ3 on the level of gene expression of the antioxidant system of *Hordeum vulgare* L. leaves when treated with bulk-ZnO (bulk) and ZnO NPs (NP) at a concentration of 300 mg.L⁻¹ (relative to the expression level of plants not treated with SkQ). Experiments were carried out in three independent replicates.

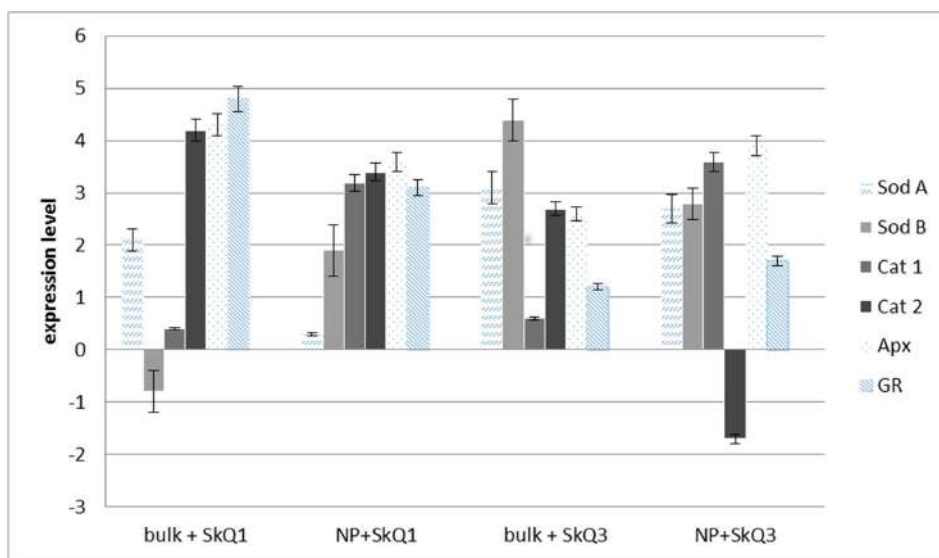


Fig. 8: Effect of SkQ1 and SkQ3 on the level of gene expression of the antioxidant system of *Hordeum vulgare* L. leaves when treated with bulk-ZnO (bulk) and ZnO NPs (NP) at a concentration of 2000 mg.L⁻¹ (relative to the expression level of plants not treated with SkQ). Experiments were carried out in three independent replicates.

Sod B genes. However, in the roots, the expression level of the studied genes increases up to 4 times, except for the *Sod* genes. With increasing ZnO concentration, the transcriptional activity of oxidative stress genes sharply decreases in leaves (up to 6 times) and increases in roots (up to 7 times), with the exception of the *Cat2* gene.

Fig. 6 shows the effect of SkQ1 and SkQ3 on the level of gene expression when exposed to ZnO NPs of different

dispersion at a concentration of 300 mg.L⁻¹ in leaves. As can be seen from Fig.7, the addition of antioxidants SkQ1 and SkQ3 leads to an increase in the transcriptional activity of all studied genes apart from *Sod*. An increase in transcriptional activity leads to an increase in the concentration of enzymes in the antioxidant system and the normalization of ROS levels.

A similar feature is observed in the leaves at a concentration of 2000 mg.L⁻¹ of ZnO. Namely, the addition

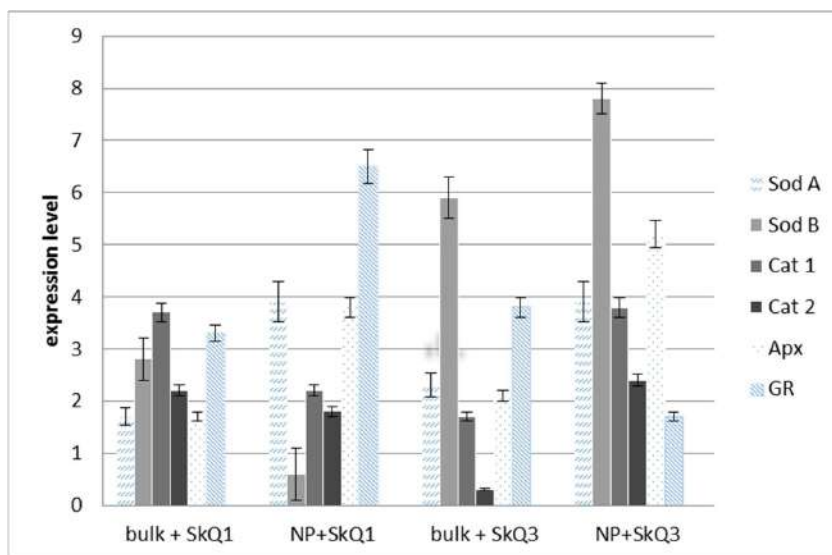


Fig. 9: Effect of SkQ1 and SkQ3 on the level of gene expression of the antioxidant system of *Hordeum vulgare* L. roots when treated with bulk-ZnO (bulk) and ZnO NPs (NP) at a concentration of 300 mg.L⁻¹ (relative to the expression level of plants not treated with SkQ). Experiments were carried out in three independent replicates.

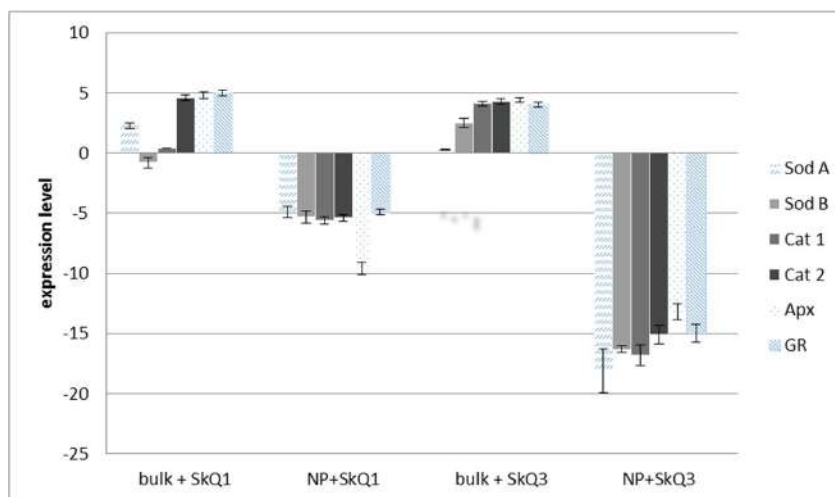


Fig. 10: Effect of SkQ1 and SkQ3 on the level of gene expression of the antioxidant system of *Hordeum vulgare* L. roots when treated with bulk-ZnO and ZnO NPs at a concentration of 2000 mg.L⁻¹ (relative to the expression level of plants not treated with SkQ). Experiments were carried out in three independent replicates.

of SkQ1 and SkQ3 leads to an increase in the transcriptional activity of oxidative stress genes (Fig. 8).

Figs. 9 and 10 show the change in the expression level of the antioxidant-related genes when treated with SkQ1 and SkQ3 under conditions of increasing the concentration of the pollutant to 2000 mg.L⁻¹. The expression level of all studied genes increases up to 8 times when treated with SkQ1 and SkQ3 when treated with ZnO at a concentration of 300 mg.L⁻¹. When the concentration of ZnO increased to 2000 mg.L⁻¹, the expression level of all the studied genes, except Cat 2, in

the leaves increased up to 4 times, and in the roots decreased by 10-18 times. Such a sharp drop in transcriptional activity indicated a significant impact of oxidative stress on the functioning of the body. In a study using SkQ1 treatment on *Arabidopsis thaliana* and *Triticum aestivum*, it was found to extend leaf lifespan, enhance overall plant longevity, and boost resistance against oxidative stress. Moreover, SkQ1 treatments promote tillering in *T. aestivum*, leading to increased crop yield. By targeting mitochondrial function and reducing ROS levels, SkQ1 significantly improves

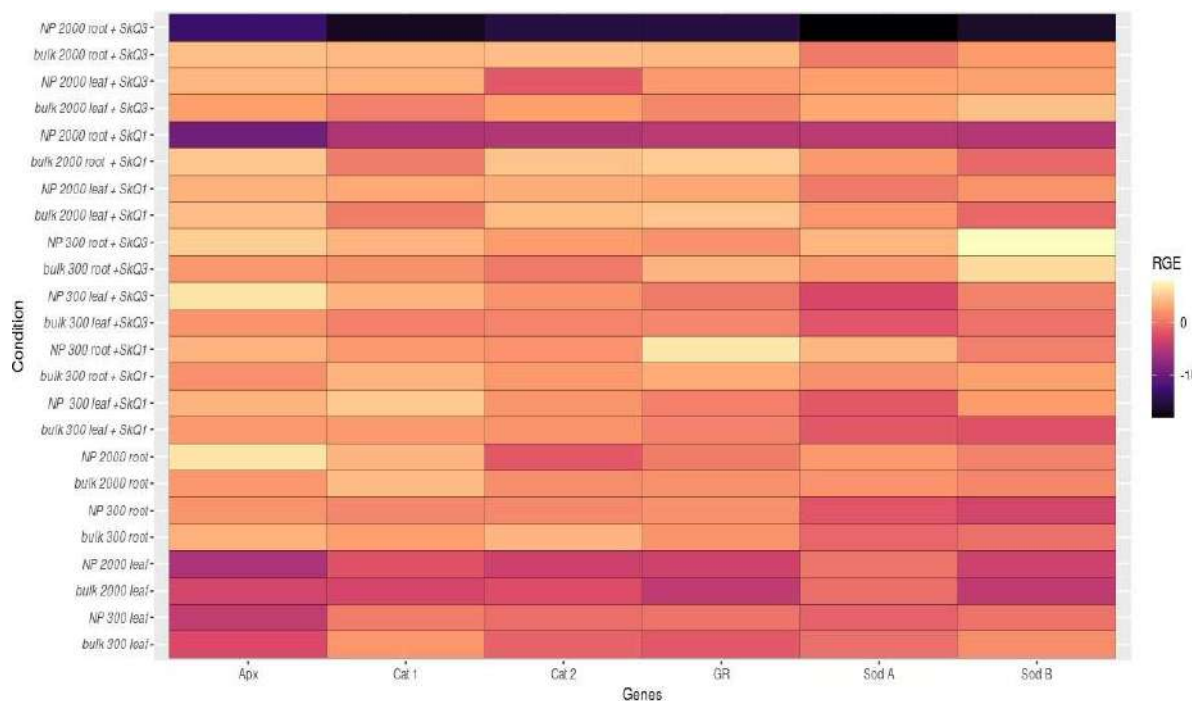


Fig. 11: Heat map of oxidative stress gene expression in leaves and roots of barley seedlings treated with bulk-ZnO and ZnO NPs under the influence of SkQ1 and SkQ3.

plant performance, particularly under stressful conditions (Dzyubinskaya et al. 2013). Our finding also aligns with the study, which has highlighted the antioxidant effect of SkQs in preventing ROS generation in mitochondria, their inhibition of non-cyclic electron transfer in chloroplasts at micromolar concentrations, and their stimulation of morphogeny in plant tissue cultures at nanomolar concentrations. SkQ1 also delayed vegetation periods and increased grain mass in wheat. However, higher concentrations of SkQs may disrupt chloroplast function (Samuilov et al. 2019). In our study, the reason for the heterogeneous changes in transcriptional activity in leaves and roots may be the fact that in the green parts of plants, chloroplasts and peroxisomes play a significant role in the generation of ROS, while the treatment of SkQ1 and SkQ3 specifically penetrated the mitochondrial matrix, and since the main suppliers of ROS in the roots are mitochondria, then the addition of an external antioxidant leads to inhibition of excessive accumulation of ROS and a decrease in the level of expression of genes of the antioxidant system.

It is interesting to note the correlation between changes in the ROS content, transcriptional activity of genes, and plant growth parameters. For better visualization, the dynamics of the expression of antioxidant defense genes are presented as a heat map (Fig. 11).

As shown above, roots respond to zinc oxide to a greater extent than leaves. This response is especially

pronounced when treated with ZnO NPs at a concentration of 2000 mg.L⁻¹. While the addition of SkQ1 and SkQ3 reduces the total ROS content in the roots by 2 times, the transcriptional activity of the antioxidant system genes is significantly reduced, and the length and weight of the roots of plants treated with SkQ1 and SkQ3 increase compared to untreated ones. Thus, all experimental data indicate an increase in plant resistance to stress factors when using these antioxidants.

In this study, Zn²⁺ ions were detected in the solutions after ultracentrifugation (30,000 rpm) and filtration, indicating the solubility of ZnO regardless of its size (bulk or nano). Interestingly, the Zn concentration in the solutions in which plants were grown was higher when bulk ZnO was added, and greater Zn accumulation in barley plants occurred when nano-ZnO was added. These data, as well as the fact that a large increase in zinc was observed in the root but not in the shoot with increasing ZnO concentration, may indicate significant adhesion of ZnO to the root surface, with nano-ZnO clearly having higher adhesion than bulk ZnO (Azarin et al. 2022).

Regarding the ecological aspect of the possible influence of SkQ on agroecosystems, the existing studies on the influence of these compounds on animals in vivo experiments did not reveal any negative effects in nanomolar concentrations. On the contrary, a geroprotective effect was demonstrated for

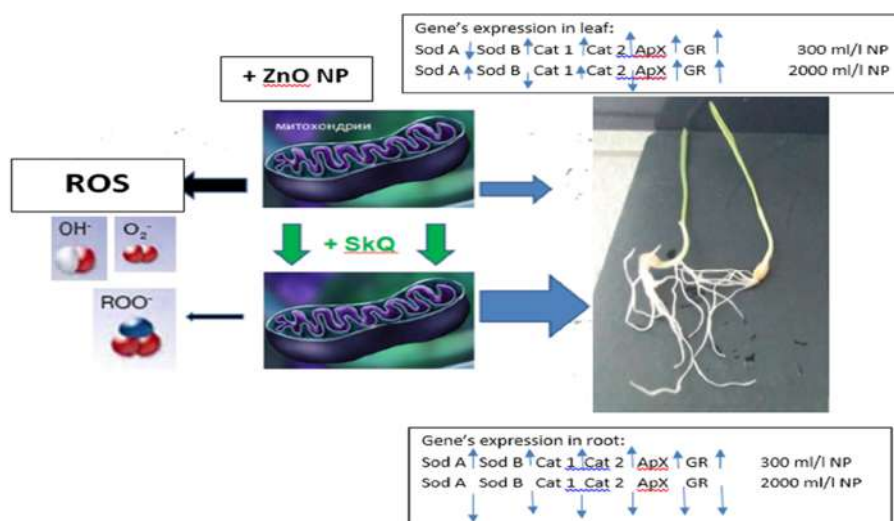


Fig. 12: Scheme of the influence of SkQ on the transcriptional activity of genes of the antioxidant system of barley seedlings.

SkQ, manifested for a wide range of living organisms. An increase in the lifespan of such a diverse range of organisms as the crustacean *Ceriodaphnia*, the insect *Drosophila melanogaster*, the fish *Nothobranchius furzeri*, mammals: mice, mole voles and hamsters was noted (Anisimov et al. 2011). When SkQ derivatives were administered to laboratory animals, it was found that in the experimental group, in contrast to the control group, some physiological signs of aging were not observed or were slightly expressed. Such a wide range of recorded effects indicates a systemic effect of SkQ group compounds (Shipounova et al. 2010). antioxidant properties also have a pronounced antibacterial effect.

In micromolar doses, quinone derivatives based on decyltriphenylphosphonium (SkQ1, SkQ3) can have an antimicrobial and cytotoxic effect, but with a decrease in the concentration of SkQ, such effects are not observed (Nazarov et al. 2024).

CONCLUSIONS

The study investigated the effects of ZnO NPs on barley seedlings, focusing on root and leaf growth as well as the transcriptional activity of antioxidant-related genes. The results showed that ZnO NPs at a concentration of 300 mg.L⁻¹ inhibited root growth and development but did not significantly affect leaf growth. However, the addition of SkQ1 and SkQ3 solutions helped to mitigate the negative effects of ZnO NPs on root growth, demonstrating increased plant resistance to NP-induced stress. Moreover, roots showed a more obvious response to ZnO exposure, showing increased expression levels of antioxidant-related genes, while leaves suffered from decreased transcriptional activity.

In particular, the expression of such genes was significantly reduced in roots treated with SkQ1 and SkQ3 solutions when exposed to higher ZnO concentrations, indirectly indicating a decrease in the production of antioxidant system enzymes. The results of the study highlight the potential of plastoquinone derivatives SkQ1 and SkQ3 in modulating the growth rate and transcriptional activity of oxidative stress genes in barley seedlings exposed to ZnO NPs, suggesting their application in strategies to alleviate ZnO NPs-induced stress in plants.

As a generalization, the scheme of the influence of plastoquinone derivatives on the transcriptional activity of the genes of the antioxidant system of barley seedlings is presented in Fig. 12.

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A Comprehensive Analysis of Land Use Types and Plant Characteristics in Carbon Sequestration in Ngiwngam

Gitsada Panumonwatee^{1†} , Rudklow Premprasit² and Savent Pampasit¹

¹Department of Natural Resources and Environment, Faculty of Agriculture Nature Resources and Environment, Naresuan University, Phitsanulok Province, 65000, Thailand

²Faculty of Social Sciences, Naresuan University, Phitsanulok Province, Thailand, 65000

†Corresponding Author: Gitsada Panumonwatee; gitsadap@nu.ac.th

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ABSTRACT

This study examined land use patterns, tree species diversity, biomass distribution, carbon sequestration, and oxygen release in Ngiwngam Subdistrict, Phitsanulok Province, Thailand. The research encompassed a total area of 6,054.72 hectares, categorized into 20 distinct land use types. Rice paddies dominated the landscape, covering over 97% of the area. A systematic sampling method using fifteen 40x40 meter plots revealed 18 tree species from 13 families, with banana trees being the most prevalent. Biomass estimation showed significant variations across land use types, with an average total biomass of 189.39 t/ha. Eucalyptus plantations exhibited the highest biomass (1,797.50 t.ha⁻¹) and carbon stock (898.75 t/ha). The study quantified carbon sequestration and oxygen release rates, finding a total sequestration of 6,944.14 t/ha across all land use types, with Eucalyptus plantations leading at 3,295.42 t.ha⁻¹. Oxygen release patterns closely mirrored carbon sequestration, totaling 5,050.28 t.ha⁻¹. The research highlights the superior performance of tree-based systems, particularly fast-growing species like Eucalyptus and Bamboo, in carbon sequestration and oxygen production compared to annual crops and grasslands. These findings underscore the potential for strategic land use planning to enhance climate change mitigation efforts and ecosystem services in the region, suggesting that increasing tree cover through various means could significantly boost the landscape's capacity for carbon storage and oxygen generation.

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INTRODUCTION

Anthropogenic climate change, primarily driven by the exponential increase in atmospheric greenhouse gas concentrations, particularly carbon dioxide (CO₂), presents an unprecedented challenge to global ecosystems and human civilization (Intergovernmental Panel on Climate Change (IPCC 2021). The relentless rise in global mean surface temperature has precipitated a cascade of interconnected environmental perturbations, including accelerated sea-level rise, intensification of extreme weather events, and profound disruptions to agricultural systems and biodiversity. In this context of the escalating climate crisis, carbon sequestration has emerged as a critical mitigation strategy, offering a potential mechanism to attenuate the rate of atmospheric CO₂ accumulation.

Carbon sequestration defined as the process of capturing and storing atmospheric carbon dioxide in long-term reservoirs, represents a vital component of the global carbon cycle management (Lal 2008). Its significance in climate change mitigation is paramount; by facilitating the removal of CO₂ from the atmosphere and its subsequent storage in terrestrial, oceanic, or geological sinks, carbon sequestration provides a means to potentially decelerate or even reverse the accumulation of greenhouse gases, thereby mitigating the intensity of global warming effects.



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Land use dynamics play a pivotal role in modulating carbon sequestration processes. The diverse mosaic of terrestrial ecosystems - encompassing forests, grasslands, agricultural lands, wetlands, and urban areas - exhibits significant heterogeneity in carbon sequestration capacity (Pan et al. 2011). Sharma et al. (2023) reported that converting natural ecosystems (forests, grasslands) to agricultural land typically results in SOC loss, and different ecosystems have varying capacities for carbon storage, which is important for determining sequestration potential. The anthropogenic manipulation of land cover and land use directly influences the global carbon cycle, simultaneously affecting both carbon emissions and sequestration rates. This intricate interplay between land use and carbon dynamics underscores the necessity for nuanced understanding and strategic management of terrestrial ecosystems in the context of climate change mitigation. Moreover, carbon sequestration increases with increasing topographic gradient. This suggests that higher elevation areas or steeper slopes may have greater potential for carbon storage (He et al. 2023).

The impact of land use changes on carbon sequestration is multifaceted and often non-linear. For instance, the conversion of primary forests to agricultural land frequently results in substantial carbon emissions, depleting both above-ground biomass and soil organic carbon pools (Don et al. 2011). Conversely, reforestation initiatives or the implementation of sustainable agricultural practices, such as conservation tillage or agroforestry, can enhance terrestrial carbon sinks (Smith et al. 2008). Urban development, while traditionally associated with increased carbon emissions, can potentially contribute to sequestration through judicious urban planning, incorporation of green infrastructure, and utilization of carbon-sequestering construction materials (Churkina et al. 2020).

At the organismal level, carbon sequestration efficiency exhibits considerable variation across plant species and functional types. Arboreal species, characterized by substantial biomass accumulation and extended lifespans, are generally regarded as highly effective carbon sinks. A mature tree can sequester several tons of CO₂ over its lifetime, with sequestration rates varying based on species, age, and environmental conditions (Nowak & Crane 2002). However, the sequestration potential of terrestrial ecosystems extends beyond arboreal components. Grasslands, for example, allocate a significant proportion of their carbon belowground, sequestering it in extensive root systems and recalcitrant soil organic matter fractions (Conant et al. 2017). Similarly, certain crops and management practices can contribute substantially to soil carbon sequestration, enhancing both climate mitigation potential and soil health (Paustian et al. 2016).

The efficacy of plants in carbon sequestering is contingent upon a complex interplay of factors, including growing rate, lifespan, and the fate of the captured carbon post-senescence. While rapid-growing species may exhibit high short-term carbon uptake rates, the long-term sequestration benefit can be limited if the captured carbon is swiftly re-released into the atmosphere through decomposition or combustion processes (Körner 2017). Therefore, a comprehensive understanding of the carbon sequestration potential across diverse plant types and ecosystems is imperative for developing effective and sustainable land use strategies to combat climate change.

Ngiwngam Subdistrict is an exemplary model of agricultural landscapes in central Thailand, making it an ideal site for studying land use patterns and their environmental impacts. Although predominantly characterized by rice paddies, the subdistrict features diverse land use types, including tree plantations, orchards, and natural vegetation, facilitating comparative analyses of carbon sequestration and oxygen release. Located in a transitional zone between lowland agriculture and upland forests, Ngiwngam offers insights into landscape-level carbon management strategies. The region's significance as an agricultural hub underscores the need to balance food production with ecosystem services and climate change mitigation. Given the limited data on land use patterns and environmental impacts in Ngiwngam, this study is crucial for local and regional planning. Additionally, like many agricultural areas in Southeast Asia, Ngiwngam is vulnerable to climate change, making it essential to understand its carbon dynamics for developing adaptive strategies. The subdistrict also presents opportunities for optimizing land use to enhance carbon sequestration while maintaining agricultural productivity, positioning it as a vital case study for sustainable landscape management.

The researchers are particularly interested in studying the carbon sequestration potential of Ngiwngam Subdistrict, Mueang Phitsanulok District, Phitsanulok Province, specifically within the areas under local administrative organizations that are members of the Local Resource Base initiative. This study aims to enhance knowledge and gain insights that complement the researchers' academic field of study. The data obtained will elucidate the carbon sequestration capacity of the area. Furthermore, this information will provide governmental agencies with a robust foundation for policy-level decision-making at the provincial scale, particularly in developing strategies for promoting plant cultivation. Such strategies would enable local farmers to optimize land use while simultaneously conserving resources and sustainably maintaining forest ecosystems.

MATERIALS AND METHODS

Study Area

The research was conducted in Ngiwngam Subdistrict, Mueang District, Phitsanulok Province, encompassing a total land area of 6,054.72 hectares. This region falls under the purview of the Plant Genetic Conservation Project initiated under the royal patronage of Her Royal Highness Princess Maha Chakri Sirindhorn. The primary objective of this study was to quantify carbon sequestration in above-ground biomass, root systems, and understory vegetation within the administrative boundaries of the local government organization in Ngiwngam.

The selection of this study area is significant due to its participation in a royal conservation initiative, which provides a unique context for examining carbon sequestration in a managed landscape. The research aims to contribute to the understanding of carbon dynamics in diverse land use types within a local administrative unit, potentially informing both conservation strategies and climate change mitigation policies at the sub-district level.

By focusing on multiple components of the ecosystem (above-ground biomass, root systems, and understory vegetation), this study adopts a comprehensive approach to assessing carbon stocks. This multifaceted examination is crucial for accurately estimating the total carbon sequestration potential of the area and understanding the distribution of carbon across different land use land cover (LULC). The findings from this localized study have the potential to provide valuable insights into carbon sequestration patterns in similar ecological and administrative contexts, thereby contributing to the broader body of knowledge on terrestrial carbon dynamics in Thailand. The LULC data was obtained from the Land Development Department (LDD) of Thailand in 2023.

Field Sampling

Field sampling was surveyed in May 2023. The study employed a systematic sampling approach to assess carbon sequestration across the study area, following established protocols (Pearson 2007). The following methods were implemented:

- 1) Tree sampling: Fifteen 40×40 meter quadrats were randomly established across the study area. Within each quadrat, all tree species were identified and measured. For each tree, the diameter at breast height (DBH, measured at 1.3 meters above ground level), total height, and crown diameter were recorded (MacDicken 1997).

- 2) Rice and Litter Sampling: Sixty 1×1 meter subplots were randomly placed within the larger quadrats to sample understory vegetation and surface litter, following the methodology outlined by Ravindranath & Ostwald (2008). These samples were collected to estimate carbon storage in understory biomass and forest floor litter.

- 3) Biomass Processing

Understory vegetation samples were oven-dried at 80°C for 48 hours or until a constant weight was achieved, as per standard protocols (Nelson & Sommers 1996). The dry weight of the biomass (DW) was determined by biomass quantity.

Data Analysis

Tree biomass estimation: Biomass components, including stem (WS), branch (WB), and leaf (WL) biomass, were estimated using species-specific allometric equations (Table 1), which related to land use type, following the approach recommended by Chave et al. (2014). Aboveground biomass (AGB) was calculated as the sum of these components ($AGB = WS + WB + WL$). Belowground biomass (RB), primarily comprising root biomass, was estimated using the widely accepted equation developed by Cairns et al. (1997): $RB = \exp(-1.0587 + 0.8836 \times \ln(AGB))$.

This comprehensive approach to biomass estimation allows for a more accurate assessment of carbon stocks across different tree components (Picard et al. 2012), providing a robust foundation for ecosystem carbon accounting. The study encompassed a diverse array of land use types, focusing on various agroforestry systems and plantations. The selected sites included orchards of tropical fruit trees such as Longan (*Dimocarpus longan*), Guava (*Psidium guajava*), Rose Apple (*Syzygium jambos*), Rambutan (*Nephelium lappaceum*), Tamarind (*Tamarindus indica*), Jackfruit (*Artocarpus heterophyllus*), Mango (*Mangifera indica*), and Santol (*Sandoricum koetjape*). Additionally, the research incorporated plantations of economically significant species like Bamboo (*Bambusa vulgaris*), Yang-na (*Dipterocarpus alatus*), Eucalyptus (*Eucalyptus* spp.), Rubber (*Hevea brasiliensis*), and Teak (*Tectona grandis*).

The study also included gardens of Lemon (*Citrus limon*), Neem (*Azadirachta indica*), Banana (*Musa* spp.), Papaya (*Carica papaya*), and Coconut (*Cocos nucifera*). This diverse selection of land use types provides a comprehensive representation of common agroforestry practices and plantation systems in the region, allowing for a thorough assessment of carbon sequestration potential across various vegetation structures (Roshetko et al. 2007, Nair et al. 2009).

Table 1: The allometric equations for each tree.

Landuse	Species	Stem (WS)	Branch (WB)	Leaf (WL)	Reference
Longan garden	<i>Dimocarpus longan</i>	0.0396	0.006003	[(28/ WS+WB)	Ogawa et al. (1965)
Guava garden	<i>Psidium guajava</i>	(DBH ² h) ^{0.9326}	(DBH ² h) ^{1.027}	+0.025] ⁻¹	
Rambutan garden	<i>Nephelium lappaceum</i>				
Tamarind orchard	<i>Tamarindus indica</i>				
Mango orchard	<i>Mangifera indica</i>				
Rubber plantation	<i>Hevea brasiliensis</i>				
Teak	<i>Tectona grandis</i>				
Rose apple garden	<i>Syzygium jambos</i>				
bamboo garden	<i>Bambusa vulgaris</i>	AGB = 0.2219DBH ^{2.2749}			Suwannapinunt et al. (1983)
Jackfruit orchard	<i>Artocarpus heterophyllus</i>	0.2903(DBH ² h)0.9815	0.11920WS ^{1.059}	0.09146 (WS+WB) ^{0.7266}	Zheng et al. (2008)
Lemon garden	<i>Citrus limon</i>				
Santol Garden	<i>Sandoricum koetjape</i>	0.0439 (DBH ² h) ^{0.8666}	0.0307 (DBH ² h) ^{0.8434}	0.0056 (DBH ² h) ^{0.9568}	Ogawa et al. (1965)
Yang-na	<i>Dipterocarpus alatus</i>	0.0509 (DBH ² h) ^{0.919}	0.00893 (DBH ² h) ^{0.977}	0.0140 (DBH ² h) ^{0.669}	Ogawa et al. (1965)
Eucalyptus garden	<i>Eucalyptus</i> spp.	0.0305 (DBH ² h) ^{0.9862}	0.0008 (DBH ² h) ^{1.2698}	0.0003 (DBH ² h) ^{1.1666}	Treepatanasuwan et al. (2008)
Neem Garden	<i>Azadirachta indica</i>	0.0410 (DBH ² h) ^{0.9148}	0.0018 (DBH ² h) ^{1.1037}	0.0023 (DBH ² h) ^{0.9388}	Viriyabuncha et al. (2004)
Teak plantation	<i>Tectona grandis</i>	0.0439 (DBH ² h) ^{0.8666}	0.0307 (DBH ² h) ^{0.8434}	0.0056 (DBH ² h) ^{0.9568}	Treepatanasuwan et al. (2008)
banana plantation	<i>Musa</i> spp.	AGB = 0.0303 (DBH) ^{2.1345}			Arifin (2001)
Papaya Garden	<i>Carica papaya</i>	AGB =10(-1.625+2.63log (DBH))			Arifin (2001)
Coconut Garden	<i>Cocos nucifera</i>	AGB = 0.666 + 12.82 (h) 0.5(ln h) ⁻¹			Pearson et al. (2007)

Carbon Stock Calculation

Carbon stocks for various biomass components were calculated using the following equations: Carbon stocks = Biomass × CF, Where CF is the carbon fraction of dry matter, assumed to be 0.47 IPCC (2006) guidelines.

Carbon Sequestration and Oxygen Release Estimation

CO₂ sequestration and Oxygen release were calculated using the molecular weight ratio of CO₂ to C (44/12) and O₂ to C (32/12), as described by IPCC (2003): CO₂ sequestration (t c.ha⁻¹) = Carbon stock (tc.ha⁻¹) × 44/12 and O₂ release (t c.ha⁻¹) = Carbon stock (t c.ha⁻¹) × 32/12

RESULTS

Land Use Land Cover

The land use patterns in Ngiwngam have been comprehensively analyzed. The subdistrict is characterized by a lowland area with structured land consolidation. The majority of the land is dedicated to agricultural purposes, benefiting from

river systems and irrigation canals that facilitate year-round agricultural activities. The total land area of the subdistrict encompasses 6,054.72 hectares. A detailed land use classification revealed 20 distinct categories, showcasing the diversity of agricultural and vegetation types in the region. These categories, listed in descending order of area coverage, are shown in Fig. 1.

This detailed land use classification provides valuable insights into the agricultural landscape and vegetation cover of the Ngiwngam Subdistrict. The predominance of rice paddies, accounting for over 97% of the total area, underscores the significance of rice cultivation in the local agricultural economy. The presence of diverse fruit orchards, plantations, and mixed cropping systems reflects the adaptation of agricultural practices to local environmental conditions and market demands. Such comprehensive land use data is crucial for informed decision-making in land management, agricultural policy formulation, and sustainable development planning at the subdistrict level (Lambin et al. 2001, Turner et al. 2007).

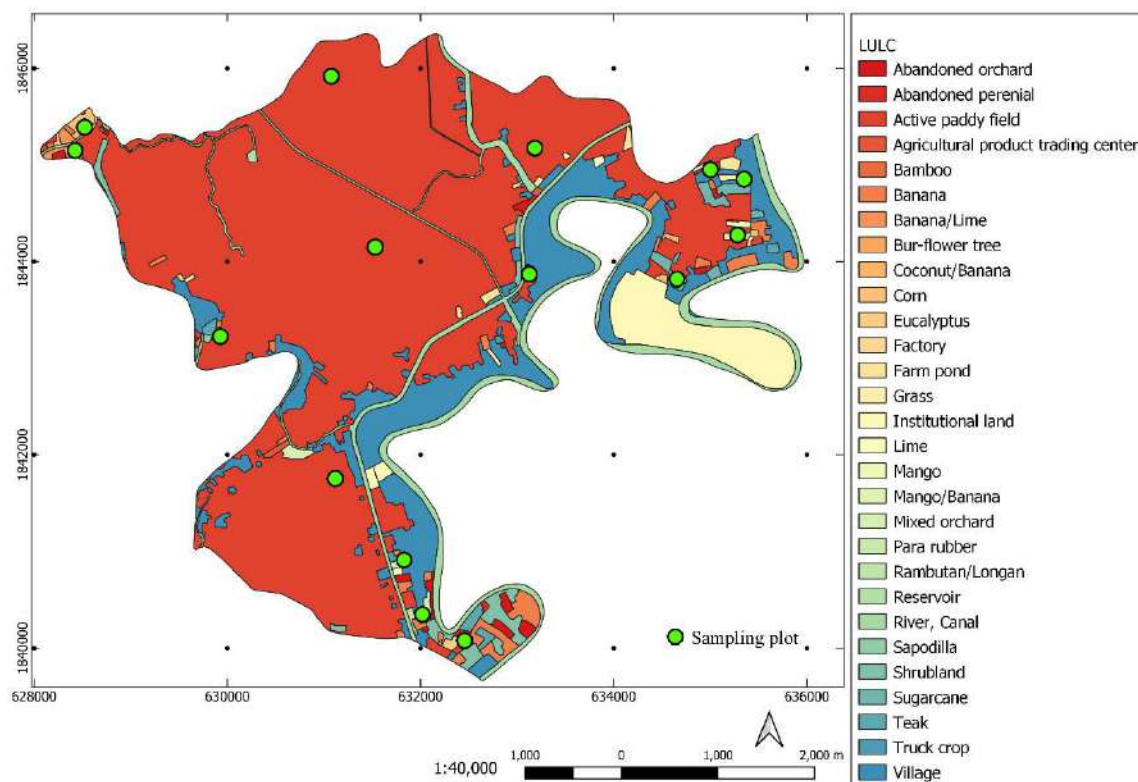


Fig. 1: Land use land cover of Ngiwngam.

Table 2: Tree species in Ngiwngam.

Id	Plant	Tree (individual)	Scientific name	Family name
1	Banana	2,091	<i>Musa spp.</i>	Musaceae
2	Mango	2,73	<i>Mangifera indica</i>	Anacardiaceae
3	Teak	240	<i>Tectona grandis</i>	Verbenaceae
4	Neem	176	<i>Azadirachta indica</i>	Meliaceae
5	Bamboo	152	<i>Bambusa Hulda</i>	Poaceae
6	Rubber tree	152	<i>Hevea brasiliensis</i>	Euphorbiaceae
7	Lime	131	<i>Citrus aurantifolia</i>	Rutaceae
8	Eucalyptus	109	<i>Eucalyptus citriodora</i>	Myrtaceae
9	Rambutan	68	<i>Nephelium lappaceum</i>	Sapindaceae
10	Papaya	64	<i>Carica papaya</i>	Caricaceae
11	Jackfruit	48	<i>Artocarpus heterophyllus</i>	Moraceae
12	Longan	44	<i>Mangifera caesia</i>	Anacardiaceae
13	Tamarind	44	<i>Tamarindus indica</i>	Fabaceae
14	Coconut	36	<i>Cocos nucifera</i>	Palmae
15	Santol	24	<i>Sandoricum koetjape</i>	Meliaceae
16	Yangna	16	<i>Dipterocarpus alatus</i>	Dipterocarpaceae
17	Rose apple	12	<i>Syzygium samarangense</i>	Myrtaceae
18	Guava	8	<i>Psidium guajava</i>	Myrtaceae

Tree Diversity

The study of tree species diversity in Ngiwngam was conducted using a systematic sampling method. Fifteen plots, each measuring 40x40 meters, were established across the study area. The survey revealed a total of 18 tree species belonging to 13 families, with an aggregate count of 3,701 individual trees. Analysis of species abundance indicated a clear dominance of certain species within the study area. The five most prevalent tree species were Banana, Mango, Teak, Neem, and Bamboo. All of the tree species data is shown in Table 2.

Notably, banana trees constituted the majority of the surveyed population, accounting for approximately 56.5% of the total tree count. This significant predominance of banana trees suggests a strong agricultural influence on the local vegetation structure, likely due to its economic importance in the region. The presence of commercially valuable species such as mango, teak, and bamboo among the top five most abundant trees further underscores the interplay between natural vegetation and agricultural practices in shaping the arboreal landscape of Ngiwngam Subdistrict. These findings provide valuable insights into the current

composition and distribution of tree species in the study area, offering a foundation for further ecological analyses and informed decision-making in local forest management and conservation efforts.

Biomass Estimation

Table 3 investigates biomass quantity and its relationship to land use patterns in Ngiwngam has yielded significant results. The study estimated an average total biomass of 189.39 t.ha⁻¹ across the area. Among the various land use categories, five stood out for their exceptionally high biomass quantities: Eucalyptus plantations led with 1,797.50 t.ha⁻¹, followed by Bamboo (466.78 t.ha⁻¹), Abandoned perennial trees (452.77 t.ha⁻¹), Teak plantations (235.59 t.ha⁻¹), and Rambutan/Longan orchards (193.01 t.ha⁻¹). These land use types also demonstrated correspondingly high carbon stock values of 898.75, 233.39, 226.39, 117.80, and 96.50 t.ha⁻¹, respectively. In contrast, the study identified five land use categories with notably low biomass quantities: Vegetable crops and Rice paddies (both at 4.44 t.ha⁻¹), Coconut/Banana plantations (3.25 t.ha⁻¹), Natural grasslands (2.88 t.ha⁻¹), and Cornfields (2.63 t.ha⁻¹). These findings underscore

Table 3: The biomass and carbon stock in the study area.

Id	LULC	Accumulation biomass [t]	biomass [t.ha ⁻¹]	Carbon stock [t.ha ⁻¹]
1	Eucalyptus	3,163.61	1,797.50	898.75
2	Bamboo	149.37	466.78	233.39
3	Abandoned perennial	796.88	452.77	226.39
4	Teak	1,960.14	235.59	117.80
5	Rambutan/Longan	247.05	193.01	96.50
6	Abandoned orchard	3,300.38	182.54	91.27
7	Shrubland	3,809.90	126.66	63.33
8	Mixed orchard	456.94	73.23	36.61
9	Coconut/Banana	388.71	59.25	29.63
10	Para rubber	46.21	57.77	28.88
11	Mango/Banana	82.69	43.07	21.53
12	Banana	1,408.21	29.63	14.82
13	Banana/lime	154.64	29.29	14.64
14	Mango	49.58	22.13	11.07
15	Lime	5.36	8.38	4.19
16	Rice paddy field	20,443.68	3.47	1.74
17	Sugarcane	62.35	3.12	1.56
18	Truck crop	2.57	1.61	0.80
19	Grass	1.65	1.03	0.51
20	Corn	6.75	0.88	0.44
Total		36,536.68	3,787.71	1,893.86
Average		1,826.83	189.39	94.69

the substantial variability in biomass accumulation across different land use types in the study area.

The total accumulation biomass for the entire study area was estimated at 36,536.68 t. The five land use categories contributing the most to the total biomass were Rice paddy fields 20,443.68 t, Grasslands interspersed with shrubs and thickets 3,809.90 t, Abandoned orchards 3,300.38 t, Eucalyptus 3,163.61 t, and Teak plantations 1,636.50 t

These results provide a detailed assessment of biomass distribution across various land use types in the study area, demonstrating significant variability in biomass accumulation. The data highlights the importance of perennial vegetation, particularly abandoned or degraded trees, and teak plantations, which contribute substantially to biomass stocks. This contrasts with agricultural lands such as vegetable crops and rice paddies, which, despite their extensive coverage, contribute relatively little to total biomass. The findings underscore the potential of certain land use practices to enhance carbon sequestration and emphasize the need for sustainable management strategies in agricultural and natural landscapes. The substantial biomass in abandoned or degraded perennial trees and fruit trees suggests an opportunity for land restoration efforts to further enhance carbon storage.

Carbon Sequestration and Oxygen Release Estimation

Our study examined carbon sequestration and oxygen release rates across 20 different land use and land cover (LULC) types in the study area (Table 4). The results reveal significant variations in these ecosystem services among different land uses, with implications for climate change mitigation and local air quality. The total carbon sequestration across all LULC types was calculated at 6,944.14 tonnes per hectare ($\text{t}\cdot\text{ha}^{-1}$), with an average of $347.21 \text{ t}\cdot\text{ha}^{-1}$. Notably, there was a wide range in sequestration rates, spanning from $1.61 \text{ t}\cdot\text{ha}^{-1}$ to $3,295.42 \text{ t}\cdot\text{ha}^{-1}$. The top five LULC types for carbon sequestration were Eucalyptus plantations ($3,295.42 \text{ t/ha}$), Bamboo stands ($855.76 \text{ t}\cdot\text{ha}^{-1}$), Abandoned perennial vegetation ($830.08 \text{ t}\cdot\text{ha}^{-1}$), Teak plantations ($431.92 \text{ t}\cdot\text{ha}^{-1}$), and Rambutan/Longan orchards ($353.85 \text{ t}\cdot\text{ha}^{-1}$)

Eucalyptus plantations demonstrated an exceptionally high carbon sequestration rate, more than three times that of the next highest category. This underscores the potential of fast-growing tree species for rapid carbon capture. Conversely, the lowest carbon sequestration rates were observed in: Rice paddy fields ($6.36 \text{ t}\cdot\text{ha}^{-1}$), Sugarcane fields ($5.72 \text{ t}\cdot\text{ha}^{-1}$), Truck crops ($2.95 \text{ t}\cdot\text{ha}^{-1}$), Grass ($1.89 \text{ t}\cdot\text{ha}^{-1}$), and Cornfields ($1.61 \text{ t}\cdot\text{ha}^{-1}$). These results highlight the

Table 4: Carbon sequestration and oxygen release estimation in the study area.

Id	LULC	Carbon sequestration [$\text{t}\cdot\text{ha}^{-1}$]	Oxygen release [$\text{t}\cdot\text{ha}^{-1}$]
1	Eucalyptus	3,295.42	2,396.67
2	Bamboo	855.76	622.37
3	Abandoned perennial	830.08	603.70
4	Teak	431.92	314.12
5	Rambutan/Longan	353.85	257.34
6	Abandoned orchard	334.66	243.39
7	Shrubland	232.21	168.88
8	Mixed orchard	134.25	97.64
9	Coconut/Banana	108.63	79.01
10	Para rubber	105.91	77.02
11	Mango/Banana	78.96	57.42
12	Banana	54.33	39.51
13	Banana/lime	53.70	39.05
14	Mango	40.58	29.51
15	Lime	15.36	11.17
16	Rice paddy field	6.36	4.63
17	Sugarcane	5.72	4.16
18	Truck crop	2.95	2.14
19	Grass	1.89	1.37
20	Corn	1.61	1.17
Total		6,944.14	5,050.28
Average		347.21	252.51

relatively low carbon sequestration potential of annual crops and grasslands compared to perennial woody vegetation.

The study also quantified oxygen release, which closely mirrored carbon sequestration patterns. Total oxygen release across all LULC types was $5,050.28 \text{ t.ha}^{-1}$, with an average of 252.51 t.ha^{-1} . The top five oxygen-releasing LULC types were estimated to have the same carbon sequestration as Eucalyptus plantations ($2,396.67 \text{ t.ha}^{-1}$), Bamboo stands (622.37 t.ha^{-1}), Abandoned perennial vegetation (603.70 t.ha^{-1}), Teak plantations (314.12 t.ha^{-1}), and Rambutan/Longan orchards (257.34 t.ha^{-1}). There is a strong positive correlation between carbon sequestration and oxygen release across all LULC types. This relationship is expected due to the stoichiometric balance in photosynthesis, where CO_2 uptake is directly linked to O_2 production.

The results demonstrate that tree-based systems, particularly fast-growing species like Eucalyptus and Bamboo, offer the highest potential for both carbon sequestration and oxygen release. Abandoned perennial vegetation also shows high rates, suggesting that allowing natural regeneration of woody vegetation can significantly contribute to these ecosystem services.

In contrast, annual cropping systems and grasslands show much lower rates of both carbon sequestration and oxygen release. This highlights a potential trade-off between agricultural production and climate regulation services. The intermediate values observed in mixed orchards and plantation crops like rubber and banana indicate that agroforestry systems might offer a balanced approach, providing both ecosystem services and agricultural products.

These findings have important implications for land use planning and climate change mitigation strategies in the region. They suggest that increasing the area under tree cover, whether through commercial plantations, agroforestry, or natural regeneration, could significantly enhance the landscape's capacity for carbon sequestration and oxygen production.

DISCUSSION

Our findings on carbon sequestration and biomass distribution across different land use types offer significant insights for land use planning strategies. For instance, the superior performance of Eucalyptus plantations in carbon sequestration ($3,295.42 \text{ t.ha}^{-1}$) compared to other land use types suggests that strategic incorporation of fast-growing tree species into the landscape could substantially enhance the region's carbon sink capacity. The comprehensive analysis of land use patterns, tree species diversity, biomass estimation, and ecosystem services in Ngiwngam provides valuable

insights into the complex interplay between agricultural practices, vegetation dynamics, and environmental services. This discussion will explore the implications of our findings, contextualize them within the broader scientific literature, and consider their significance for sustainable land management and climate change mitigation strategies.

The predominance of rice paddies, covering over 97% of the total area, underscores the critical role of rice cultivation in the local agricultural economy. This monoculture dominance is consistent with the broader agricultural landscape of Thailand, where rice is a staple crop and a significant export commodity (Sirisupluxana et al. 2017). However, the extensive coverage of a single crop type raises concerns about biodiversity, ecosystem resilience, and long-term sustainability. The diversity of other land use types, including various fruit orchards and plantations, suggests attempts at agricultural diversification. This aligns with global trends towards more diverse agricultural landscapes, which can enhance ecosystem services and economic resilience (Kremen & Miles 2012). The presence of mixed cropping systems, albeit in smaller proportions, indicates potential pathways for increasing agricultural diversity and sustainability in the region.

The survey of tree species diversity revealed a total of 18 species from 13 families, with a clear dominance of banana trees. This finding reflects the strong agricultural influence on the local vegetation structure. The prevalence of commercially valuable species such as mango, teak, and bamboo among the most abundant trees further emphasizes the interconnection between economic priorities and vegetation composition. The dominance of banana trees (56.5% of the total count) is particularly noteworthy. While bananas are an important crop with significant economic value, their prevalence raises questions about the overall ecological balance and biodiversity of the area. A high abundance of a single species can increase vulnerability to pests and diseases, potentially threatening both agricultural productivity and ecosystem stability (Altieri et al. 2015). The presence of teak and neem among the top five most abundant species is encouraging from both economic and ecological perspectives. Teak is a valuable timber species with high carbon sequestration potential (Kenzo et al. 2014), while neem is known for its multiple uses in agriculture and medicine, as well as its ecological benefits (Lokanadhan et al. 2012). The inclusion of these species in the landscape suggests opportunities for agroforestry systems that could enhance both economic returns and ecosystem services.

The biomass estimation results reveal significant variability across different land use types, with implications

for carbon sequestration and climate change mitigation strategies. The exceptionally high biomass quantities observed in Eucalyptus plantations ($1,797.50 \text{ t.ha}^{-1}$) align with previous studies highlighting the rapid growth and high biomass accumulation potential of Eucalyptus species (Forrester et al. 2010). However, it is crucial to consider the ecological implications of large-scale Eucalyptus plantations, including potential impacts on water resources and biodiversity (Ferraz et al. 2013). The high biomass quantities in bamboo stands (466.78 t.ha^{-1}) and abandoned perennial vegetation (452.77 t.ha^{-1}) underscore the carbon sequestration potential of these land use types. Bamboo has gained attention as a fast-growing, renewable resource with significant carbon sequestration capabilities (Song et al. 2011). The high biomass in abandoned perennial vegetation suggests that natural regeneration processes can contribute substantially to carbon storage, highlighting the potential benefits of allowing some agricultural lands to revert to natural vegetation (Chazdon et al. 2016). The low biomass quantities observed in annual crops such as rice paddies, vegetable crops, and corn fields (ranging from 2.63 to 4.44 t.ha^{-1}) highlight a critical challenge in balancing food production with climate change mitigation objectives. These findings align with global patterns where annual cropping systems generally have lower biomass and carbon sequestration potential compared to perennial vegetation (Lal 2004).

The analysis of carbon sequestration and oxygen release rates across different land use types provides crucial insights into the climate regulation services of the landscape. The wide range in sequestration rates (1.61 to $3,295.42 \text{ t.ha}^{-1}$) emphasizes the significant impact that land use decisions can have on the carbon balance of the region.

The exceptional performance of Eucalyptus plantations in both carbon sequestration ($3,295.42 \text{ t.ha}^{-1}$) and oxygen release ($2,396.67 \text{ t.ha}^{-1}$) aligns with their high biomass accumulation. This finding supports the potential role of fast-growing tree plantations in rapid carbon capture strategies (Paquette & Messier 2010). However, it is essential to balance these benefits against potential ecological drawbacks and consider the long-term sustainability of such plantations.

The high sequestration rates observed in bamboo stands, abandoned perennial vegetation, and teak plantations offer promising avenues for enhancing the carbon sink capacity of the landscape. These findings support the argument for integrating more perennial vegetation and agroforestry systems into agricultural landscapes as a strategy for climate change mitigation (Jose & Bardhan 2012). The low carbon sequestration and oxygen release rates in annual cropping systems highlight a significant challenge in reconciling food production with climate regulation services. This trade-

off is a central issue in sustainable land management and requires innovative approaches to increase the carbon storage potential of agricultural lands without compromising food security (Smith et al. 2013).

In Ngiwngam, the average total biomass is 189.39 t.ha^{-1} , with Eucalyptus plantations showing the highest biomass at $1,797.50 \text{ t.ha}^{-1}$. This level of biomass is comparable to findings from other regions in Southeast Asia. For instance, a study conducted in mixed agricultural landscapes in central Vietnam reported an average biomass of 172.5 t.ha^{-1} (Chazdon & Guariguata 2016), indicating a similar capacity for biomass accumulation in these agricultural systems. However, the Eucalyptus plantations in Ngiwngam demonstrate a notably higher biomass than the average of $1,250 \text{ t.ha}^{-1}$ reported for similar plantations in Sumatra (Forrester et al. 2010). This disparity may be attributed to several factors, including differences in plantation age, management practices, soil fertility, and local climatic conditions.

In terms of carbon sequestration, Ngiwngam's total carbon sequestration rate of $6,944.14 \text{ t.ha}^{-1}$ across all land use types exceeds the average of $5,500 \text{ t.ha}^{-1}$ reported for agricultural landscapes in northern Thailand (Don et al. 2011). This suggests that Ngiwngam Subdistrict may possess above-average carbon sequestration potential compared to other regions, highlighting its significance in regional carbon management strategies. Such findings align with broader observations across Southeast Asia, where land use practices significantly influence carbon dynamics. For example, studies have shown that agroforestry systems and well-managed tree plantations can enhance carbon storage capabilities (Jose & Bardhan 2012, Roshetko et al. 2007).

Overall, the results from the Ngiwngam Subdistrict underscore the potential for strategic land use planning to optimize biomass and carbon sequestration while maintaining agricultural productivity. This comparative analysis emphasizes the importance of local conditions and management practices in determining the effectiveness of carbon sequestration strategies across different agricultural landscapes in Southeast Asia.

Implications for Sustainable Land Management and Climate Change Mitigation

The findings of this study have several important implications for sustainable land management and climate change mitigation strategies in the Ngiwngam Subdistrict and similar agricultural landscapes:

1. **Diversification of Agricultural Systems:** The dominance of rice paddies, while economically important, presents risks in terms of biodiversity loss and limited ecosystem

services. Encouraging a more diverse agricultural landscape, including the integration of tree-based systems and agroforestry, could enhance both ecological resilience and economic stability (Tscharntke et al. 2012).

2. **Promotion of Perennial Vegetation:** The high biomass and carbon sequestration potential of perennial vegetation, including abandoned orchards and tree plantations, suggests that increasing the cover of woody perennials could significantly enhance the carbon sink capacity of the landscape. This could be achieved through targeted reforestation, agroforestry initiatives, and policies that incentivize the maintenance of tree cover on agricultural lands (Chazdon et al. 2016).
3. **Sustainable Management of High-Performing Species:** While Eucalyptus plantations show exceptional performance in terms of biomass accumulation and carbon sequestration, their widespread adoption should be approached cautiously. Balancing the carbon benefits with potential ecological impacts requires careful planning and management strategies (Ferraz et al. 2013).
4. **Enhancement of Carbon Storage in Agricultural Systems:** Given the low carbon sequestration rates in annual cropping systems, there is a need for practices that enhance soil organic carbon and above-ground biomass in these systems. Conservation agriculture, cover cropping, and the integration of trees into agricultural landscapes are potential strategies to address this challenge (Lal 2004).
5. **Leveraging Natural Regeneration:** The high biomass and carbon sequestration potential of abandoned perennial vegetation highlights the value of natural regeneration processes. Policies that allow for and incentivize the natural recovery of degraded or abandoned agricultural lands could provide a cost-effective approach to enhancing carbon sinks (Chazdon & Guariguata 2016).
6. **Balancing Multiple Ecosystem Services:** The study underscores the need to consider multiple ecosystem services in land use planning. While carbon sequestration is crucial for climate change mitigation, other services such as biodiversity conservation, water regulation, and food production must also be considered in a holistic approach to landscape management (Bennett et al. 2009).
7. **Policy Implications:** The findings suggest a need for policies that incentivize land use practices that enhance carbon sequestration and other ecosystem services. This could include payments for ecosystem services, carbon credits for agroforestry and reforestation initiatives,

and support for sustainable agricultural practices that increase soil carbon storage (Pagiola et al. 2005).

CONCLUSIONS

This study highlights the significant carbon sequestration potential of the Ngiwngam Subdistrict, with an average total biomass of 189.39 t.ha⁻¹ and a total carbon sequestration rate of 6,944.14 t.ha⁻¹ across diverse land use types. Eucalyptus plantations emerged as the most effective in biomass accumulation, indicating their suitability for enhancing carbon storage in agricultural landscapes. These findings suggest that strategic land use planning, including the expansion of tree cover and the implementation of agroforestry practices, could substantially enhance carbon sequestration and improve ecosystem services in the region.

RECOMMENDATIONS FOR FUTURE RESEARCH AND POLICY

1. **Targeted Reforestation:** Identify areas within the Ngiwngam Subdistrict that are suitable for reforestation with native tree species to maximize biodiversity and carbon storage.
2. **Agroforestry Initiatives:** Promote agroforestry systems that integrate trees with crops to optimize land use while enhancing carbon sequestration and soil health.
3. **Longitudinal Studies:** Conduct long-term monitoring of biomass and carbon dynamics to assess the effectiveness of different land management practices over time.
4. **Policy Development:** Encourage local policymakers to incorporate findings into land use policies that prioritize sustainable practices aimed at increasing carbon sinks while supporting agricultural productivity.

By adopting these strategies, Ngiwngam Subdistrict can play a pivotal role in regional efforts to mitigate climate change through enhanced carbon management practices. This revised conclusion succinctly summarizes your study's key findings while providing clear recommendations for future research and policy initiatives related to carbon sequestration and land use management.

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Spatio-Temporal Changes of Chlorophyll Concentration with Environmental Parameters Over the Gulf of Mannar, India

M. Tholkapiyan^{1†} , Veeramalai Sankaradass², C. Hariharasudhan¹, M. Priyanka¹ and Kalaivani Ravisundar³

¹Department of Civil Engineering, Chennai Institute of Technology, Chennai, Tamil Nadu, India

²Department of Computer Science and Engineering, Chennai Institute of Technology, Chennai, Tamil Nadu, India

³Department of ECE, Saveetha Engineering College, Chennai, Tamil Nadu, India

†Corresponding author: M. Tholkapiyan; m.tholkapiyan@gmail.com

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ABSTRACT

The present study investigated the spatio-temporal analysis of chlorophyll concentration with the variation of environmental parameters such as Aerosol optical depth (AOD) and sea surface temperature (SST) over the Gulf of Mannar (GOM) located in the southeastern part of India. The data derived from several satellites already provide information about the aquatic environment throughout the global waters. This study utilized the standard algorithms for the retrieval of *Chl-a* from the Moderate-resolution Imaging Spectroradiometer (MODIS) satellite sensor for a period of about 2002 to 2020. The monthly spatio-temporal variability of the *Chl-a* concentration with the changes in SST and AOD were analyzed over the Gulf of Mannar for the year 2011. This shows that many areas in the Gulf of Mannar have high chlorophyll concentrations of about 2 to 5 mg.m⁻³ in the single-day images for several months during 2011. Also, the variation in the SST is observed for several months during 2011, ranging from 28 to 31.5°C. The AOD values fall in the range of 0.09 to 0.12. In addition, the time series analysis of *Chl-a*, SST, and AOD were also studied for two different stations over the GOM. The result of the time series analysis explains that high chlorophyll concentration was observed during the colder months with low values in SST and AOD.

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INTRODUCTION

Chlorophyll-a (*Chl-a*) is an important water quality index in both marine and coastal environments. *Chl-a* in the ocean is the indicator of phytoplankton biomass (Gitelson et al. 1993) and primary productivity (Cannizzaro & Carder 2006, Huot et al. 2007). Phytoplankton are microscopic plants that form the base of the marine food web, and their growth and distribution are influenced by various environmental factors. *Chl-a* is the most abundant pigment in phytoplankton (Patissier et al. 2014). *Chl-a* is the biological component accessible to remote sensing (via ocean color) and provides a key metric for assessing the health and productivity of marine ecosystems globally (Gurlin et al. 2011). *Chl-a* absorbs sunlight and carbon dioxide from the earth and supports the primary production (Hu et al. 2012), which is good for overall ocean health. It is responsible for the appearance of green pigments in plants and algae (Platt & Sathyendranath 2008). When the chlorophyll concentration increases, the water color shifts slightly varies from blue to green. This indicates an increase in the phytoplankton population (Simon & Shanmugam 2012). Regular monitoring is necessary for assessing the blooms and implementing effective management strategies to avoid the deterioration of water quality (Le et al. 2013).

The extension of three different one-dimensional OCDMA codes into a three-dimensional OCDMA system for improved performance. The three distinct codes are the non-mapping, MS, and MD. The extensions are such that the spectral domain utilizes the non-mapping code, time spreading uses the



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MS code, and spatial domains extend with the MD code (Gupta et al. 2024).

The growth of phytoplankton is often limited by the availability of nutrients such as nitrogen, phosphorus, and silica. Areas with upwelling or high nutrient input typically have higher chlorophyll concentrations. The increase in nutrient supply primarily increases the phytoplankton growth rate (Banse et al. 1996). The *Chl-a* concentration is mainly affected by environmental and climatic factors such as rainfall, currents, seasonal atmospheric cycles, inland river water inflow, local wind regimes, water column stratification, and sea surface temperature (*SST*) volatilities (Tian et al. 2016).

Sea Surface Temperature (*SST*) is the important parameter that affects the phytoplankton growth rates and community composition. Extreme temperatures can inhibit phytoplankton growth or lead to harmful algal blooms. Variations in *SST* can affect the marine ecosystem in various ways (Brochier et al. 2013). According to the Intergovernmental Panel on Climate Change (IPCC), global sea surface temperature is expected to rise by approximately 0.4 to 1.1°C by 2025. Change in surface temperature affects the plant and animal species present in the ocean. An increase in *SST* may decrease the *Chl-a* concentration and affect the circulation pattern that brings nutrients from the deep sea to surface water. An excessive increase in *SST* destroys the growth of phytoplankton, and another factor that affects the growth is the amount of nutrients loaded with freshwater from river discharge (Nuridin et al. 2013). Changes in sea surface temperature and *Chl-a* concentration may lead to global warming (Gunasinghe et al. 2024, Mondal & Lee 2024).

The *Chl-a* concentration can also be affected when the change in salinity occurs due to the evaporation of seawater (Al-Tae 2018). When the wind speed increases, the evaporation rate also increases, which causes a change in salinity and *Chl-a* concentration (Mahpeykar & Khalilabadi 2021). Aerosols are rich sources of nutrients that increase the production of marine environment and their carbon sequence (Paytan et al. 2009). Aerosols contain a variety of natural and synthetic compounds, including mineral dust, sea salt crystals, bacteria, and other microscopic particles. Aerosols are solid or liquid particles suspended in the atmosphere with a diameter of approximately 0.001 to 100 µm (Giles et al. 2019). Aerosol particles not only affect the radiative forcing of the Earth's air system but also influence climate and cause environmental problems such as acid rain and haze (Li et al. 2018, Huang & Ding 2021). High concentrations of aerosols cause serious threats to human health (Morozova & Mironova 2015). The properties of aerosols include parameters such as aerosol optical depth (AOD), single-

scattering albedo, scattering phase function, and absorbing optical depth. AOD is the most important parameter, which is defined as the integral of the aerosol extinction coefficient in the vertical direction. This describes the attenuation effect of aerosols on light and is an important indicator of the level of air pollution.

Estimating *Chl-a* concentration using the remote sensing data is challenging in turbid and productive waters. It is difficult to monitor the changes in the *Chl-a* concentrations through field measurement and cost high. This can be overcome by using satellite measurements through remote sensing techniques, which are of low cost with high efficiency. Several studies have been conducted to monitor the water quality on a large scale using remote sensors (Le et al. 2013, Tong et al. 2022, Koponen et al. 2007). Satellite sensors provide reliable global ocean coverage of *Chl-a* and *SST* at relatively high spatial and temporal resolution. The spatial and temporal distribution can be effectively measured from space (Ishizaka 2010).

Several satellites like Coastal Zone Color Scanner (CZCS), Sea-viewing Wide Field-of-View sensor (SeaWiFS), Moderate Resolution Imaging Spectrometer (MODIS), Medium Resolution Imaging Spectrometer (MERIS) and VIIRS, etc. are used to derive *Chl-a* products from the ocean and coastal waters. NASA CZCS, launched in 1978, is designed to monitor ocean color. Ocean and Land Color Instrument (OLCI) onboard the sentinel-3 satellite is introduced to monitor water quality in turbid lakes. OLCI satellite includes 21 spectral bands, improved signal-to-noise ratio (SNR), cameras for mitigation of sun glint, a global spatial resolution of 300 m, daily revisit times, and enhanced coverage. Several research has attempted to map *Chl-a* concentration based on OLCI images in various turbid lakes (Pahlevan et al. 2020, Shen et al. 2020).

Satellite remote sensing is the best way to view the entire ocean surface and to monitor *Chl-a* at high spatial and temporal resolution (Brewin et al. 2021). To derive *Chl-a*, remote sensing has employed empirical algorithms (Dierssen 2010) and semi-analytical algorithms (IOCCG 2006). The empirical algorithm, known as the ocean color algorithm, uses absorption properties of *Chl-a* and remote sensing reflectance to eliminate *Chl-a* from space (O'Reilly & Werdell 2019, O'Reilly et al. 1998). The semi-empirical algorithm uses physical models of light interactions with water to eliminate the *Chl-a* (Werdell et al. 2018).

Remote sensing reflectance (*R_{rs}*) is determined from the inherent optical properties (IOPs) of water and optically active water constituents (OAC) (Gordon et al. 1988, Mobley 1999). Estimation of *Chl-a* from *R_{rs}* can be analytically approached by forward or inverse modeling or by empirically

associated variability of R_{rs} with one or more wavebands (Schalles 2006). When the phytoplankton increases in the ocean, the shift of reflectance is observed from blue to green wavelengths caused by the combination of increased absorption and particulate backscattering process (Morel & Prieur 1977). This observation is then converted to the most widely used Ocean Color (OC) algorithm, in which several variations occur for specific sensors (O'Reilly et al. 1998, O'Reilly & Werdell 2019). The empirical algorithms are specially designed to estimate $Chl-a$ from waters with high algal biomass and identify algal blooms based on peak height methods (Matthews et al. 2012).

Several studies have been conducted to understand the variability of $Chl-a$ in coastal waters using satellite sensors. Chen et al. (2013) presented $Chl-a$ concentration retrieval in the coastal waters of Bohai, Yellow, and East China Sea. The in-situ data were collected and are used to assess the performance of HY-1C in retrieving the $Chl-a$. The result of this study shows that the algorithm based on blue-green band ratios performed well, and the broadband channel of HY-1C CZ1 can retrieve $Chl-a$ in turbid waters. Alexander et al. 2010 made a comprehensive study on the algorithm for retrieving the $Chl-a$ from the coastal waters. The two and three-band algorithms are tested with the bands that matched the spectral channels of MERIS.

In this study, the changes in $Chl-a$ concentrations were analyzed with SST and AOT using MODIS satellite images. The study is conducted in the Gulf of Mannar, where the coastal waters are dominated by Chlorophyll-a and are

monitored using satellite sensors. Here, the OC3 algorithm is used for the retrieval of $Chl-a$ from R_{rs} . The analysis includes the spatio-temporal analysis of $Chl-a$ with AOT and SST variation using the monthly images over the year 2011. Further, the study extended to the time series analysis of $Chl-a$ with the changes of environmental parameters such as AOD and SST analyzed over the years 2000-2020.

STUDY AREA

Gulf of Mannar (GOM) ($8.47^{\circ}N$, $79.02^{\circ}E$) is located in the Indian Ocean between Southeastern India and Western Sri Lanka. GOM extends from Tuticorin to Rameswaram Island in the SE-NE direction. There are 21 islands situated at an average distance of about 8 km from the coast and running almost parallel to the coastal line. GOM has a rich and diverse fauna compared to other regions. GOM region is enriched with various habitats such as Coral reefs, mangroves, sea grasses, seaweeds, etc. GOM is a biologically rich area and is identified with more than 4223 species of flora and fauna. The Gulf of Mannar typically exhibits moderate to high levels of chlorophyll-a present in the nearshore waters along the Indian coast.

The stations used for the analysis are shown in Fig. 1. The study area of station 1, with a latitude of 9.7138 and longitude of 79.1568 , is located near the coastal region, which is shallow water, and station 2, with a latitude of 9.3199 and longitude of 79.2026 , is located near the Pamban bridge. In the Gulf of Mannar, the water temperature is influenced by the atmospheric temperature. From January to April, the

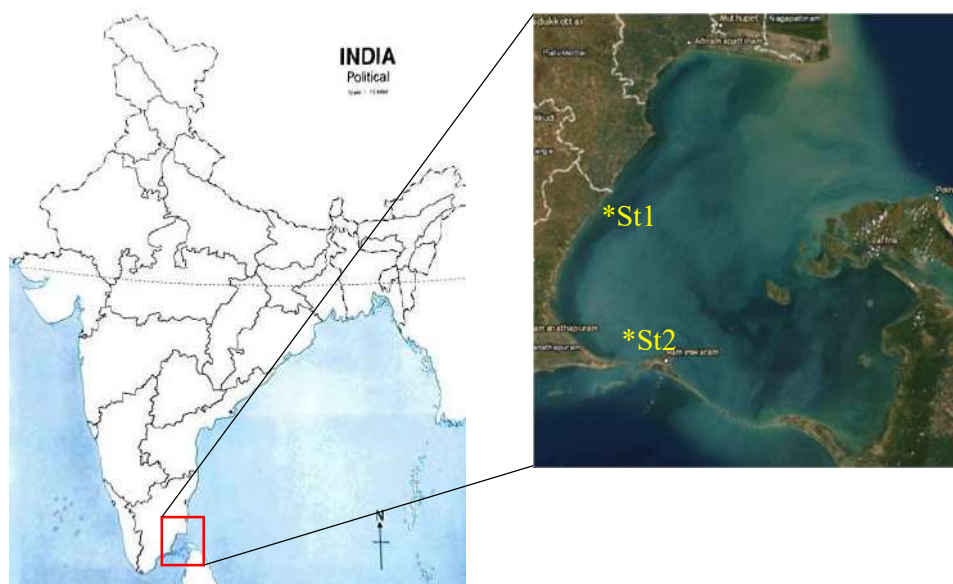


Fig.1: The location of the study area over the Gulf of Mannar in the southeast coastal waters of India (Left side). The data from stations st-1 and st-2 are used for the time series analysis (right side).

temperature steadily increases, with a highest peak of 32°C in May. And till August, the temperature gradually decreases. Between September and October, the temperature again increases and falls between December and January. The water temperature may exceed the atmospheric temperature during summer in shallow regions closer to land.

In the Gulf of Mannar, the *Chl-a* concentration is high during the northeast monsoon. Because, cold water has higher *Chl-a* than warm water, which contains nutrients that come from the deep ocean (Balasubramanian & Shanmugam 2015). *SST* are mostly cooler than the nearby waters where the ocean current causes upwelling, and the *Chl-a* concentration is high. The Gulf of Mannar, being a tropical region, has a warm climate with low change in surface temperature (Tholkapiyan 2012). It is easier for the deep water to rise to the surface when the surface water is cold.

MODIS Aqua satellite data is used for accurately mapping the *Chl-a* concentration in the coastal water of the Gulf of Mannar. MODIS is a satellite-based sensor used to measure climatic conditions. Terra (EOS AM, launched in 1999) and Aqua (EOS PM, launched in 2000) satellites are the two MODIS sensors. MODIS sensors can capture data in 36 spectral bands ranging in wavelength from 0.4 μm to 14.4 μm and at varying spatial resolutions. The captured data from the top of the atmosphere provides Rrs after undergoing atmospheric correction. This Rrs dataset is used to monitor the measurements in large-scale global dynamics, including piles of earth cloud cover and processes occurring in the

ocean, land, and the lower atmosphere. MODIS sensor plays a major role in predicting global changes accurately.

Using the MODIS Aqua sensor, the daily images for the two stations of the Gulf of Mannar are collected over the year 2000 to 2020 from the Ocean Color website. The collected data is then processed, and atmospheric correction is carried out using the ACOLITE software (Pahlevan et al. 2021). The ACOLITE processor was used to correct the atmospheric effects using the dark spectrum fitting (DSF) (proposed by Vanhellemont & Ruddick 2018, Vanhellemont 2019) over SWIR-based exponential extrapolation. Sun glint effect can also be removed using the ACOLITE processor. Using the satellite images, the *Chl-a* is estimated with good spatial resolutions.

MATERIALS AND METHODS

In this study, *Chl-a* concentration was analyzed with *SST* and *AOD* using the Moderate-resolution imaging spectroradiometer (MODIS) satellite sensor over a 20 years from 2000-2020. The standard atmospheric correction procedure (Bailey et al. 2009) is used to perform the atmospheric correction process in which the top-of-atmosphere radiance received by sensors is converted to surface reflectance. OC3 Chlorophyll algorithm is used to retrieve the *Chl-a* from the remote sensing reflectance (R_{rs}), which is developed based on the semi-analytical and bio-optical remote sensing reflectance, R_{rs} . The sea surface temperature (*SST*) measurement and Aerosol optical

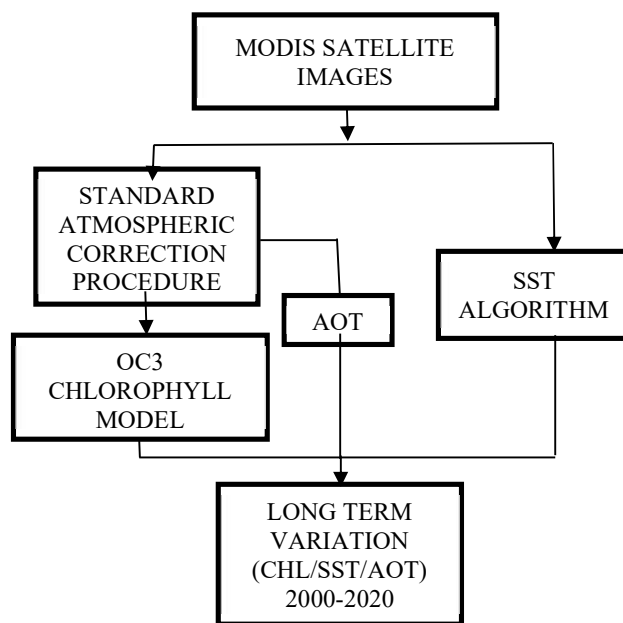


Fig. 2: The schematic block diagram represents the methodology for the estimation of *Chl-a* concentration, *AOT*, and *SST* for understanding their long-term changes by processing the MODIS satellite sensor data from 2000-2020.

depth (AOD) are also derived for this analysis from the MODIS satellite sensor. The variation in the chlorophyll concentration, Aerosol optical depth measurement, and surface temperature analysis is presented in this study. Fig. 2 represents the schematic diagram of the methodology.

Atmospheric correction is a crucial step in remote sensing for accurately estimating surface properties like chlorophyll-a (*Chl-a*) concentrations from satellite data (Gordon et al. 1998). Atmospheric correction aims to remove or correct the effects of the atmosphere on the observed radiance, which includes scattering and absorption of light by atmospheric particles and gases. The atmospheric correction algorithm is the estimation of spectral aerosol reflectance $\rho_a(\lambda)$, subtracted from total measured signals $R_{rs}(\lambda)$. NASA Ocean Biology Processing Group (OBPG) implemented an algorithm that (Gordon & Wang 1994) requires an assumption of negligible water-leaving reflectance in the NIR of the spectrum (i.e., $R_{rs}(\text{NIR}) = 0 \text{ sr}^{-1}$).

Ocean Color remote sensing involves the estimation of concentration in the ocean by measuring the variation in the spectral quality of the water surface. OC is obtained from the incident light interactions with other substances present in water (*Chl-a* concentration, suspended sediments, and CDOM). These substances are quantified by water leaving radiance, which is measured in the visible portion of electromagnetic radiation. OC algorithm is the band-ratio-based algorithm used for the retrieval of *Chl-a* from the remote sensing reflectance, R_{rs} . In the empirical OC algorithm, *Chl-a* has log-normal distribution, which means the model is trained on log-transformed (with base 10) in situ measured *Chl-a*. OC3 algorithm uses a quadratic polynomial with a single explanatory variable. For the OC3 algorithm, the numerator is the maximum of the bands $R_{rs} 443$ and $R_{rs} 488$, and the denominator with $R_{rs} 551$. The OC3 algorithm uses a three-band blue-green reflectance ratio is defined as:

$$R = \log_{10} \left[\frac{R_{rs443} > R_{rs488}}{R_{rs551}} \right] \quad \dots(1)$$

$$Chl-a = 10^{0.28 - 2.753R + 1.457 R^2 + 0.659 R^4}$$

where, *Chl-a* is Chlorophyll-a concentration $\text{mg} \cdot \text{m}^{-3}$, R is Blue-green band ratio. OC-3 algorithm has been used to retrieve low as well as high *Chl-a* concentrations and is also useful in monitoring the waters from estuarine regions using spatial resolution sensors (Morel & Maritorena 2001).

SST maps are based on observations by the MODIS Aqua satellite. The temperature estimated from the satellite measurements is known as brightness temperature (BT). *SST* measured at the sea surface is not the same as the satellite-derived *SST* because of atmospheric interference. So, for the identification or removal of clouds and aerosols' contribution to satellite radiance, an atmospheric correction procedure

was followed. The bands that provide more information for the correction procedure are the atmospheric windows at the wavelength range of $\lambda = 3.5\text{--}4.1 \mu\text{m}$ and $\lambda = 9.5\text{--}12.5 \mu\text{m}$ (McMillin 1975). Although the two spectral channels provide BT, the magnitudes of the retrievals are different based on the atmospheric transmissivity. The difference in brightness temperature is defined as the temperature drop of *SST* and other BT (McMillin 1975, Barton 1995), which provides the linearized relation in terms of the multi-channel sea surface temperature equation. The equation is as follows:

$$SST = aT_i + \gamma(T_i - T_j) + c \quad \dots(2)$$

where γ is the coefficient of differential absorption, T_i and T_j are the brightness temperature obtained from the two channels. MODIS Aqua/Terra has two bands in the transmission window. The robust algorithm is as follows:

$$SST = a_0 + a_1 T_{3.95} + a_2 (T_{3.95} - T_{4.05}) + a_3 (\sec(\theta) - 1) \quad \dots(3)$$

Here, the coefficients of the equation, such as a_0 , a_1 , a_2 , and a_3 , are obtained from Kilpatrick et al. (2015). The above method has been applied to produce satellite-derived *SST* for the temporal and spatial analysis and the *SST* retrieval from the MODIS images.

RESULTS AND DISCUSSION

This section describes the results in two subsections: one is the spatio-temporal analysis, and the second one is the time series analysis. The spatio-temporal analysis of *Chl-a* concentration, AOD, and *SST* are presented for one year (2011) with monthly images. The time series analysis is carried out to investigate the possibility of a significant trend in the *Chl*, *SST*, and AOD over 19 years.

Spatiotemporal Analysis

The above figures represent the *Chl-a*, *AOT*, and *SST* measurements over the Gulf of Mannar by MODIS satellite sensor for the 6-month duration from January to June 2011. The variability of *Chl-a* concentration with the changes in *SST* and *AOT* over the Gulf of Mannar is presented in this study. Fig. 3a. Represents the *Chl-a* concentration, and the color bar represents the relative changes corresponding to the dimensional trend for the *Chl-a* concentration. Fig. 3b represents the Aerosol optical depth measurement, and the color bar indicates the relative change for the aerosol measurement. Fig. 3c represents the surface temperature, and the color bar indicates the relative changes with respect to sea surface temperature.

The *Chl-a*, *AOT*, and *SST* measurements are obtained from the global MODIS ocean color satellite. Data collection

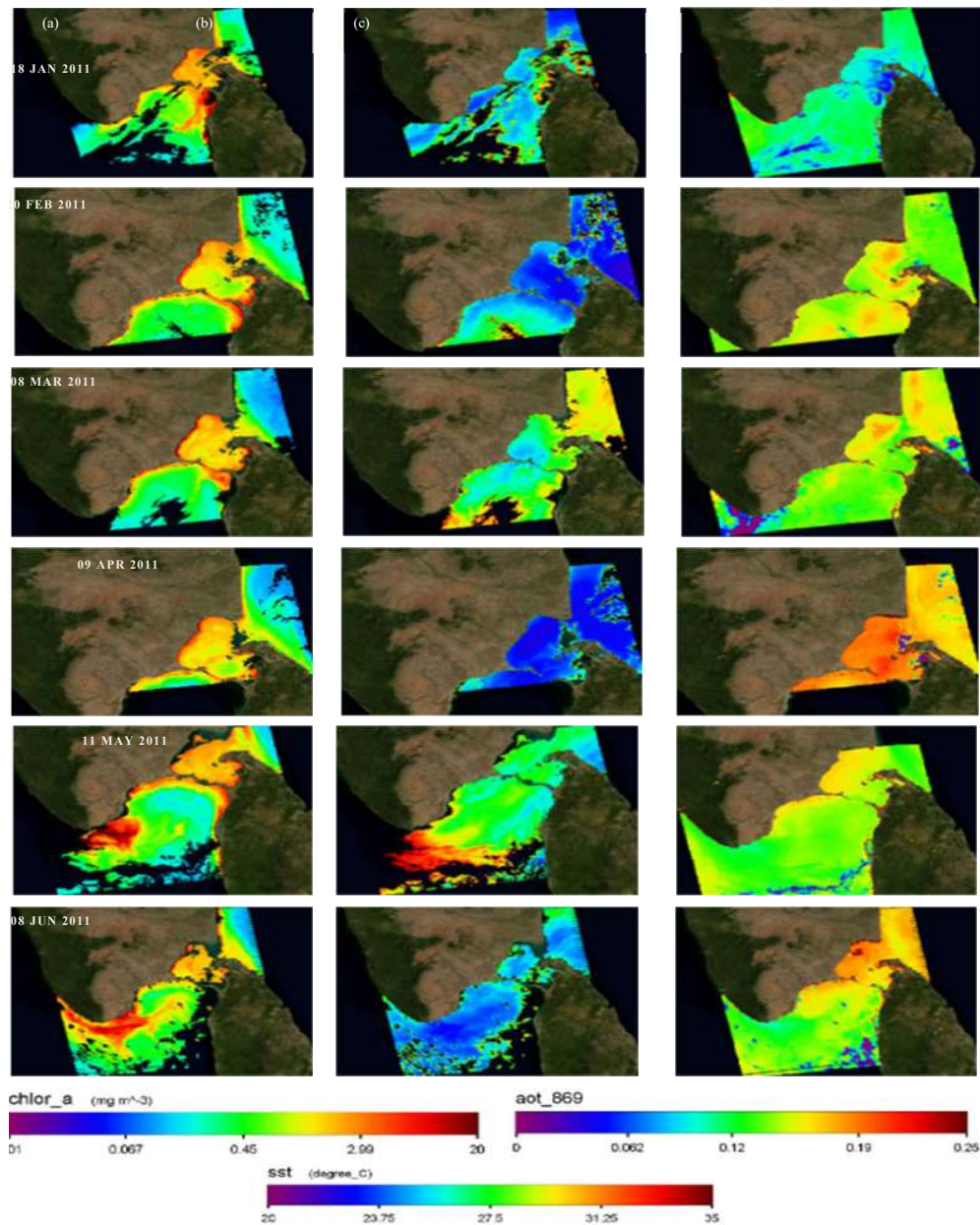


Fig. 3: (a) *Chl-a* concentration trend over the Gulf of Mannar by using a MODIS satellite sensor for the duration of 6 months from January to June 2011, (b) Aerosol optical depth, and (c) Sea surface temperature measurement during the study period. The color bar scale represents the relative changes corresponding to the dimensional trend for the *Chl-a* concentration, AOT, and SST values.

from satellites involves frequent measurements over time with large spatial coverage, which is useful for assessing long-term changes. The *Chl-a* concentration in the Gulf of Mannar shows different seasonal variability for different regions. The *Chl-a* concentration and SST are highly dependent due to the difference in gaining the heat between shallow and deeper portions of the sea. The deeper sea observes high temperatures. The surface water in the middle

region gains a lower temperature than the water near the shore. An increase in surface temperature in shallow water increases the concentration of *Chl-a*, while an increase in surface temperature in the deeper water tends to decrease the concentration of *Chl-a*. During summer, in shallow regions closer to land, the water temperature may exceed even the atmospheric temperature. GOM is characterized by high salinity and surface temperature during the northeast

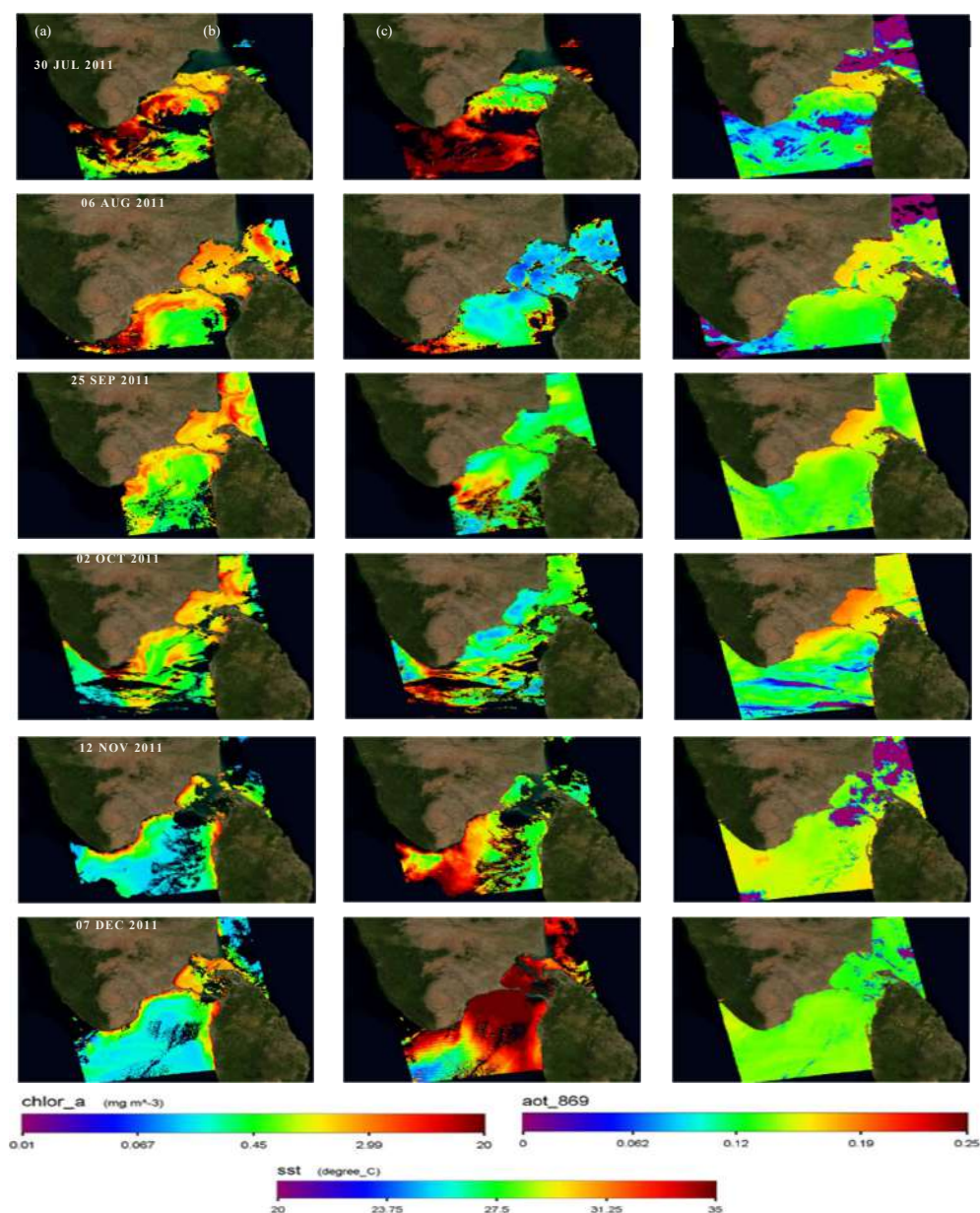


Fig. 4: (a) *Chl-a* concentration trend over the Gulf of Mannar by using a MODIS satellite sensor for the duration of 6 months from July to December 2011, (b) Aerosol optical depth measurement during the study period, and (c) Sea surface temperature measurement during the study period. The color bar scale represents the relative changes corresponding to the dimensional trend for the *Chl-a* concentration, AOT, and SST values.

monsoon. When more heat is absorbed in the ocean, surface temperature increases, and the ocean circulation patterns transport warm and cold water around the globe. From Fig. 3a, very low numbers of chlorophyll concentrations appear in light green, and high chlorophyll concentrations appear in dark orange. The observations were taken from the MODIS sensor on NASA's Aqua satellite. The satellites measure the temperature of the top millimeter of the ocean surface. From Fig. 3c, the coolest water temperature appears

in blue, and the warmest temperature appears in red. In the Gulf of Mannar, the Northeast Monsoon is steadiest in January and is weak by March. The wind becomes light and variable toward the end of April.

The northern part of GOM experiences high surface pigment concentration. The waters are very shallow compared to other surrounding waters of the island. Thus, the water contains other substances like suspended particles and dissolved organic matter in addition to *Chl-a*. From the

above figure, *Chl-a* concentration is high during January which is due to the decrease in the *AOT* and the *SST*. *SST* is high during April. Thus, the *Chl-a* concentration and the *AOT* measurements are reduced due to the high surface temperature. During the months of March to May, the *Chl-a* concentration was reduced when compared with January, which may be due to surface heating during summer. From February to May, there was minimal variation in chlorophyll concentration. During June, the *Chl-a* concentration is low which is due to the increase in the surface temperature. The highest spatially mean *Chl-a* concentration is observed in January, and the lowest is observed in June.

Spatial and temporal analysis shows that *SST* and *Chl-a* concentration in this area is greatly influenced by the monsoon. *Chl-a* is an indicator of photosynthesis present in the ocean. *Chl-a* concentration is influenced by climatic factors such as surface temperature and winds. In GOM, *Chl-a* concentration is high during the Northwest monsoon compared to the Southwest monsoon. The *Chl-a* concentration is high along the coast due to the nutrient-rich run-off from land via rivers. Also in near shore, *Chl-a* variability was high and decreases with distance towards offshore. *Chl-a* concentration is affected by rainfall and

river discharge. During the southwest monsoon, *SST* is low compared to the Northwest monsoon, while *Chl-a* in the Northwest monsoon is higher than the Southwest monsoon. After the SW monsoon, the southwest coasts have higher surface pigment concentrations. In the SW monsoon, satellite data were not available, in which these waters are rich in *Chl-a* concentration. During the Southwest monsoon period from July to September, high wind speed and a decrease in surface temperature are observed around the southeastern coast. Hence increase in chlorophyll concentration is observed from August to October 2011.

Fig. 4. represents the *Chl-a*, *AOT*, and the *SST* measurements over the Gulf of Mannar by MODIS satellite sensor for the 6-month duration from July to December 2011. The color bar scale represents the relative changes corresponding to the dimensional trend for the *Chl-a* concentration, Aerosol depth measurement, and surface temperature values. During July, the surface temperature measurement was very low. So, the reduction in the *Chl-a* concentration and the aerosol optical depth measurement is observed during July which is due to the decrease in the surface temperature level. Fig. 4a indicates that the *Chl-a* concentration is high during August and September. In

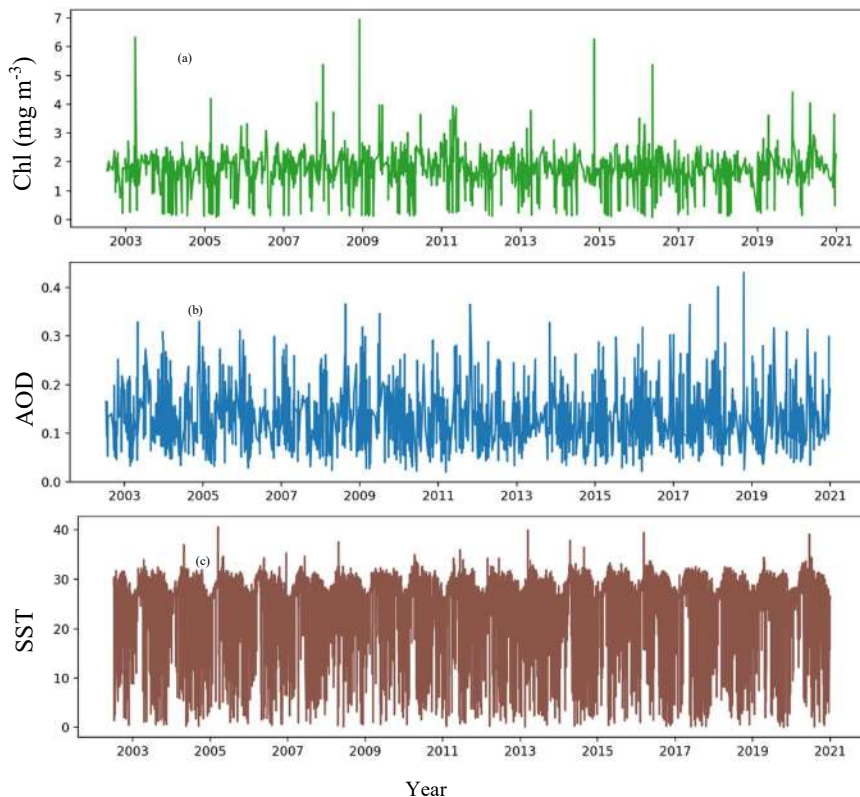


Fig. 5: Variation of *Chl-a*, *AOD*, and *SST* from MODIS data for location (st-1) over the Gulf of Mannar from 2002 to 2020. (a)Time series representation of *Chl* data (b) time series representation of *AOD* data (c) Time series representation of *SST* data.

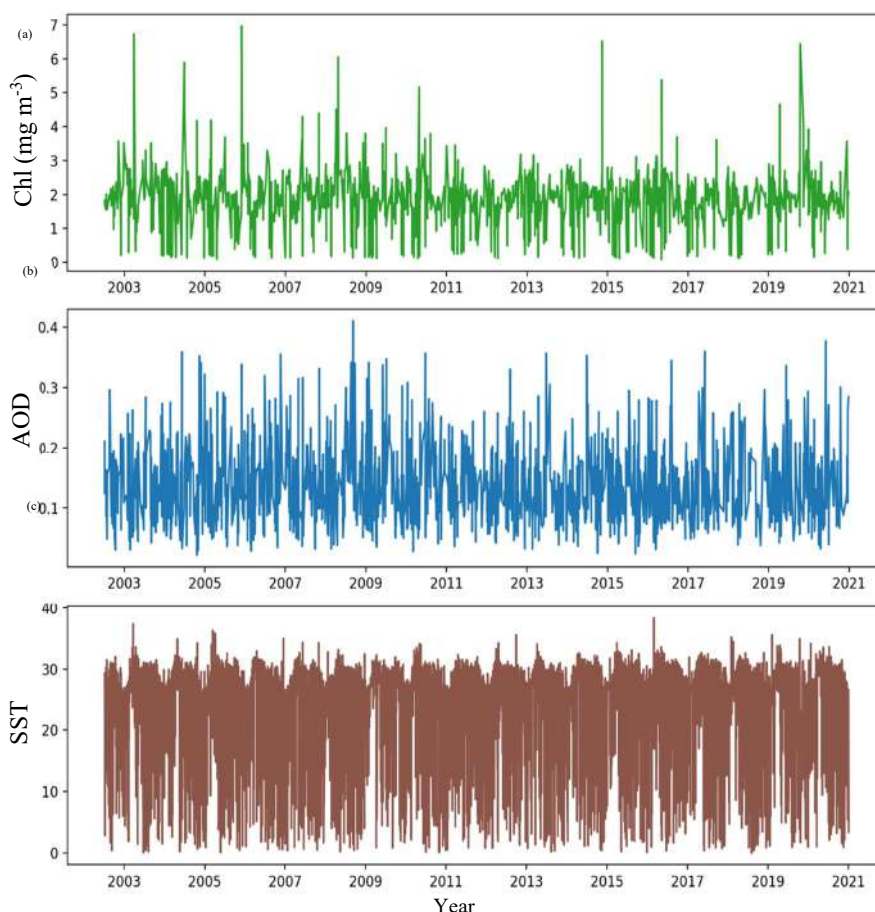


Fig. 6: Variation of *Chl*, *AOD*, and *SST* from MODIS data for location (st-2) over the Gulf of Mannar from 2002 to 2020. (a) Time series representation of *Chl* data (b) time series representation of *AOD* data (c) Time series representation of *SST* data.

August due to high *Chl-a* concentration, the aerosol optical depth measurement is low and an average range of surface temperature measurement. High *Chl-a* concentration in September shows an average range of *AOT* and surface temperature measurements. Increased levels of *Chl-a* concentration and the aerosol optical depth measurement observed during December experienced average surface temperature. During the month of August-October, the southwest monsoon period, results in a high *Chl-a* concentration, which shows the low range of *AOT* and average surface temperature measurements. High *Chl-a* concentration in the open ocean waters is observed due to upwelling rather than other particles. From Fig. 4b very high level of optical depth measurement is obtained during December. So, due to increased aerosol measurement, the *Chl-a* concentration increases with low surface temperature.

Long-Term Time Series Analysis

The time series distribution of *Chl-a* concentration, *AOD*, and *SST* data are obtained from MODIS sensors for the

daily data from 2002 to 2020. The time series distribution of the long-term changes in *Chl-a* concentration was unevenly distributed over the years in station 1. The *Chl-a* concentration was high over station 1, which may be due to the upwelling in the coastal region. Over the study period of about 19 years, monthly *Chl-a* showed a range of 1.8 to 2.7 mg.m^{-3} during the cooler months of January and February and about 1.5 to 2 mg.m^{-3} during the warmer months of July and August, indicating higher *Chl-a* concentrations during the latter period. This is likely due to coastal waters being calmer during the summer than in winter. Throughout the years, during warmer months, the *Chl-a* concentration is about 2 mg.m^{-3} , ranging from 1.8 to 2.2 mg.m^{-3} , and during the colder months, the average *Chl* concentration of about 2.4 mg.m^{-3} , ranging from 2 to 2.7 mg.m^{-3} , was observed.

The time series distribution of *SST* increases during the warmer months and decreases during colder months which may be due to the change in climatic conditions, as shown in Figure 5c. The annual average *SST* was observed during 2011 at about 28.3°C. The overall analysis shows that GOM

experiences an average *SST* of about 27.5°C. *SST* shows a minimum in July and reaches a maximum in April. The time series distribution shows that the *AOT* values increase in linear were observed during the beginning of every year and gradually decrease to minimum values at the end of the year. The average range of *AOT* is about 0.15. High nutrients are observed during summer in which the temperature recorded is also high. The *Chl-a* concentration values are observed as high during the winter season.

The time series distribution of *Chl-a* concentration, *AOD*, and *SST* data for station 2 is obtained from MODIS sensors for the daily data from 2002 to 2020 (Fig. 5). The time series distribution of the long-term changes in *Chl-a* concentration, *AOD*, and *SST* were unevenly distributed over the years in station-2. This station is located near the Pamban bridge, in which the moving of ships occurs, which will affect the *Chl-a* concentration. The *Chl-a* concentration was low in this area which is due to several disturbances that occur in the water. In the western region, *chlorophyll-a* tends to be low, which is due to the strong tidal mixing that causes the residence time of algae in the photic zone. Whereas in the east region, *chlorophyll-a* will be higher. Monthly average *Chl-a* concentration ranges from 1.5 to 2.2 mg.m⁻³. The distribution of *SST* in the east begins to rise in November to April, with an increase in March, and decreases in May to October, with the lowest *SST* in August. The monthly long-term variation for the *SST* from 2002 to 2020 shows that high surface temperature with an average of about 30°C (Fig. 6). Due to high sea surface temperature the *Chl-a* concentration for this area gets reduced. The monthly variation data of *AOT* shows an average of about 0.15 to 0.18.

CONCLUSIONS

Understanding the variations in *Chl-a*, *SST*, and *AOD* in the GoM is crucial, as these parameters significantly influence marine ecosystems and living organisms. The GoM's environmental conditions are particularly unique due to the presence of coral reefs, seagrass beds, and mangroves, which serve as essential habitats for various marine species by providing spawning, feeding grounds, and shelter. This study examines the variability of *Chl-a* in relation to environmental parameters using a satellite-based correction procedure for estimating *Chl-a* concentration, bypassing the need for additional validation steps with in-situ data, as previous research has established the accuracy of satellite-derived measurements. By analysing MODIS satellite data from 2011, we explored the spatio-temporal variability of *Chl-a*, *AOD*, and *SST* across the GoM. Our results indicate that *Chl-a* concentrations are generally lower in coastal regions than in open waters. Additionally, a time-series analysis

of *Chl-a* from 2002 to 2020 revealed correlations between *Chl-a*, *AOD*, and *SST*, showing that increases in *SST* and *AOD* tend to coincide with decreases in *Chl-a* and vice versa. Seasonal fluctuations in *SST*, *AOD*, and *Chl-a* concentrations further suggest that changes in seawater temperature and salinity play a significant role in regulating *Chl-a* levels.

The study also concludes that *Chl-a* concentration decreases with increasing water depth and distance from the shoreline and that *Chl-a* exhibits a strong correlation with physical parameters such as *SST* and salinity. The findings of this study provide valuable insights for the remote sensing community, contributing to the understanding of marine ecosystems and can aid in identifying potential fishing zones. Future work should focus on expanding the spatial coverage of this research and incorporating in-situ or ground truth data to validate satellite observations in other regions where satellite data may be limited.

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Trend Analysis of Meteorological Parameters in the Perspective on Climate Change in Kolkata District During 1901-2019

Kanchan Paira^{1†}, Moatula Ao², Brihaspati Mondal² and Grace Bahalen Mundu²

¹Department of Geography, Hijli College, Kharagpur, Paschim, Medinipur-721306, West Bengal, India

²P.G. Department of Population Studies, Fakir Mohan University, Vyasa Vihar, Nuapadhi, Balasore-756 089, Odisha, India

†Corresponding author: Kanchan Paira, pairakanchan002@gmail.com

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ABSTRACT

Analysis of temporal dynamics of climatic parameters is indispensable for advancing the “Sustainable Development Goals (SDGs)-11 and 13”. This study aims to assess the trend of temperature and rainfall in Kolkata District using CRU (Climate Research Unit) data from 1901 to 2019. Statistical methods such as anomaly index, CV (“coefficient of variation”), and PCI (“Precipitation Concentration Index”) were employed along with ITA (Innovative trend analysis) techniques, Mann-Kendall test, and Spearman’s Rho tools. These measures are widely used in climate and environmental research to recognize the trend of climate change. The Mann-Kendall and Spearman’s Rho tools both reveal that the seasonal (summer and winter) and yearly temperatures are rising significantly ($P < 0.01\%$). In contrast, the result shows a significant rise in annual and monsoon rainfall and a notable upward trend in temperature throughout all months. Trend analysis shows a rising trend of temperature, as well as, rainfall in Kolkata District, whereas PCI shows moderate (16 years), high (79 years), and very high (24 years) concentrations of precipitation. The current research proposes increasing government efforts to decrease greenhouse gas emissions and enhance urban environmental conditions by regulating air pollution emissions. These measures, if implemented effectively, can significantly lessen the effect of the changing climate and enhance the quality of life in Kolkata. Effective land use management and stringent population control policies are essential for achieving SDG 13 and promoting inclusivity, safety, resilience, and sustainability in Kolkata (SDG 11).

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INTRODUCTION

Climate change, a significant global environmental phenomenon, carries profound implications for human existence, economic growth, and ecosystems (Das & Bhattacharya 2018). It is a phenomenon by which all sectors of life in the present day experience the extreme barbarity of climatic theatricality (Prarikeslan et al. 2023). According to “Human Development Report-2007-08”, climate change is the most significant phenomenon that humanity is currently confronting since the beginning of the 21st century. There is a possibility of an unprecedented catastrophe in the growth of human civilization if we fail to address this challenge. Numerous studies have confirmed a warming trend on Earth (Jones et al. 2013, Levites et al. 2012, Miller 2012) between 1951 and 2012, with a decadal rate of 0.12°C (IPCC 2013). This warming has already begun to impact the atmosphere, affecting both ocean and land.

The “Intergovernmental Panel on Climate Change” (Stocker et al. 2013) asserts that the key reason for global warming is anthropogenic activity. Specifically the emission of various greenhouse gases (GHGs) such as carbon dioxide and methane from the burning of fossil fuels. The concentration of carbon dioxide (CO_2) has risen by 40% since the pre-industrial era, primarily due to these emissions (Stocker

et al. 2013, Huang et al. 2015). IPCC's fourth assessment report (2007) reiterates that climate change is expected to intensify risks and large-scale disasters. This could also contribute to systematic change due to human activities such as the emission of Greenhouse gases. The average temperature of the earth has also increased by 0.74 °C from the pre-industrial era due to the substantial concentration of CO₂ in the atmosphere (UNFCCC 2007).

Climate change is a universally recognized reality. Short to large-scale effects have been observed on climate instability, natural resources, and humans. According to the IPCC (2014), the temperature of the earth might increase from 1.1 to 6.4 °C, and the sea level could rise by 28–79 cm as well by 2100. As a result, weather patterns will become less predictable, and intense climatic disasters such as floods, storms, droughts, and heat waves will take place more regularly. The IPCC anticipated that the projected decline in freshwater levels could negatively impact people in Central, South, East, and South-East Asia.

Climatic change hurts human beings, particularly the displaced people or refugees in India, Southeast Asia, and the Indian subcontinent (Lonergan 1998, Kolmannskog & Skretteberg 2009). It also raises the risk of rising sea levels and alters fundamental living patterns. Recently, research on climate change investigated the health implications of biodiversity loss via changing the microbiome, which might lead to neurologic illnesses, autoimmune, and inflammatory (Ray & Ming 2020). The severe impact of heat waves also poses serious distress in the form of disease, which affects more men than women. Nevertheless, all age groups are vulnerable to heat-associated poor health (Li et al. 2014). In addition, epidemiological studies demonstrated that a slight increase in the maximum or average daily temperature is associated with premature death. Numerous studies have demonstrated the adverse effects of environmental pollution on health (Majra & Gur 2009, Bush et al. 2011, Dhara et al. 2013, Sahu et al. 2013).

A study conducted in Dinhata, West Bengal, by Das & Bhattacharya (2018) examined temperature changes from 1980 to the present and reported that the overall annual, pre-monsoon, and monsoon rainfall has decreased, whereas the post-monsoon rainfall has increased over time. The study also revealed that the summer, winter, and average yearly temperatures have risen, whereas the annual minimum temperature was increased with the increase of the monthly minimum temperature. Using similar statistical tools to measure the change in temperature over Tripura, Tomar et al. (2017) found that except in January and December months, the temperature over two stations increased considerably between 1969 and 2014. Jaswal (2010) found a substantial rising trend of temperature in winter between 1970 and

2007. District-level analysis in Andhra Pradesh (during 2011-2020) done by Kavitha et al. (2023) pinpointed that monthly and seasonal rainfall exhibited an increasing trend, whereas the monthly highest rainfall was found to be varied during the last decade. Choudhuri et al. (2012) revealed that maximum temperatures at Mid-altitude Meghalaya demonstrated a considerable increasing trend, whereas minimum temperatures showed an inconsequential declining trend. A study in Sagar Island by Mandal et al. (2013) between 1982 and 2010 reported a considerable increase in the inter-annual mean and maximum temperature, as well as a consistent decline in yearly rainfall. Kothawale & Rupa (2005) conducted a trend study in India. They discovered that the mean annual temperature has grown considerably over the last century. Therefore, studying micro-regional climate variables is essential in detecting climate change, managing water resources and regional planning (Kavitha et al. 2023), public health, and economic setup. Extensive study on climate change by different researchers was observed across various regions worldwide, primarily focusing on temperature and precipitation. They have set off an alarm bell worldwide. From the ongoing discussion, trend analysis studies conducted so far in urban areas of India are not found to be very conclusive. Many of the urban areas in developing countries are vulnerable to climate change due to city location, existing infrastructure, population, economic losses (UN Habitat 2011), and social issues like poverty and hunger (Gasper et al. 2011). Additionally, the urban poor- i.e., slum dwellers in developing countries are indeed increasingly vulnerable (UN Habitat 2011). Despite these risks, many cities have not addressed this due to the lack of steady-state and relevant city policies and action plans. Hence, studying the trend of climatic scenarios at a micro-level, especially in an urban region, is indispensable for understanding and designing mitigation plans as well as the implementation of cost-effective and sustainable adaptation measures.

Therefore, due to the dearth of studies at the micro level, the primary objective of our study is to examine the temporal trend of meteorological parameters in Kolkata, India.

MATERIALS AND METHODS

Study Area

According to the “Intergovernmental Panel on Climate Change”, the Kolkata metropolitan area and its surroundings are warming tremendously. According to the atlas, the annual mean temperature of Kolkata might increase by almost 4.5°C between 2081 and 2100, compared to the pre-industrial period, if the emission of greenhouse gases is not restrained (Basu 2021). During the period 1950 to 2018, Kolkata Metropolitan reported the maximum increase in

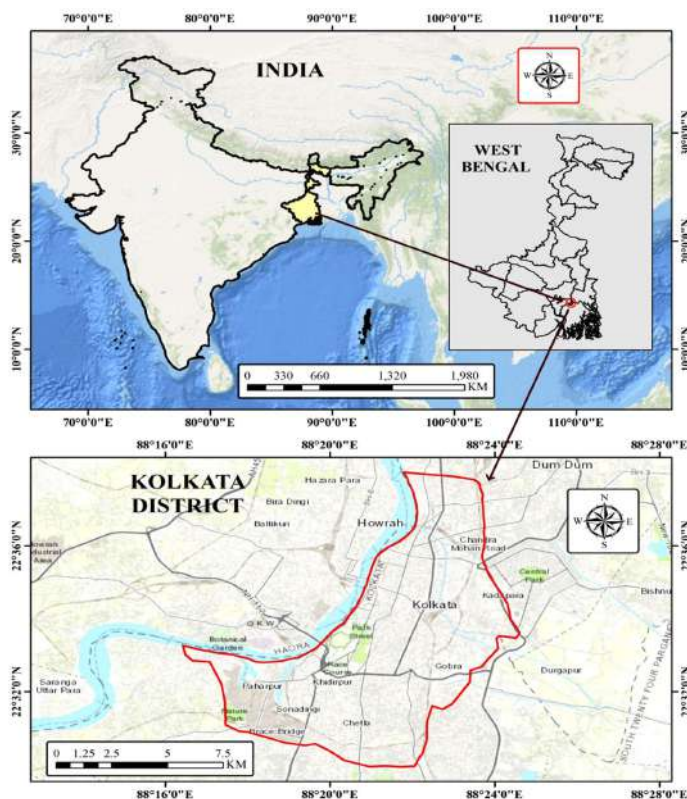


Fig. 1: Location map of the study area.

surface air temperature. Kolkata district is currently facing huge demographic pressure. A rapid population growth of 3.94% between 1991 and 2001 is visible in the Kolkata district. Additionally, the district was home to a population of 4,496,694 with a density of 24,306 people per sq. km (Census 2011), solidifying its status as one of the largest and densest cities. The greenery quotient of Kolkata is also one of the lowest in the country (Dhara 2019), and the city is experiencing water logging due to unplanned urban growth and massive population growth within the city (Paul 2021). A study on Kolkata by Shackleton et al. (2023) revealed a significant correlation between temperature and summer cholera, while they identified rainfall as the primary factor driving monsoon cholera. The meteorological condition, particularly the rising temperature and the present level of air pollutants in Kolkata also has the potential to impact public health drastically. Therefore, the city was selected in this study to investigate the trend of long-term climate parameters for understanding climate change by which its possible adverse impacts on the city can be minimized.

Data Source

Temperature and rainfall data have been accumulated from the Climate Research Unit, followed by Harris et al. (2020)

for the period from 1901 to 2019 (119 years). The station of CRU TS 4.04 temperature is CALCUTTA/ALIPO, which is centered at 22° 33' 54.22" N and 88° 21' 30.76" E (Fig. 1).

Data Analysis Tools

The current study examined rainfall and temperature data using trend and variability analysis. The variability was analyzed by applying the Precipitation Concentration Index/PCI (Oliver 1980 modified by De Luis et al. 2011), Coefficient of Variation/CV (Hare 2003), and anomalies (Agnew & Chappel 1999, Woldeamlak & Conway 2007, Viste et al. 2012, Gebreet et al. 2013, Asfaw et al. 2018). Nevertheless, non-parametric tests were used for trend analysis and detection. The MK test, a widely used method for identifying trends in climatological variables (Mekonnen & Woldeamlak 2014, Tabari et al. 2015, Gebremedhin et al. 2016), as well as the non-parametric Spearman's Rho test, were used in this investigation. Since there is a likelihood of outliers being present in the dataset, the non-parametric MK test is useful because its statistic is based on the positive or negative (+ or -) signs rather than the values of the random variable, and, therefore, the trends examined are less affected by the outliers (Birsan et al. 2005). Furthermore, World Meteorological Data has been extensively recommended for this test for free exercise to the public to

measure trends (Shi et al. 2013) and to identify the statistically significant trends of long-term data. The Spearman's rho (SR) test is a rank-based non-parametric statistical test that can also be used to detect monotonic trends in a time series (Lehmann 1975, Sneyers 1990). Therefore, the power of both methods relies on the size of the sample, pre-ascribed significance level, magnitude of the trend, and degree of variation within a time series. That is, the higher the absolute magnitude of the trend, the tests become more powerful with the increase in sample size, tests become more powerful, and the power of the tests decreases with the increase of the degree of variation within a time series. So, two tests have identical power in detecting a trend (Yue et al. 2002).

Sen's (2012) Innovative Trend Analysis (ITA) was also used for trend analysis. Recently, the ITA method has been applied extensively for trend detection of meteorological and hydrological parameters in different parts of the world (Dabanlı et al. 2016, Tabari et al. 2017, Zhou et al. 2018, Ahmas et al. 2018, Caloiero et al. 2018). Some of the researchers believed that the ITA method has more

advantages and is also more practical in detecting hydro-meteorological series trends than the MK test (Dabanlı et al. 2016, Wu & Qian 2017). The study's location map was constructed using the QGIS 3.28, and data analysis was done using SPSS (v21.0) and Excel spreadsheets (Fig. 2).

RESULTS

Temperature Trend in Kolkata District (1901-2019)

Annual temperature ranged between 25.19 to 28.02°C, with a mean value of 26.77 ($\pm 0.42^\circ\text{C}$). CV was found to be highest in March with 4.73% and lowest in June with 1.26%. The mean annual temperature was found to be largely constant (CV=1.56%) than the mean winter and mean summer temperature, whereas the mean summer was more consistent (CV=2.77%) than the winter mean temperature (CV=3.39%) (see Table 1).

Mann-Kendall and rho statistics both demonstrated a substantial ($p < 0.01$) increasing tendency in the average annual ($\square = 0.33$, $\rho = 0.46$), summer ($\square = 0.23$, $\rho = 0.34$),

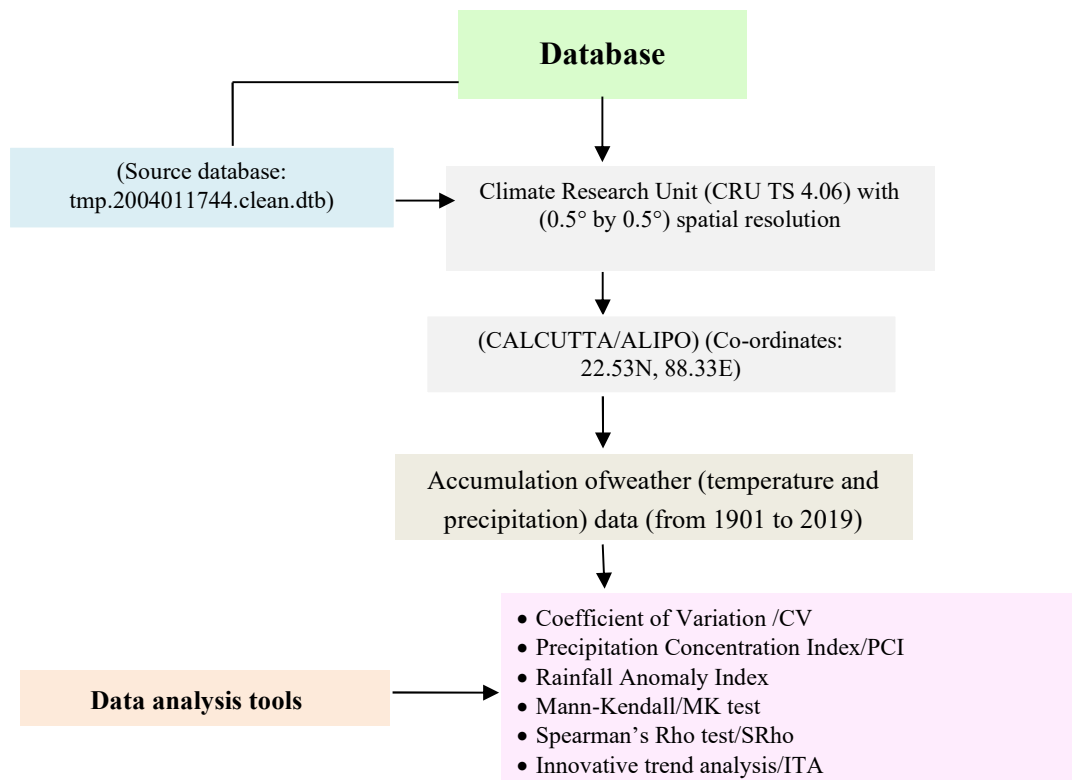


Fig. 2: Data source and analysis techniques.

Table 1: Trend of seasonal and annual temperature.

Seasonal/Annual	Minimum(°C)	Maximum(°C)	Mean(°C)	SD	CV	ρ	τ
Summer	25.73	31.86	29.61	.82	2.77	.34***	.23***
Winter	16.98	23.06	20.92	.71	3.39	.33***	.24***
Annual	25.19	28.02	26.77	.42	1.56	.46***	.33***

τ , Kendall's tau, ρ , spearman's Rho

Note: significance value *** $p < 0.01$, ** $p < 0.05$

and winter ($\tau = 0.24$, $\rho = 0.33$) temperatures (see Table 1). Correspondingly, the Mann-Kendall trend statistics indicate a significant ($p < 0.01$) rising trend in the average temperature of every month except January, while the result of rho shows a noteworthy ($p < 0.05$ and $p < 0.05$) increasing trend in every month except January (Table 2).

The average annual temperature was observed to be rising in the anomaly graph (Fig. 3). It is evident from the

graph that the temperature trend line indicates a gradual but steady increase in temperature over time (Fig. 3), and the association between anomaly and time revealed a positive relationship ($r = +0.47$). The result of ITA in the case of temperature has confirmed the ascendancy of increasing trend throughout the considered period, as the scatter points are aggregates mostly above the trendless line (Fig. 4).

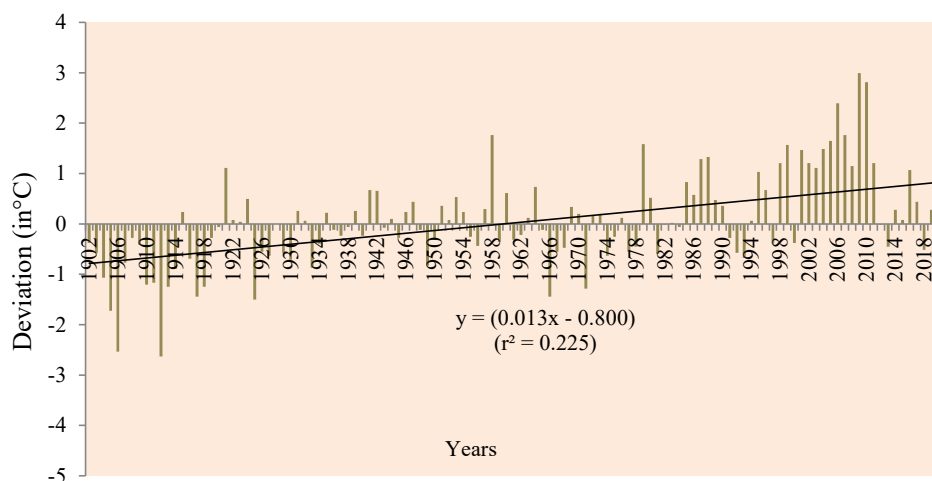


Fig. 3: Temperature anomaly.

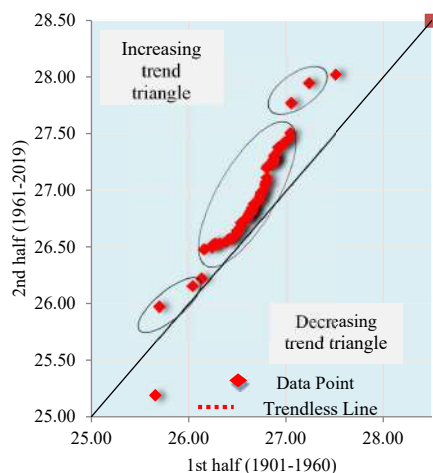


Fig 4: ITA graph of temperature.

Table 2: Status of monthly temperature and trend statistics.

Months	Minimum(°C)	Maximum(°C)	Mean(°C)	SD	CV	ρ	τ
January	15.67	22.1	19.90	0.83	4.17	.05	0.04
February	19	25.9	22.69	0.97	4.27	.32***	.23***
March	17.1	29.8	27.46	1.30	4.73	.28***	.19***
April	21.5	33.76	30.41	1.24	4.08	.23**	.17***
May	21.5	32.7	30.97	1.10	3.55	.29***	.21***
June	28.3	31.8	30.35	0.69	2.27	.23**	.16***
July	28.4	30.4	29.25	0.37	1.26	.25***	.19***
August	27.7	29.9	28.92	0.39	1.34	.49***	.36***
September	27.38	29.9	29.09	0.37	1.27	.24***	.16***
October	26.1	29.90	27.86	0.56	2.01	.30***	.28***
November	20.83	26.8	24.11	0.90	3.73	.38***	.27***
December	16.29	23.3	20.17	0.90	4.46	.33***	.25***

τ , Kendall's tau; ρ , spearman's Rho

Note: significance value *** $p < 0.01$, ** $p < 0.05$

Rainfall trend in Kolkata District (1901-2019)

The annual rainfall, which varied between 889.50mm to 2056.62 mm, with an average of 1474.66 ± 230.66 mm, was a consistent feature throughout the long-term period from 1901 to 2019. However, in the monsoon season, the amount of rainfall was found to be highly variable, ranging between 658.60mm to 1654.90 mm. The average rainfall was 1109.29mm (± 202.39) (Table 3)

The monthly highest mean rainfall was recorded in August, measuring 311.46 ± 83.83 mm. In contrast, the lowest average monthly rainfall was observed in December, with a value of 6.94 ± 14.51 mm. In September 1986, the region received the highest recorded rainfall, reaching a maximum of 614.70 mm. Contrariwise, months such as

January to April and October to December recorded the lowest rainfall, with a minimum of 0 mm, as shown in Table 4. Highest rainfall was observed during the monsoon season (Fig. 5).

The annual data of rainfall revealed higher consistency (CV=15.64%) than the remaining seasons, whereas the consistency of monsoonal rainfall is highest (CV=18.24%) among the seasons (see Table 3). The consistency was minimum and maximum, respectively, in August (CV=26.91%) and December (CV=209.07%). As a result, August experienced more consistent rainfall (Table 4).

Both MK and rho statistics demonstrated a considerable ($p < 0.05$) rising trend of monsoonal ($\rho = 0.18$, = 0.12) and annual ($\rho = 0.21$, = 0.14) rainfall. However, no significant

Table 3: Trend of seasonal and annual rainfall.

Measures	Rainfall [mm]				
	Pre-monsoon rainfall [mm]	Monsoon rainfall [mm]	Post-monsoon Rainfall [mm]	Winter rainfall [mm]	Annual Rainfall [mm]
Minimum	55.9	658.60	5.50	0.00	889.50
Maximum	450.90	1654.90	322.40	160.20	2056.62
Mean	199.53	1109.29	125.28	40.55	1474.66
SD	78.77	202.39	70.72	30.51	230.66
CV	39.47	18.24	56.44	75.24	15.64
ρ	0.09	0.18**	0.14	0.10	0.21**
	0.07	0.12**	0.09	0.07	0.14**

τ , Kendall's tau; ρ , spearman's Rho

Note: significance value *** $p < 0.01$, ** $p < 0.05$

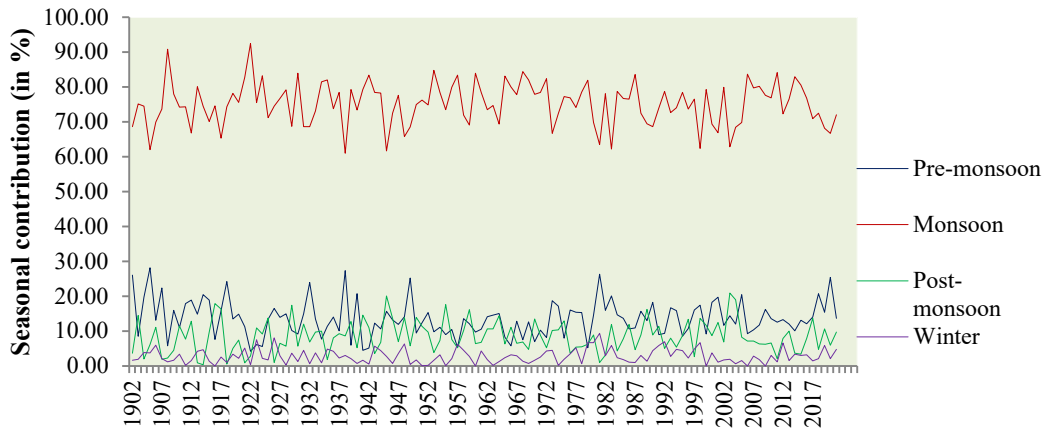


Fig. 5: Seasonal distribution of rainfall.

association with time was found for the remaining seasons (see Table 1). Considering the monthly rainfall, September exhibited a noteworthy ($p < 0.01$) increasing ($\square = 0.18$, $\rho = 0.27$) trend. Additionally, December also demonstrated

a substantial ($p < 0.05$) upward trend in rho statistics (see Table 4). The graph depicting the deviation in rainfall showed an upward trajectory in the amount of precipitation received each year. The correlation analysis between the duration

Table 4: Status of monthly Rainfall with trend statistics.

Months	Minimum (mm)	Maximum (mm)	Mean (mm)	SD	CV	ρ	τ
January	0.00	56.10	11.38	13.51	118.71	.032	.021
February	0.00	15.6	22.23	23.11	103.95	.003	.005
March	0.00	148.3	30.89	31.19	100.97	.029	.029
April	0.00	189.80	57.35	40.09	69.90	.123	.089
May	8.20	283.40	111.28	55.30	49.69	.067	.047
June	43.70	592.90	253.17	107.59	42.49	-.057	-.037
July	111.60	551.60	303.31	93.66	30.87	.127	.086
August	147.40	553.50	311.46	83.83	26.91	.109	.069
September	18.60	614.70	241.34	94.90	39.32	.27***	.18***
October	0.00	322.40	111.62	67.44	60.41	.109	.065
November	0.00	77.40	13.66	18.62	136.31	.146	.097
December	0.00	96.87	6.94	14.51	209.07	.174**	.122

τ , Kendall's tau, ρ , spearman's Rho

Note: significance value *** $p < 0.01$, ** $p < 0.05$

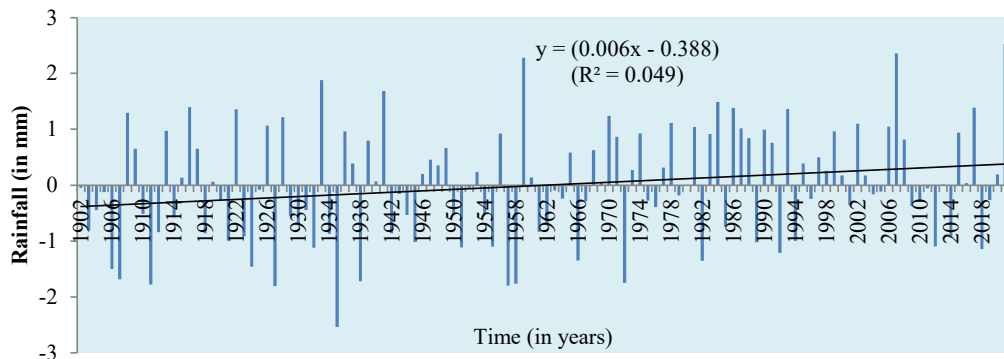


Fig. 6: Rainfall anomaly.

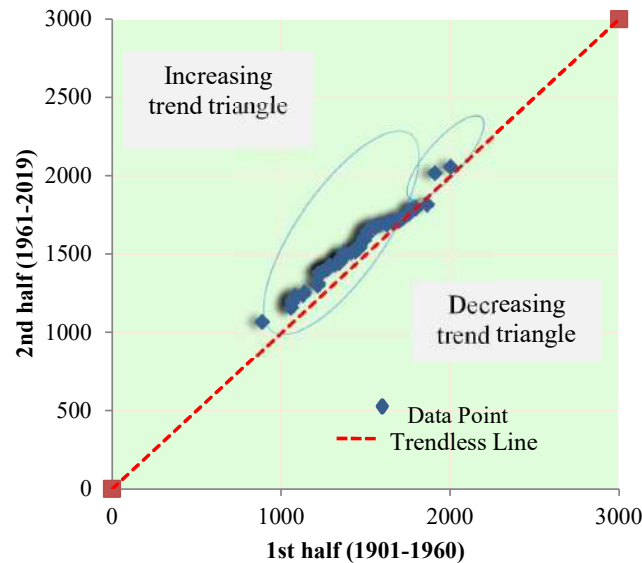


Fig. 7: ITA graph of rainfall.

(in years) and deviation from the average rainfall showed a positive correlation ($r=+0.22$). The trend of rainfall indicates a moderate and consistent rise over time (Fig. 6).

Innovative trend analysis (ITA) recognized the prevalence of increasing tendency throughout the study period, as most of the scatter points aggregated around the 1:1 line. Fig. 7 shows that most data points fall inside the positive trend triangle. The study duration (1901-2019) showed that the mean precipitation concentration index (PCI) was determined to be 17.45. This indicates that there were no years with little precipitation and, importantly, no uniform distribution of rainfall concentration ($PCI < 10$). From total study years, 16 have been found in moderate index, 79 have been found in high concentration index and the remaining 24 years have been observed to be very high (see Table 5). The PCI score confirms that the consistency and influence of monsoonal winds remain constant in Kolkata. Additionally, the PCI scores also increased by pre-monsoonal rainfall, largely due to intense thunderstorms (referred to locally as Kaal Baisakhi), also increased the PCI scores.

Table 5: Precipitation Concentration Index.

Index	Description	Number of years (1901-2019)
<10	Low concentration (almost uniform)	0
11-15	Moderate concentration	16
16-20	High concentration	79
≥ 21	Very high concentration	24

Note: Average PCI (1901–2019) = 17.45 (implied high concentration of Rainfall)

DISCUSSION

The present research aimed to analyze and understand the trends and fluctuations of two major meteorological characteristics, namely temperature, and rainfall in Kolkata from 1901 to 2019. The MK and Spearman's Rho tests were used to examine the weather parameters' monthly, seasonal, and annual trends. The overall results demonstrated a noticeable change in the research region's temperature and precipitation patterns throughout the analysis. The results of the analysis indicate that the annual and seasonal (winter and summer) temperatures, as well as the temperature throughout all the months, are rising significantly ($p < 0.01$). However, temperatures in January do not demonstrate any considerable trend. A previous study by Goswami (2018) indicates that the long-term mean minimum temperature data of Kolkata shows a declining trend in January that intensifies the cold spell during winter, resulting in a non-significant trend of temperature during this month of every year. The study's use of innovative trend analysis for statistical purposes also revealed an increasing temperature trend, as shown in the graph, where most scatter points were concentrated on the side of the positive trend triangle. Out of all the months, June had a consistent temperature. This intensifying temperature amplifies the risk of heat-associated diseases and water shortages in urban regions (Paul 2021, Ravindra et al. 2024). Throughout the research period, monsoonal and annual rainfall rose significantly. Regarding monthly distribution, rainfall amplified drastically only in September and December months. The highest CV was observed during the winter season, which implies more inter-annual variability for this season, whereas the lowest inter-annual variability

was observed in the monsoon season as a significant amount of rain occurs almost every year during this season due to the Indian Summer Monsoon (De et al. 2023)

The investigation of ITA and precipitation anomaly demonstrated a positive correlation between precipitation and slope across the duration of the research. The analysis found no persistent year with a low concentration of precipitation. However, the study noticed a high precipitation concentration for 79 years and a very high concentration for 24 years. Precipitation during the monsoon was found to be more consistent as the study shows less CV throughout the monsoonal months, i.e. June (42.49%), July (30.87%), August (26.91%), and September (39.32%). The variation between the maximum and minimum rainfall levels was significant during the monsoon season. A study by Hunt & Fletcher (2019) suggests that the central Bay of Bengal (BoB) is the region of strongest synoptic organization. Hence, in the present study, the variation that is observed in monsoon season rainfall may be attributed to the modulation by synoptic-scale tropical low-pressure areas (LPAs), the strongest of which is known as monsoon depressions. In addition, the Indian summer monsoon is a part of global circulation and exhibits variability in a variety of time scales, such as intra-seasonal, inter-annual, and inter-decadal, which may be connected to climate variations of a more global scale (Naidu et al. 2011). The present study's findings on temperature aligns with the other study done by various researchers in different parts of India taking different historical periods and found that temperature is significantly rising with time (Kothawale & Rupa 2005, Jaswal 2010, Choudhuri et al. 2012, Mandal et al. 2021, Das & Bhattacharya 2018).

The present study shows that monsoonal rainfall rose significantly throughout the study period, which may cause Pluvial flooding in Kolkata. Although this flooding is produced by various factors such as the natural slope, increasing concretization, indiscriminate dumping, and logging of drainage lines (Paul 2021), situated low in the Ganga delta is also a reason which makes the city more vulnerable to climate change (Randhawa 2024). Besides, the unassuming flooding at the high tide in Hoogly River is a recurring hazard in Kolkata (Dasgupta et al. 2013). Hence, more intense rainfall especially during monsoon, can lead to pervasive and severe flooding and bring the city to a standstill for many days.

The investigation on long-term trends of temperature on a monthly, seasonal, and annual basis, as well as the distribution of precipitation in Kolkata, indicates that various human-induced causes such as loss of vegetation and expansion of heat absorbing surfaces, livelihood, and

consumption trend together with fuel burning and emission rates affect the magnitude of pollution in the city (Das 2022) and extensively linked with constant increase of surface temperature. Since the 1960s, India has seen a rapid rise in carbon dioxide (CO₂) emissions, mainly as a result of several anthropogenic activities for economic development. These activities also indirectly contribute to global warming. The increased range of these short-term climates forcing (SLCPs) pollutants traps excess heat and results in local climate warming. Raising temperatures and air pollution have a severe impact on public health since they aggravate respiratory and cardiovascular disorders, as well as a variety of chronic illnesses, including diabetes, cerebrovascular and renal disease.

Therefore, the present study suggests more government investments and initiatives to reduce greenhouse gas emissions and improve environmental quality by managing air pollutants in the area. Because over emission of greenhouse gases by human activities has led to the phenomenon of global warming and resulted in climate change (Prarikeslan et al. 2023). The urban planners, policymakers, and environmental managers in Kolkata should identify the root causes of climate change-related vulnerabilities the city may face, including natural system and socio-economic factors. Focusing more on a time-bound qualitative approach could help in assessing climate risk and adaption needs of the city, including: 'The City Resilience Index' (CRI), the 'Urban Adaptation Support Tool', and the 'UKCIP Adaptation Wizard' (Metroeconomica 2004), 'Climate Smart Cities Assessment Framework' (NIUA 2021). Additionally, multi-pronged approaches such as improving drainage systems, creating green spaces, and Adapting building codes to future climate settings and severe weather events (European Environment Agency 2023) could make the city more climate-friendly and less susceptible to climate change. IPCC (2007), after evaluating the deviation and distribution of climatic parameters, has also recommended developing an adaptation and preparedness plan at the regional level to deal with the changing climate. This will help in determining a sustainable method to formulate a rural-urban ecological conservation and environmental plan. In addition, management of land use and strict policies regarding population control could also be necessary to fulfill SDG 13 and make the city more inclusive, safe, resilient, and sustainable (SDG 11).

Strengths, Limitations, and Future Research Directions

This study is limited to the coarse resolution of CRU data, which may not capture small-scale variations of climatic parameters completely. Additionally, this study examined

rainfall and temperature parameters, which may not express Kolkata's complex climate change trends. Adding humidity, air pressure, urban heat island effects, and other microclimates may assist in a better understanding of climate change in the study area.

To improve the accuracy of the variation of Observational station data, high-resolution satellite images and machine learning-based modeling are needed to improve the accuracy and also to capture the fine-scale variation and climate change.

CONCLUSIONS

The primary aim of this study was to explore the temporal fluctuations in monthly, seasonal, and yearly meteorological factors in the city of Kolkata, India. The findings indicate a significant increase in both seasonal (winter and summer) and yearly temperatures, as well as rainfall. The monthly data clearly shows a consistent upward trend in temperature across all months. The statistical evaluation of the innovative trend also indicated an upward tendency in both temperature and rainfall. The PCI shows a significant amount of precipitation, ranging from high to extremely high levels, throughout the majority of the years included in the research. Both the Mann-Kendall and Spearman's Rho statistics detected a substantial upward trend in the average summer, winter, and annual temperature. Furthermore, a considerable upward trend was discovered in the annual and monsoon season rainfall. The "United Nations World Meteorological Organization" demonstrated the risk of climate change, stating that "we are the first generation to fully understand it and the last generation to be able to do something about it". Our study's greatest strength is its contribution to the existing body of literature. We specifically focus on the temporal variation of climatic parameters in Kolkata, where various factors such as overpopulation, changes in social needs and demand, and the increase in factories with inadequate technological advancements have directly and indirectly contributed to the unpredictable nature of the environment. Given the urgency of the situation, immediate action is crucial to diminish the consequences of climatic issues.

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Nutrient Dynamics and Recovery Potential in the Tukad Badung River, Indonesia

I. Made Wahyu Wijaya^{1†} , I. G. D. Yudha Partama¹, I. Ketut Sumantra² , Kailas Deoram Ahire³ and Fransiskus Vebrian Kenedy⁴

¹Regional Planning and Rural Area Study Program, Postgraduate Program, Universitas Mahasaraswati Denpasar, Jalan Kamboja No. 11 A, Denpasar, Bali, 80233, Indonesia

²Agrotechnology Study Program, Faculty of Agriculture and Business, Universitas Mahasaraswati Denpasar, Jalan Kamboja No. 11 A, Denpasar, Bali, 80233, Indonesia

³Department of Environmental Science, K.R.T. Arts, B.H. Commerce, A.M. Science (KTHM) College, Nashik, Maharashtra, India

⁴Environmental Engineering Study Program, Faculty of Engineering, Universitas Mahasaraswati Denpasar, Jalan Kamboja No. 11 A, Denpasar, Bali, 80233, Indonesia

†Corresponding author: I. Made Wahyu Wijaya: wijaya@unmas.ac.id

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ABSTRACT

This study explores the spatiotemporal variations in nitrogen and phosphorus pollutants in the Tukad Badung River, an essential water source for Bali's communities, increasingly impacted by agricultural, domestic, and industrial discharges. Bi-daily sampling at six strategically selected sites along the river's 18-kilometer stretch revealed substantial fluctuations in water quality, with downstream sites consistently exhibiting elevated pollutant concentrations. Ammonia concentrations varied from 1.5 to 4.2 mg.L⁻¹, nitrate levels ranged from 5.0 to 11.6 mg.L⁻¹, and total phosphorus concentrations spanned 0.5 to 2.5 mg.L⁻¹, all of which were highest during afternoon sampling, likely due to reduced flow and increased anthropogenic inputs. Total suspended solids (TSS) exhibited temporal and spatial variability, ranging from 80 to 127 mg.L⁻¹, with the highest concentrations observed at midstream sites, suggesting localized sedimentation from human activities. The nutrient dynamics displayed marked temporal variations, with concentrations rising during afternoon hours, reflecting shifts in human activity and changes in river flow conditions. Furthermore, the study assessed nutrient recovery technologies, such as precipitation and adsorption, which were able to recover up to 80% of extractable nutrients. These findings not only characterize the pollution trends but also highlight the potential of nutrient recovery techniques in reducing dependency on synthetic fertilizers. This research emphasizes the need for integrated watershed management and adaptive recovery strategies to mitigate nutrient pollution and enhance the sustainability of river ecosystems for future generations.

INTRODUCTION

Rivers are vital ecosystems that support numerous communities worldwide, providing essential water resources for domestic, agricultural, and industrial use. However, rapid urbanization and industrialization have significantly impacted the health of river systems, leading to widespread pollution that threatens water quality and ecosystem integrity (Khonok et al. 2022, Wu et al. 2023). Nevertheless, the swift process of urbanisation and industrialization has resulted in concerning levels of pollution in numerous river systems, posing a threat to both the quality of water and the health of ecosystems. In particular, the Tukad Badung River, located in Bali, Indonesia, faces increasing pollution from agricultural runoff, domestic wastewater, and industrial discharges (Wijaya et al. 2023). This has resulted in elevated levels of nitrogen and phosphorus, key contributors

to eutrophication and degradation of aquatic habitats (Ciawi et al. 2022).

The Tukad Badung River covers approximately 30 kilometers and has a watershed area of 37.7 square kilometers. It is an important water source for nearby villages (Nyoman Wiarta et al. 2008). Despite its significance, the river is under constant threat from the influx of solid and liquid waste generated by various human activities within its watershed. The increased flow of pollutants, particularly nitrogen and phosphorus compounds, poses a serious threat to the river's ecological balance and the welfare of the downstream community (Ciawi et al. 2022). An interdisciplinary research effort is essential to address the complex challenges of river pollution (Rey-Martínez et al. 2024a, Romero et al. 2021). The present study aims to investigate the complex interactions of nitrogen and phosphorus pollution in the Tukad Badung River, with a focus on identifying methods to extract these nutrients from the stream water. This research is driven by a strong commitment to both environmental stewardship and the ideals of the circular economy.

This study is centred around an innovative method for preserving rivers and managing their resources. This project aims to explore the patterns of nitrogen and phosphorus levels in stream water and develop innovative methods for recovering nutrients. Its goal is to establish a connection between environmental conservation and the sustainable use of resources. Recent studies on nutrient recovery from polluted waters have highlighted innovative approaches to managing nutrient-rich wastewater, with potential for restoring water quality and reducing the environmental impact of nutrient discharges (Betti & Nurhayati 2022, Katkaew & Chamchoi 2024, Li et al. 2024). Methods such as nutrient precipitation, adsorption, and biological treatments have been shown to recover valuable resources, including nitrogen and phosphorus, thus contributing to the circular economy (Antunes et al. 2022, Zarei, 2020). However, while these methods have been explored in various contexts, there remains a significant gap in applying such strategies to river ecosystems, particularly in Southeast Asia, where nutrient management is often insufficient or overlooked (Sauvé et al. 2021). Given the current state of rivers globally, which is characterised by an unparalleled degree of pollution, it is crucial to urgently devise efficient and scalable measures to protect these precious ecosystems (Suwarno et al. 2014, Vigiak et al. 2023, Wijaya et al. 2023). This research aims to fill that gap by investigating nutrient dynamics in the Tukad Badung River and exploring recovery techniques in alignment with circular economy principles. By focusing on the potential for sustainable nutrient recovery, this study contributes to a growing body

of work on managing river pollution through innovative, resource-efficient approaches. While previous studies have primarily concentrated on wastewater treatment plants, few have examined the spatial and temporal variations of nutrients in river systems, especially in regions with limited pollution control infrastructure (Rey-Martínez et al. 2024b). This study thus positions itself at the intersection of river pollution management and sustainable resource recovery, offering novel insights into the application of circular economy principles in river ecosystems. By integrating meticulous data collection, analysis, and experimentation, this research seeks to provide a valuable contribution to the growing field of river conservation and sustainable water management, as well as promote the concepts of the circular economy worldwide (Díaz et al. 2024, Piash et al. 2021).

Incorporating circular economy concepts into river management involves not only mitigating pollution but also recovering valuable resources such as nitrogen and phosphorus, which can be transformed into fertilizers or bioenergy substrates (Slootweg 2020, Zvimba et al. 2021). Such strategies not only reduce dependency on synthetic fertilizers but also enhance resource efficiency and support long-term ecological sustainability. Therefore, this study advocates for integrated watershed management strategies that embrace both pollution control and resource recovery, ensuring the resilience of river ecosystems while contributing to sustainable development goals.

MATERIALS AND METHODS

The methodology employed in this study involves a systematic approach to data collection, analysis, and experimentation aimed at elucidating nitrogen and phosphorus trends in the Tukad Badung River and exploring methods for nutrient recovery from stream water. The research is structured into distinct phases, each designed to achieve specific objectives with a methodological rigor. Before commencing fieldwork and laboratory experiments, meticulous preparations were undertaken to ensure the smooth execution of the research activities. Additionally, a comprehensive inventory of equipment, materials, and personnel was conducted to facilitate efficient data collection and analysis throughout the research period.

Sampling Points

Sampling points were strategically selected along an 18-kilometer stretch of the Tukad Badung River, divided into upstream, midstream, and downstream zones (Fig. 1). This zoning approach enables the capture of spatial variations in nutrient concentrations along the river's course. Six sampling points were designated, with two points in each zone, to

ensure adequate coverage of the river's spatial dynamics. The selection of sampling points adhered to established guidelines outlined in SNI 03-7016-2004 Procedure for Water Quality Sampling in River Drainage Areas for Monitoring Purposes, ensuring consistency and reliability in data collection. The sampling coordinates are provided in Table 1.

The selection of these locations aimed to represent the diverse pollution sources within the river's watershed and to reflect variations in water quality as influenced by agricultural runoff, domestic wastewater, and industrial effluents.

- **Upstream locations (Sampling Points 1 and 2)** were chosen to capture the relatively cleaner water entering the river before major pollution sources.
- **Midstream locations (Sampling Points 3 and 4)** represent areas influenced by both upstream

contributions and localized pollution from nearby settlements and industries.

- **Downstream locations (Sampling Points 5 and 6)** capture the cumulative effects of pollution, with higher concentrations of nutrients likely due to agricultural runoff, domestic wastewater, and reduced dilution from lower flow conditions.

This design ensures that the sampling points span the river's pollution gradient, enabling a thorough analysis of nutrient dynamics along the river. The rationale for selecting these six points is supported by the literature on river pollution dynamics, which emphasizes the need for a spatially representative sampling strategy to understand variations in nutrient concentrations and their impact on river ecosystems (Betti & Nurhayati 2022, Ciawi et al. 2022).

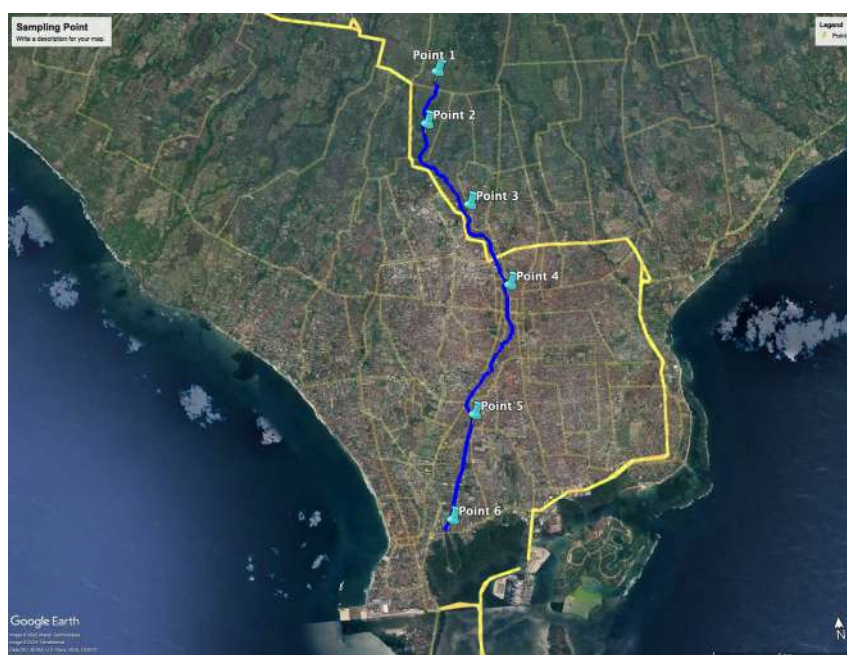


Fig. 1: Sampling points in Tukad Badung River.

Table 1: Sampling point coordinates.

No	Sampling Point	Coordinates		Zone
		Latitude	Longitude	
1	Point 1	8°34'57.98"S	115°11'43.69"E	Upstream
2	Point 2	8°35'54.35"S	115°11'27.07"E	Upstream
3	Point 3	8°37'27.85"S	115°12'7.48"E	Midstream
4	Point 4	8°39'0.50"S	115°12'45.12"E	Midstream
5	Point 5	8°41'21.13"S	115°11'53.05"E	Downstream
6	Point 6	8°43'16.77"S	115°11'18.22"E	Downstream

Sample Collection

Sampling activities were conducted twice daily, at 08:00 am and at 07:00 pm, to capture diurnal variations in nutrient levels and peak domestic wastewater conditions. The 08:00 am represents the morning sampling, when domestic and industrial discharges are typically lower, and the water quality is less impacted by human activities. The 07:00 pm was selected for the afternoon sampling to capture peak pollution times, as this period often coincides with higher domestic wastewater inputs and lower river flow due to reduced hydrological activity. Additionally, nutrient concentrations such as ammonia and phosphate tend to be elevated in the afternoon due to the accumulation of pollutants throughout the day and reduced water flow, which limits dilution. This diurnal variation in sampling times is consistent with findings from other studies that demonstrate the significant impact of human activity on water quality, particularly during peak hours when domestic wastewater and industrial effluents contribute to higher nutrient concentrations (Conley et al. 2009). These time intervals help provide a comprehensive view of the temporal dynamics of nutrient concentrations within the river. At each sampling point, water samples were collected using standardized techniques outlined in the SNI 6989.57-2009 standard for surface water sampling methods. A total of five litres of water was collected from each sampling point during each sampling event, ensuring an adequate sample size for subsequent laboratory analysis.

Laboratory Analysis

Upon collection, water samples were transported to the laboratory for a comprehensive analysis. The key water quality parameters include total suspended solids (TSS), ammonia (NH_3^-), nitrite (NO_2^-), nitrate (NO_3^-), total nitrogen (Total N), and total phosphorus (Total P) were analysed by following the standard methods (APHA/AWWA/WEF 2017). Spectrophotometric analyses were conducted using state-of-the-art equipment and methodologies to ensure accuracy and precision in measuring nutrient concentrations.

Data Analysis

Data collected from both field sampling and laboratory experiments were subjected to rigorous statistical analysis to identify spatial and temporal trends in nitrogen and phosphorus concentrations along the Tukad Badung River. Additionally, graphical representations such as maps and trend charts were generated to visualize spatial and temporal variations in nutrient concentrations, aiding in the interpretation of research findings. The culmination of data analysis and experimentation enabled the interpretation of findings and the formulation of evidence-based

conclusions. The implications of the observed nutrient trends and the efficacy of nutrient recovery strategies were critically evaluated in the context of river conservation and sustainable water management. Insights derived from this study contribute to the body of knowledge surrounding nutrient dynamics in river ecosystems and provide valuable guidance for policymakers, environmental practitioners, and stakeholders involved in water resource management and conservation efforts.

An examination of nitrogen and phosphorus levels at six specific sampling locations along the Tukad Badung River yields useful information about the spatial and temporal changes in nutrient pollution within the river ecosystem. The morning sample session at 08:00 am and the afternoon sampling session at 07:00 pm exhibited clear patterns in nutrient levels, indicating fluctuations throughout the day and potential sources of pollution within the watershed. The following analysis provides a comprehensive examination of each metric, followed by a discussion of its impact on water quality and aquatic life.

RESULTS AND DISCUSSION

The nutrient concentrations in the Tukad Badung River are varied across different sampling points, with higher concentrations observed downstream. This spatial variation can be attributed to a combination of natural processes and anthropogenic activities within the watershed. The land use map of Denpasar City, shown in Fig. 2, illustrates the land use surrounding the sampling points. Upstream areas (Sampling Points 1 and 2) generally exhibited lower nutrient concentrations, likely due to limited human activity and agricultural runoff. However, as the river flows downstream, nutrient concentrations increase, particularly at Sampling Points 5 and 6, which are more heavily influenced by urban areas and home industrial effluents. The increased nutrient concentrations in these areas could be linked to runoff from agricultural lands, wastewater discharges from domestic and industrial sources, and reduced dilution capacity in stagnant flow conditions. The land use in the surrounding watershed, including agriculture, urbanization, and industrial zones, significantly contributes to the nutrient dynamics observed in these areas (Ciawi et al. 2022). The overall measured parameter concentration is shown in Table 2.

Nutrient concentrations in the Tukad Badung River demonstrated clear temporal variations, with higher values recorded during afternoon sampling (07:00 pm) compared to morning sampling (08:00 am). This pattern can be explained by several factors. Diurnal biological activity, such as increased microbial decomposition of organic matter, typically leads to elevated nutrient levels in the afternoon.

Additionally, the discharge of domestic wastewater tends to be higher during peak hours, contributing to increased nutrient loads in the afternoon. Furthermore, reduced flow rates during the afternoon decrease the river's ability to dilute pollutants, exacerbating the accumulation of nutrients. These temporal dynamics are influenced by both ecological processes, such as biological activity and sedimentation, as well as socio-economic factors, including human activities

that lead to higher discharges of domestic and industrial waste during afternoon hours (Conley et al. 2009).

Total Suspended Solids (TSS)

The Total Suspended Solids (TSS) values exhibited substantial variability across different sampling stations and time points, reflecting both spatial and temporal fluctuations in sediment loads and water quality dynamics. Spatially,

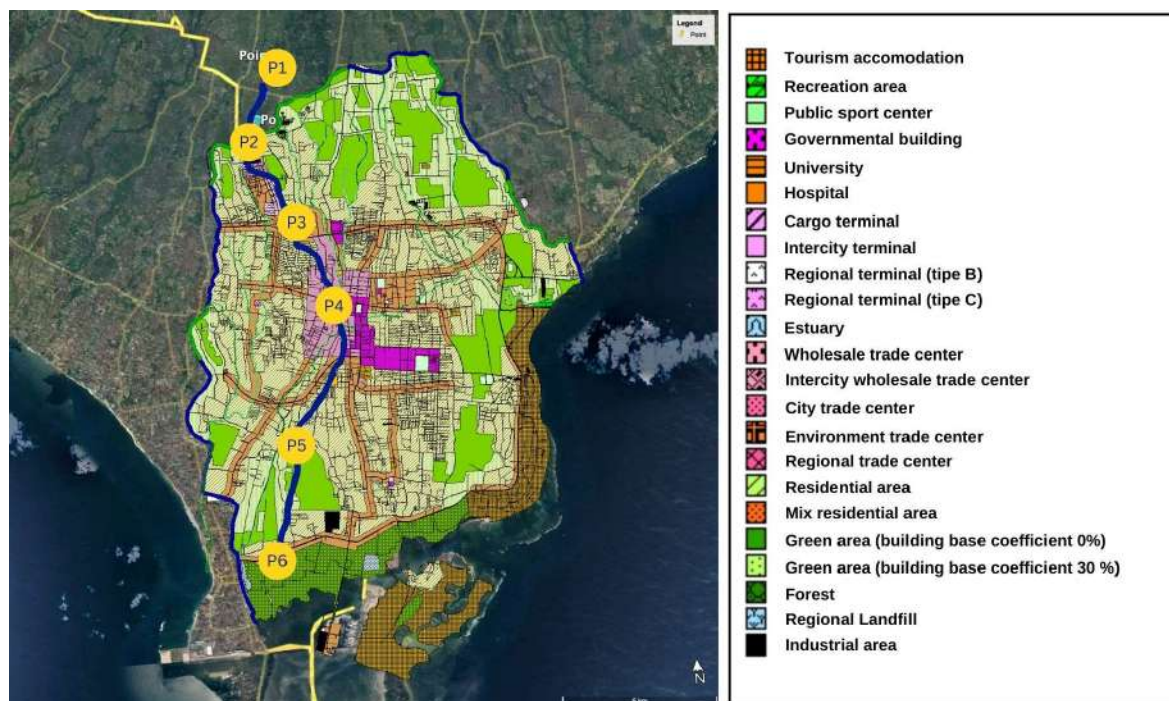


Fig. 2: Land use map of Denpasar City surrounding the sampling points.

Table 2: The concentration of measured parameters (mg/L).

Parameters	Sampling Time	Sampling Points					
		1	2	3	4	5	6
Total Suspended Solids	08.00 am	12.00	19.00	20.00	18.00	9.00	12.00
	07.00 pm	18.00	20.00	32.00	14.00	17.00	10.00
Ammonia (NH ₃ -N)	08.00 am	0.57	0.00	0.09	0.25	1.54	1.96
	07.00 pm	0.62	0.00	1.27	0.19	2.00	0.68
Nitrite (NO ₂ -N)	08.00 am	0.09	0.00	0.03	0.13	0.10	0.16
	07.00 pm	0.77	0.00	0.70	0.93	0.73	0.91
Nitrate (NO ₃ -N)	08.00 am	1.22	1.39	1.23	1.91	1.41	1.19
	07.00 pm	4.58	1.89	9.21	2.90	3.40	3.45
Total Phosphate	08.00 am	0.01	0.07	0.14	0.28	0.65	0.49
	07.00 pm	0.14	0.00	0.48	0.31	0.70	0.52
Total Nitrogen	08.00 am	1.89	1.39	1.36	2.29	3.06	3.31
	07.00 pm	5.97	1.89	11.17	4.02	6.14	5.03

TSS concentrations (as shown in Fig. 3) demonstrated notable differences between sampling locations. Sample Points 3 and 4, situated in the central section of the stream, consistently recorded higher TSS levels than the upstream (Sample Points 1 and 2) and downstream (Sample Points 5 and 6) sites. This spatial distribution suggests that sedimentation and erosion in the middle reaches of the river are concentrated in particular zones, likely influenced by anthropogenic activities such as agriculture, construction, and urbanization (Baiyin et al. 2024, Silva-Gálvez et al. 2024, Yuan et al. 2023). The TSS levels exhibited temporal variation between morning and afternoon sampling sessions. Generally, TSS concentrations tended to increase during the afternoon sessions. This pattern may be attributed to sediment resuspension and heightened water flow from adjacent land areas, which likely occurred during daylight hours. (Noor et al. 2023, South & Nazir 2016, Wang & Liu 2023). The fact that sediment transport processes within the river ecosystem vary during the day emphasises the dynamic nature of these processes. It also emphasises the need to consider changes over time when assessing water quality.

Elevated TSS (total suspended solids) concentrations can have substantial consequences for the hydrodynamics of water streams and the biodiversity of aquatic ecosystems. Excessive sedimentation can hinder water clarity, leading to a decrease in the amount of light that can penetrate the water and lowering the ability of aquatic plants to carry out photosynthesis (Khonok et al. 2022, Soedjono et al. 2018, Suantara et al. 2020). Consequently, this can disturb the habitats at the bottom of the river and modify the natural equilibrium of the river ecosystem. In addition, water containing silt can suffocate aquatic environments, such as

gravel beds and riffles, which are vital for fish reproduction and the habitat of macroinvertebrates. In addition, high levels of total suspended solids (TSS) can lead to greater turbidity, which decreases visibility for aquatic creatures and hinders predator-prey interactions (García-Avila et al. 2023, Rowland et al. 2021, Ural-Janssen et al. 2024). Suspended silt particles can obstruct the respiratory organs of fish, disrupt the feeding process of filter-feeding animals, and inhibit the growth of fish embryos and larvae. As a result, elevated levels of total suspended solids (TSS) can harm the diversity of aquatic life, causing a decrease in populations and degradation of the environment over a period (Dory et al. 2024, Qiu et al. 2024, Sumantra et al. 2023).

Multiple reasons can lead to elevated levels of Total Suspended Solids (TSS) in river water, such as soil erosion originating from agricultural fields, construction sites, and deforested areas (Wiegers & Larsen 2024, Zarei, 2020). Urban runoff, which includes particles from roads, rooftops, and paved surfaces, can increase the concentration of total suspended solids (TSS) in river systems. In addition, natural phenomena such as bank erosion, streambed scouring, and the suspension of sediment during periods of high flow can increase the amount of silt in rivers (Darji et al. 2022, Nugraha et al. 2020). Human activities, such as clearing land, mining, and inappropriate waste disposal, can worsen sedimentation problems by increasing the pace at which soil erodes and silt is carried into bodies of water. Insufficient soil conservation methods, such as inappropriate land management and deforestation, can exacerbate sedimentation issues, resulting in diminished water quality and the loss of habitats in river ecosystems (Kaown et al. 2023).

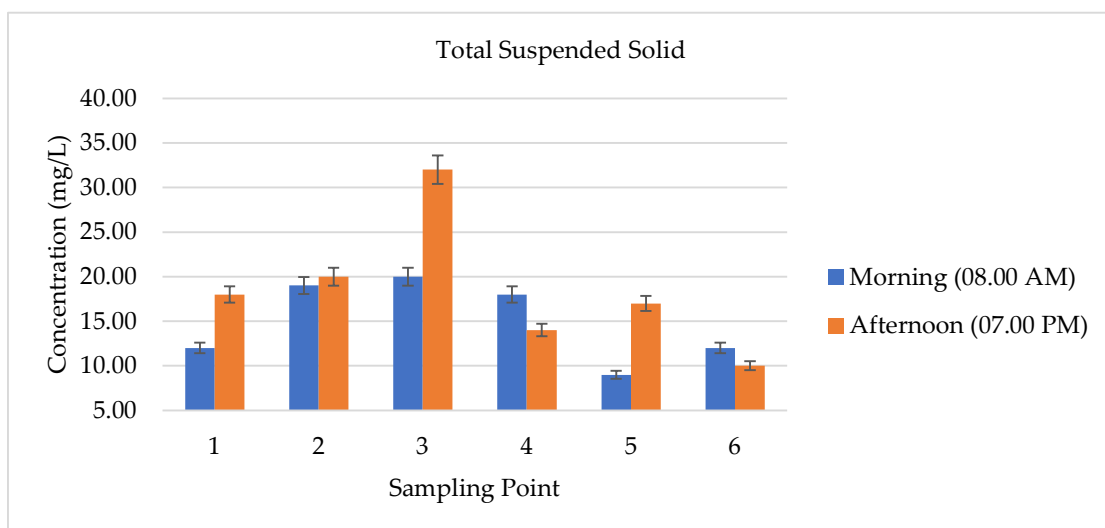


Fig. 3: Total suspended solid (TSS) concentration trend.

The examination of TSS concentrations in the Tukad Badung River emphasises the fluctuation in sediment loads across space and time and the possible consequences of elevated TSS levels on the quality of water and aquatic organisms. To tackle sedimentation problems, it is necessary to implement holistic approaches to managing watersheds. These approaches should incorporate steps to conserve soil, plan land use effectively, and implement erosion control procedures (Darji et al. 2022). By doing so, we may reduce the amount of sediment that is transported to water bodies and protect river ecosystems for future generations.

Nutrient Concentration

The analysis of nutrient dynamics in the Tukad Badung River highlights distinct temporal and spatial variations in ammonia, nitrate, and phosphorus concentrations. These parameters exhibited similar trends across sampling points, with higher concentrations found downstream and during afternoon sampling sessions. The increases in these nutrient concentrations are likely driven by anthropogenic influences, such as urban wastewater discharges and agricultural runoff. Notably, total phosphorus and ammonia concentrations were highest at downstream sites, reflecting the cumulative effects

of human activities, while nitrate concentrations showed a more consistent increase from upstream to downstream. These trends suggest that nitrogen and phosphorus are primarily introduced into the river through domestic and industrial waste, as well as agricultural activities that contribute to nutrient loading. According to Table 2, the trends comparison between each nutrient concentration is provided in Fig. 4.

The levels of ammonia showed noticeable variations in both space and time, indicating the impact of specific locations and daily changes in pollution levels. Geographically, according to Fig. 4, there were significant differences in ammonia concentrations among the sampling stations. Elevated concentrations of ammonia were consistently detected at the downstream sample points (Sample Points 5 and 6) in comparison to the upstream (Sample Points 1 and 2) and midstream (Sample Points 3 and 4) locations. The observed arrangement of locations indicates that there are specific sources of pollution, such as industrial waste or urban runoff, that are causing higher levels of ammonia in the lower parts of the river (Fig. 2). Temporally, ammonia concentration varied between the morning and afternoon sampling. Typically, the levels of ammonia were greater

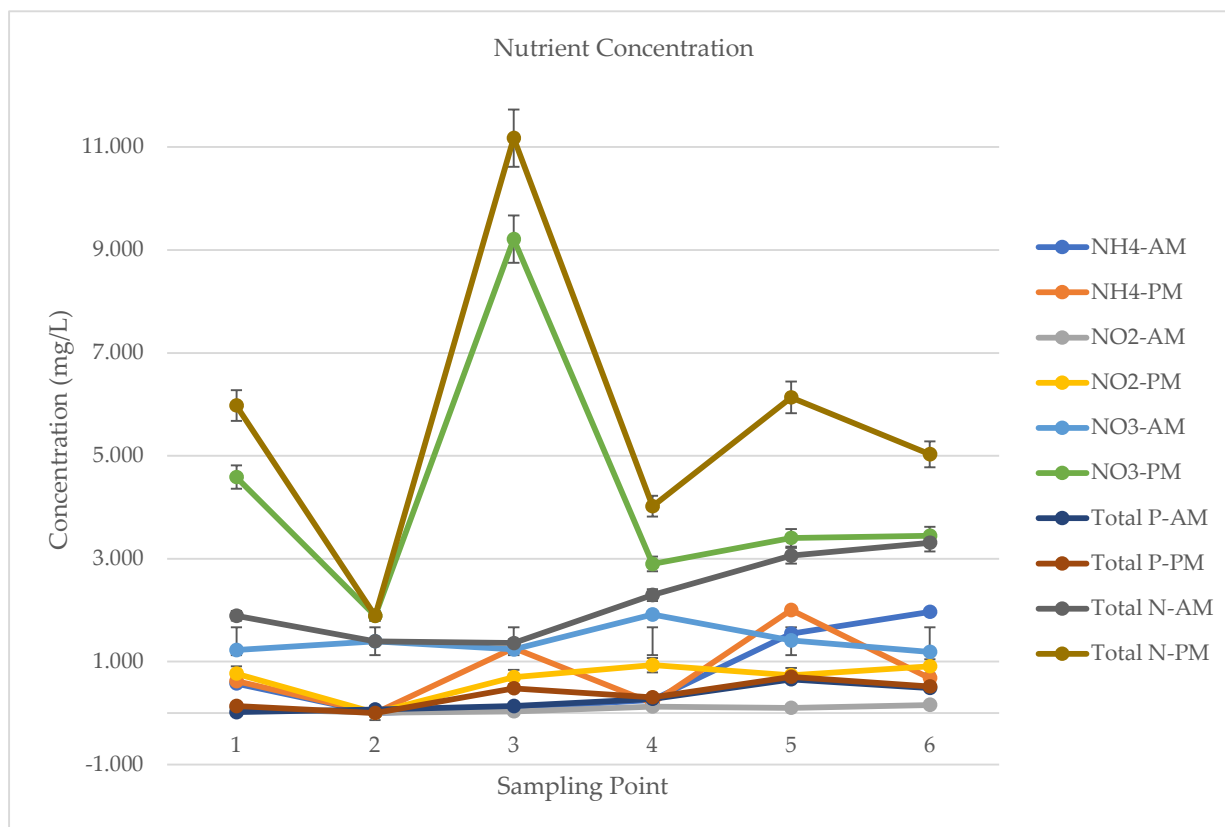


Fig. 4: Nutrient parameters concentration trend.

during the afternoon sampling sessions at all sample locations. The diurnal pattern seen can be explained by the increased biological activity and decomposition of organic matter during daylight hours, resulting in enhanced ammonia production and its release into the water column (Conley et al. 2009, Ezzati et al. 2023). In addition, lower water flow rates and sluggish circumstances in the afternoon might lead to an increase in ammonia levels and a decrease in the ability to dilute it.

Elevated concentrations of ammonia can adversely affect water quality and the species residing in aquatic ecosystems. Elevated levels of ammonia are highly detrimental to aquatic creatures, particularly fish and invertebrates (Bartelme et al. 2017, Kim et al. 2010, Pejman Sereshkeh et al. 2024). It impairs respiratory function, damages the gills, inhibits growth, and increases the organism's vulnerability to diseases. Furthermore, elevated ammonia concentrations can disrupt the normal functioning of physiological systems in aquatic organisms, including osmoregulation and metabolic activities. (Chen et al. 2024, Gu et al. 2024). These changes may result in decreased survival rates and impaired reproductive success. Increased ammonia concentrations alter the chemical composition of water, influencing pH levels and changing nutrient cycling in aquatic ecosystems. Ammonia poisoning can indirectly impact other aquatic organisms by disrupting food webs and community dynamics. (Astals et al. 2018, Ha et al. 2023, Muscarella et al. 2021). Consequently, increased levels of ammonia can have substantial negative impacts on the diversity of aquatic life and ecosystems health, especially in vulnerable regions like spawning grounds and nursery areas.

Numerous factors contribute to increased ammonia levels in river water. Industrial discharges, sewage effluents, and agricultural runoff exemplify point sources of pollution that contribute elevated concentrations of ammonia to aquatic systems. (Trap et al. 2024, Vystavna et al. 2023). Ammonia pollution problems can be exacerbated by ineffective wastewater treatment methods and incorrect organic waste disposal, leading to localized contamination hotspots. Furthermore, natural processes, including the decomposition of organic matter and nutrient cycling in aquatic environments, might facilitate ammonia generation. (Patterson, 2003, Serra et al. 2023, Yates et al. 2022). An excess of organic materials, such as rotting vegetation or animal waste, can stimulate microbial proliferation and ammonification, resulting in increased ammonia emission into the water. Moreover, the existence of stagnant water, diminished water movement, and inadequate dissolved oxygen levels can exacerbate ammonia accumulation and impede the natural processes that alleviate pollution, thus

intensifying the problem. (Ural-Janssen et al. 2024). To tackle this dilemma, it is essential to deploy comprehensive solutions that target both direct and diffuse sources of contamination. This encompasses enhancing wastewater treatment techniques and advocating for sustainable land use practices to diminish fertilizer inputs and save river habitats for future generations.

The study reveals distinct geographical and temporal patterns in nitrite and nitrate levels, indicating particular localized influences and daily fluctuations in nutrient loads.. Geographically, the levels of nitrite and nitrate showed significant differences among the locations where samples were taken (Fig. 4) Elevated concentrations of nitrite and nitrate were regularly detected at the sampling points downstream (Sample Points 5 and 6) in comparison to the upstream (Sample Points 1 and 2) and midstream (Sample Points 3 and 4) regions. Specific pollution sources, such as agricultural runoff or industrial discharges, have an impact on the lower parts of the river.

The concentrations of nitrite and nitrate fluctuated during the morning and afternoon sample sessions. The concentrations of nitrite and nitrate were generally higher during the afternoon sampling sessions at all sampling points. Human activities, including agriculture and urban runoff, contribute supplementary nutrients to the river during daylight, elucidating the observed diurnal variation in nutrient levels. This leads to a heightened concentration of nutrients in the water column. Moreover, reduced water flow rates and sluggish conditions in the afternoon may result in nutrient accumulation and diminished dilution capacity (Busico et al. 2024, Flynn et al. 2023).

High levels of nitrite and nitrate in water can have serious consequences for water quality and the organisms that live in it. Nitrite and nitrate are essential nutrients for plant development (Begam et al. 2024, Sorokin et al. 2012). However, when present in excessive amounts, they can lead to eutrophication, algal blooms, and oxygen depletion in aquatic habitats. Excessive nitrogen inputs can trigger algae growth, resulting in the production of thick mats of algae and the eventual depletion of oxygen due to microbial decomposition (van Wijk et al. 2024, Yu et al. 2014). Elevated levels of nitrites can also have a detrimental effect on aquatic organisms, including fish and invertebrates, by interfering with their respiratory processes and causing methemoglobinemia, a condition that reduces their blood's ability to carry oxygen (Carneiro Marques et al. 2023, Ma et al. 2016, Silvestrini et al. 2024). Nitrate, on the other hand, indirectly affects aquatic ecosystems by stimulating algal proliferation and altering water chemistry, which can result in shifts in pH levels and nutrient cycle mechanisms.

Moreover, increased concentrations of nitrates might pose hazards to human well-being by polluting supplies of potable water, especially in regions that depend on underground water reserves. Several factors contribute to elevated levels of nitrite and nitrate in river water. Agricultural practices, including fertilizer application and livestock husbandry, elevate nitrogen compound concentrations in aquatic bodies via surface runoff and leaching mechanisms. (Arcas-Pilz et al. 2023). Furthermore, the process of urbanisation and industrialization can lead to the contamination of nitrite and nitrate due to the release of nitrogen-rich pollutants in sewage effluents, industrial discharges, and stormwater runoff.

The total nitrogen concentrations in the Tukad Badung River exhibited clear spatial and temporal variations. During the morning sampling at 08:00 am, total nitrogen concentrations ranged from 1.36 mg/L at Point 3 to 3.31 mg.L⁻¹ at Point 6, with the highest levels observed downstream. The concentrations were relatively lower upstream, particularly at Points 1 (1.89 mg.L⁻¹) and 2 (1.39 mg.L⁻¹), indicating that anthropogenic influences such as agricultural runoff and wastewater discharge likely contribute to higher nitrogen levels as the river progresses downstream. In contrast, the afternoon sampling at 07:00 pm showed a noticeable increase in nitrogen concentrations across all points, with values ranging from 1.89 mg.L⁻¹ at Point 2 to 11.17 mg.L⁻¹ at Point 3. The highest recorded concentration of total nitrogen (11.17 mg.L⁻¹) was observed at Point 3, indicating a significant accumulation of nitrogen in the midstream area, potentially due to increased human activities such as wastewater discharge and biological decomposition during the afternoon. The nitrogen concentrations at Points 5 and 6 (6.14 mg.L⁻¹ and 5.03 mg.L⁻¹, respectively) remained elevated, although slightly lower than at Point 3.

These temporal variations suggest that total nitrogen concentrations are influenced by both natural processes, such as biological cycling, and socio-economic factors, including peak human activity in the afternoon, leading to higher nutrient discharges. The increase in nitrogen concentrations during the afternoon can likely be attributed to both reduced water flow during off-peak hours, which limits dilution, and increased domestic wastewater contributions, which typically peak in the afternoon. Atmospheric deposition and biological nitrogen fixing are natural processes that can also add nitrite and nitrate to aquatic habitats. Nevertheless, human activities have greatly increased nitrogen pollution problems, resulting in extensive deterioration of water quality and disturbances to ecosystems (Panjwani et al. 2021, Stein & Klotz, 2016, Zhou et al. 2024). The amalgamation of inadequate nutrient management practices, substandard waste disposal, and deficient wastewater treatment infrastructure exacerbates

the issue of nitrite and nitrate contamination. This poses considerable issues for river conservation and water resource management. The analysis of nitrite and nitrate concentrations in the Tukad Badung River underscores the complex dynamics of nitrogen pollution and its impact on water quality and the associated ecosystems. A well-coordinated strategy is required to tackle food sources from both point and non-point origins (Lavallais & Dunn 2023). This can be achieved by implementing effective nutrient management practices and promoting sustainable land use practices. It can protect river ecosystems and ensure the availability of clean and healthy water resources for current and future generations.

The research uncovers noticeable regional and temporal patterns in the levels of total phosphorus, which indicate the presence of localised impacts and daily variations in nutrient loads. Downstream sampling stations (Sample Stations 5 and 6) consistently exhibited higher total phosphorus levels compared to upstream (Sample Points 1 and 2) and midstream (Sample Points 3 and 4) locations. The overall phosphorus levels fluctuated between the morning and afternoon sampling periods. Total phosphorus concentrations were generally higher during afternoon sampling sessions at all sampling stations. Human activities, including agriculture and urban runoff, contribute supplementary nutrients to the river during daylight, elucidating the observed diurnal variation in nutrient levels. This results in an increased concentration of nutrients in the water column (Ezzati et al. 2023, Lu et al. 2024, Xu et al. 2018). In addition, reduced water flow rates and sluggish circumstances in the afternoon can lead to the accumulation of nutrients and a decrease in the ability to dilute them.

High levels of total phosphorus can have a significant impact on the water quality and the organisms that live in it (Conley et al. 2009, Smith & Myers 2024). Excessive amounts of total phosphorus, an essential nutrient for the growth of algae, can cause eutrophication, algal blooms, and oxygen depletion in aquatic habitats. Elevated phosphorus concentrations can trigger the rapid growth of algae, leading to the development of thick mats of algae that block sunlight from reaching native plants and reduce biodiversity (Correll, 1998, Suryawan et al. 2024, Zhang et al. 2023). Furthermore, the proliferation of algal blooms can result in a reduction in oxygen levels due to the breakdown of organic matter by microorganisms. This can create hypoxic conditions that are detrimental to aquatic animals (Hu et al. 2023).

In addition, elevated quantities of total phosphorus can disrupt the chemical composition of water, causing fluctuations in pH levels and disrupting nutrient cycle systems. Water acidification can have detrimental effects on

aquatic creatures, compromising their metabolic functions and reproductive success (Liu et al. 2024, Rey-Martínez et al. 2024b). Phosphorus pollution also poses an indirect effect on human health by contaminating drinking water sources, especially in regions that depend on surface water for their supply of safe drinking water (Panasiuk, 2012, Proskynitopoulou et al. 2024, Silva et al. 2023). Agricultural practices, including the application of fertilizers and the rearing of livestock, can lead to increased phosphorus levels in water bodies through surface runoff and leaching (Koulouri et al. 2024, Zhu et al. 2023). Urbanisation and industrialization further lead to the contamination of phosphorus through the release of phosphorus-rich pollutants in sewage effluents, industrial discharges, and stormwater runoff.

Natural processes, such as the weathering of phosphorus-rich rocks and the resuspension of silt, can affect phosphorus inputs in aquatic environments. Nonetheless, human activities have markedly exacerbated phosphorus pollution, leading to substantial degradation of water quality and disruption of ecosystems. Inefficient nutrient management, improper waste disposal, and insufficient wastewater treatment facilities worsen phosphorus contamination issues. (Galeano et al. 2023, Galligan & McClanahan 2024, Ruijter et al. 2016). These factors provide considerable challenges for river conservation and water resource management. The examination of overall phosphorus levels in the Tukad Badung River highlights the intricate nature of nutrient contamination patterns and their effects on the water quality and aquatic ecosystems. It is necessary to implement comprehensive management strategies that address both specific and diffuse sources of contamination. This includes improving nutrient management and promoting sustainable land use practices to protect river ecosystems and ensure the availability of clean and healthy water resources for current and future generations.

Potential of Nutrient Recovery from Streamwater

The possibility of nutrient recovery from the Tukad Badung River is intricately linked to the tenets of the circular economy. By extracting nutrients like nitrogen and phosphorus from contaminated water, these essential resources can be reused for agricultural applications, thereby diminishing the need for synthetic fertilizers. Nutrient recovery techniques, such as precipitation and adsorption, have demonstrated efficacy in extracting these nutrients from water, with recovered phosphorus and nitrogen being utilized as fertilizers for agriculture or substrates for bioenergy generation.. Studies have demonstrated that such nutrient recovery can result in up to 80% of extractable nutrients being recovered from wastewater (Hofmann et al. 2024a).

Implementing these technologies in the Tukad Badung River could significantly reduce dependence on chemical fertilizers, lower environmental impacts, and contribute to a more sustainable and resource-efficient agricultural system. Quantitative forecasts indicate that recovering and repurposing a portion of the nutrients from the river might substantially diminish nutrient loads in agricultural runoff, thus enhancing water quality and mitigating downstream eutrophication.

This study provides valuable insights to support the initiative of nutrient recovery from stream water as part of a circular economy model for wastewater treatment. By analysing the spatial and temporal variations of nutrient pollutants along the Tukad Badung River, the study identifies opportunities for the sustainable management and utilisation of these nutrients to minimise environmental impact and enhance resource efficiency. The findings highlight the significant concentrations of nutrients in the river, including nitrogen and phosphorus. These nutrients, if properly recovered, can serve as valuable resources for various applications, such as agricultural fertilisers, bioenergy production, and aquaculture feed (Hofmann et al. 2024a, Winkler & Straka, 2019, Xiao et al. 2016). By implementing technologies for nutrient recovery, such as nutrient adsorption, precipitation, and biological processes, wastewater treatment facilities can extract these nutrients from stream water and convert them into valuable products, thereby closing the nutrient loop and reducing dependency on synthetic fertilizers and other external inputs (Lin et al. 2016, Marcińczyk et al. 2022, Rey-Martínez et al. 2024b, Sari et al. 2023, Xiao et al. 2016). The alternatives of the nutrient recovery technologies have been summarized in Table 3.

The research delineates the spatial distribution of nutrient hotspots throughout the river, highlighting prospective sites for nutrient recovery facilities. By strategically positioning nutrient recovery facilities in proximity to regions with elevated nutrient concentrations, such as urban centers or industrial districts, wastewater treatment operators can enhance resource recovery and reduce transportation expenses related to nutrient extraction and delivery (Soedjono et al. 2017, Sumantra et al. 2022, Wijaya et al. 2017). Furthermore, the temporal variations in nutrient concentrations revealed by the study underscore the dynamic nature of nutrient pollution in river ecosystems. Establishing real-time monitoring systems and adaptive management strategies allows wastewater treatment plants to modify nutrient recovery procedures according to variations in nutrient loads. This method optimizes resource recovery efficiency and reduces environmental effects. This study offers critical data and insights to guide the planning and execution of nutrient recovery activities within a circular

Table 3: Summaries of the nutrient recovery technology.

Technology	Description	Case Studies	Recovery Efficiency
Nutrient Precipitation	Chemical processes that form insoluble compounds from dissolved nutrients (e.g., struvite precipitation for phosphorus recovery).	Germany: Struvite precipitation in wastewater treatment plants (WWTPs) has been widely adopted for phosphorus recovery.	Up to 90% phosphorus recovery (Hofmann et al. 2024b)
Adsorption	Use of adsorbents (e.g., biochar, activated carbon) to remove nutrients from water.	India: Application of biochar in decentralized treatment systems in rural areas to remove nitrogen from wastewater.	Up to 80% nitrogen recovery (Xiao et al. 2016)
Membrane Filtration	Use of membranes to selectively filter out nutrients from water.	USA: Membrane bioreactors (MBRs) are used in wastewater treatment plants for nitrogen and phosphorus removal.	70-85% nitrogen and phosphorus recovery (Wang et al. 2016)
Biological Processes	Microbial processes such as nitrification-denitrification and algae-based systems.	China: Algae-based systems for nutrient recovery in urban wastewater treatment.	50-80% nutrient recovery (Rey-Martínez et al. 2024b)
Electrochemical Methods	Electrochemical processes for nutrient extraction and recovery.	Sweden: Electrochemical cells for nitrogen removal from wastewater and agricultural runoff.	60-70% nitrogen recovery (Galeano et al. 2023)
Constructed Wetlands	Engineered wetland systems are designed to naturally treat wastewater by supporting nutrient uptake via vegetation.	Brazil: Constructed wetlands in rural areas for removing nitrogen and phosphorus from agricultural runoff and domestic wastewater.	50-80% nutrient removal (Darji et al. 2022)
Anammox (Anaerobic Ammonium Oxidation)	Biological process using bacteria to convert ammonia directly into nitrogen gas, bypassing traditional nitrification.	Netherlands: Anammox process for nitrogen removal in full-scale wastewater treatment plants.	90-95% nitrogen recovery (Kim et al. 2010)
Phytoremediation	Use of plants to absorb or remove nutrients, particularly nitrogen and phosphorus, from water.	USA: Wetland plants used in the Everglades to reduce nutrient loading from agricultural runoff.	50-70% nutrient removal (García-Avila et al. 2023)

economy framework for wastewater treatment. (Antunes et al. 2022, Sauvé et al. 2021, Slootweg, 2020, Zvimba et al. 2021). These projects extract essential nutrients from stream water and transform them into reusable goods like fertilizers or biofuels, so promoting resource conservation, environmental protection, and sustainable development in the Tukad Badung River basin and beyond.

CONCLUSIONS

This study highlights nutrient pollution in the Tukad Badung River, driven by agricultural runoff, domestic wastewater, and industrial discharges. Nutrient recovery technologies, such as precipitation, adsorption, and biological processes, offer promising solutions to reduce nutrient levels, minimize dependence on synthetic fertilizers, and enhance water quality. To improve watershed management, it is essential to integrate these recovery technologies into local infrastructure. Policymakers should focus on decentralized wastewater treatment systems and promote sustainable agricultural practices to reduce nutrient runoff. Strengthening regulatory frameworks for wastewater treatment will further support these efforts. However, challenges remain, including the scalability of recovery methods and the need for cost-benefit analysis to assess their feasibility for large-scale implementation. Future research should address

these issues to ensure the practical application of nutrient recovery technologies. In conclusion, this study contributes to sustainable river management by demonstrating the potential of nutrient recovery to address nutrient pollution. The findings offer valuable insights for

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Depth-wise Distribution of Microplastics Around Teluk Lerong Intake, the Mahakam River: Implications for Water Treatment Processes

Dwi Ermawati Rahayu[†], Ratu Fortuna P. T. Putri, Ika Meicahayanti, Searphin Nugroho and Fahrizal Adnan

Department of Environmental Engineering, Faculty of Engineering, Mulawarman University, Samarinda, East Kalimantan, 75123, Indonesia

[†]Corresponding author: Dwi Ermawati Rahayu; dwiermarahayu@gmail.com

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ABSTRACT

The Mahakam River is an essential raw water source for the Water Treatment Plant (WTP) in the Regional Drinking Water Company of Samarinda City. This means its quality is crucial, specifically due to several activities usually conducted in the river and the density of the surrounding community. One of the most critical issues related to surface water quality is microplastics. This study aimed to assess the abundance, size, and types of microplastics present in the water column of rivers at various depth levels. Samples were collected from the intake using a composite sampling method. The samples were processed by first filtering with 180 µm nylon, removing organic substances with H₂O₂, separating by density, conducting a second filtration with different nylon sieves, and then performing microscopic observation. The identification of polymers was carried out using FTIR. The results indicated the presence of microplastics in all tested samples. The highest abundance of microplastics, 25.4 particles per liter, was in the size range of 180-250 µm at a depth of 0.5 meters. The abundance of microplastics decreased as the depth of the river increased. The most common types of microplastics detected were fibers, fragments, and films. It is crucial to map the distribution of microplastics, mitigate their occurrence, and reduce degradation and fragmentation to increase WTP performance.

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INTRODUCTION

The Mahakam River is the second-longest river in Indonesia, flowing through several regencies and cities such as Samarinda City, Kutai Kartanegara Regency, and West Kutai Regency. It has a length and area of 920 km and 149,277 km², respectively (Badan Pusat Statistik 2014) and is reported to be the source for 13 rivers and supplies several lakes along the rivers, such as Jempang, Semayang, and Melintang Lakes (Noor et al. 2013). The essential function of the river to the people of Samarinda City is related to its usage as the main source of raw water used by the PDAM's WTP, which has more than 156,278 customers (BPS 2019). It is important to note that the Cendana WTP has the most significant supply and utilizes the raw water of the Mahakam River through the Lerong Bay Intake.

This means it is essential to determine the quality of this river due to the effect of raw water on processed products. It is pertinent to understand that the quality also depends on the watershed. However, the Mahakam River watershed is located in an area with several domestic, commercial, and industrial activities, which means it is vulnerable to both intentional and unintentional dumping of liquid and solid wastes. This is indicated by the habit of some residents throwing garbage into the river (Dewi 2016), which accumulates and causes silting (Tambunan 2014). Therefore, monitoring the raw water's physical, chemical, and biological quality is crucial to ensure that the treated water produced is safe for consumption. Meanwhile, one of the critical issues usually related to surface water quality, which requires adequate monitoring, is microplastic content, even though there



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has been no regulation concerning acceptable standards up to the present time. Microplastic is the smallest part of plastic experiencing degradation through the physical, mechanical, and biological fragmentation process (Corcoran 2021, Klein et al. 2018), and also usually < 5 mm in size (Azizah et al. 2020, Ayuningtyas 2019). It was further divided into macroplastics, meso-plastics (large microplastics), microplastics (small microplastics), and nano-plastics with a size of > 5.00 mm, $\leq 5 - > 1$ mm, ≤ 1 mm - > 0.1 μ m, and ≤ 0.1 μ m, respectively (Azizah et al. 2020) [14-17]. This is necessary because the rivers in urban areas typically have the potential to contain higher microplastics, which are transported upstream (McCormick et al. 2016) to the seashore (Łabuz 2021).

However, there are limitations to research on the freshwater environment (Wagner et al. 2014, Bellasi et al. 2020), so monitoring, fate, and transport of the microplastic presence need to be researched in the upstream areas, namely lakes and rivers (Rogowska et al. 2021). The presence of microplastics in surface water can be caused by the solid wastes dumped into the river, based on the activities in the watershed. This was confirmed by the findings of Scherer et al. (2020) that the high microplastic content in the water and sediment samples originated from industrial activities. Harpah et al. (2020) also reported that microplastics in the Sei Sikambing River, Medan, were due to domestic or household wastes. Additionally, the highest levels of microplastic contamination were found in water treatment plants that utilized raw water from rivers near industrial areas and densely populated regions that discharged untreated domestic waste (Pivokonsky et al. 2018). The presence of microplastic waste in the sea and freshwater has become a crucial issue in recent years.

Some recent research showed that different types and sizes of microplastics are present in several water areas in Indonesia, as indicated by those found in water (Ayuningtyas 2019, Kapo et al. 2020, Sutanhaji et al. 2021, Sembiring et al. 2020, Alam et al. 2019a), water sedimentation (Azizah et al. 2020, Firdaus et al. 2020, Sembiring et al. 2020, Alam et al. 2019a), aquatic biota (Bahri et al. 2020, Sembiring et al. 2020, Yudhantari et al. 2019), and aquatic product samples such as salt. Furthermore, Shen et al. (2020) also showed that microplastics detected in freshwater worldwide ranged between zero and millions of particles per cubic meter.

Several previous studies have discussed the depth factor (Lestari et al., 2020), indicating that microplastic particles tend to be distributed in the surface column of rivers. Research by Wijaya and Trihadiningrum (2019) analyzed both vertical and horizontal distributions, revealing that vertical distribution tends to fluctuate. Similarly, Alam et al.

(2019b) examined the distribution of microplastics (MPs) in river water and sediment samples. Napper et al. (2023) also investigated microplastics, focusing on their distribution in air, water, and sediment.

However, these studies have not thoroughly analyzed the effect of depth factors on the abundance and size of microplastics. It is important to note that microplastics present in raw water can be transported into Water Treatment Plants (WTPs) and subsequently into the drinking water supply system for public consumption. This concern was confirmed by research conducted by Pivokonsky et al. (2018) in the Czech Republic and by Ferraz et al. (2020) in Brazil, both of which demonstrated the presence of microplastic contamination in both raw and treated water.

This implies that conventional WTPs are not effective in removing microplastics from raw water, thereby posing risks to human health. These risks include toxic effects, nerve damage, and chronic inflammation (Kumar et al., 2021; Liu et al., 2020), which may occur through exposure via bioaccumulated food chains, biomagnification, water consumption, and other pathways (Wong et al., 2020; Akdogan & Guven, 2019). This means it is necessary to conduct this research to determine microplastics' presence in the Mahakam River. The process involved identifying the raw water from the Mahakam River retrieved from the Teluk Lerong intake to be used in the WTP. Moreover, microplastics' abundance, type, and size were analyzed based on the river depth.

MATERIALS AND METHODS

An overview of the water sampling, sample preparation, microplastic abundance calculation, and identification methods used in the study of microplastics in Mahakam River water samples is given in Table 1. The methods refer to the NOAA microplastic analysis standards (NOAA 2015). 2015), which are also referred to by several researchers (Lestari et al. 2020, Eamrat et al. 2022, Rodrigues et al. 2018, Sharma 2019, Ayuningtyas 2019).

RESULTS AND DISCUSSION

Microplastic Abundance Based on Depth and Size

The results showed that all the water samples from Mahakam River were contaminated by microplastics that ranged between 500 μ m, 500 μ m > $\times$ 250 μ m, and 250 μ m > $\times$ $\geq$ 180 μ m at different depths, as shown in Fig. 1. It was discovered that the highest were those between 250 μ m > $\times$ 180 μ m with an average abundance of 25.4 particles/L found at a depth of 0.5 m. This means the smaller microplastics are

Table 1: Sampling details.

Process	Details
Water Sampling (Pivokonsky et al. 2018), (Standar nasional Indonesia, 2004)	• Location: Mahakam River, Teluk Lerong Intake (Cendana WTP) • Sampling Frequency: Three times in 24 hours (every 8 h) • Depths: 0.5, 1, and 1.5 meters • Total Volume: 9 liters (composite sample) • Method: Grab sampling (1-liter samples) • Equipment: The horizontal van Dorn water sampler system is a sturdy transparent tube with a double cover and a durable steel release system. This tool is suitable for sampling in rivers with different depths horizontally
Sample Preparation	• Initial Filtering: 500 mL filtered through a 180 µm nylon sieve with a vacuum pump • Heating: Sieve placed in a porcelain dish, covered with aluminum foil, heated at 90°C for 24 hours • Residue Transfer: Solid transferred to a beaker with distilled water • Dissolving Organic Matter: 20 mL of 0.05 M Fe(II) and 20 mL of 30% H₂O₂ added, left for 5 min (Pico et al. 2019) • Heating: Beaker heated at 75°C until organic matter disappears (NOAA, 2015) • NaCl Addition: 6 g of NaCl per 20 mL sample was added and stirred at 75°C • Density Separation: The sample was placed in a density separator for 24 hours; solids were removed, remaining water and microplastics were transferred to a beaker • Filtration: Filtered using nylon sieves (500 µm, 250 µm, 180 µm) in petri dishes (Ayuningtyas 2019). • Contamination prevention measures are by limiting exposure of sample materials to MP sources, such as air, synthetic textiles, and plastic materials. Nylon materials are rinsed several times with distilled water. Samples should be covered between uses, and polymer-free clothing use be worn while performing tests
Microplastic Abundance Calculation	Microplastic abundance is calculated using the formula: $\text{Microplastic abundance} = \frac{\text{number of microplastic particles (particles)}}{\text{The volume of filtered water (m}^3\text{)}}$ • Calculated for each size category (500 µm, >500 µm to 250 µm, >250 µm to 180 µm) • Data was collected three times to analyze the effect of sieve size and depth on microplastic abundance. • Due to the data distribution being normal (R^2 0.95, P-value 0.059 > 0.05), a correlation test was used to determine the influence of depth on the abundance and size of microplastics
Identification of Microplastic Types	• Microplastics identified based on shape observed under a microscope (Azizah et al. 2020, Hanif et al. 2021): Fiber: Elongated shape, Film: Transparent, thin, soft. Fragment: Hard, rigid • Analysis Method: FTIR analysis is used to determine polymer types (e.g., PP, PE, PVC) (Shim et al. 2017) by comparing spectra with a known sample

found on the river's surface, while the bigger ones that are >500 µm are lesser and found in the deeper part.

The abundance was observed to decrease as the size and depth of the river increased, as indicated in Fig. 1.

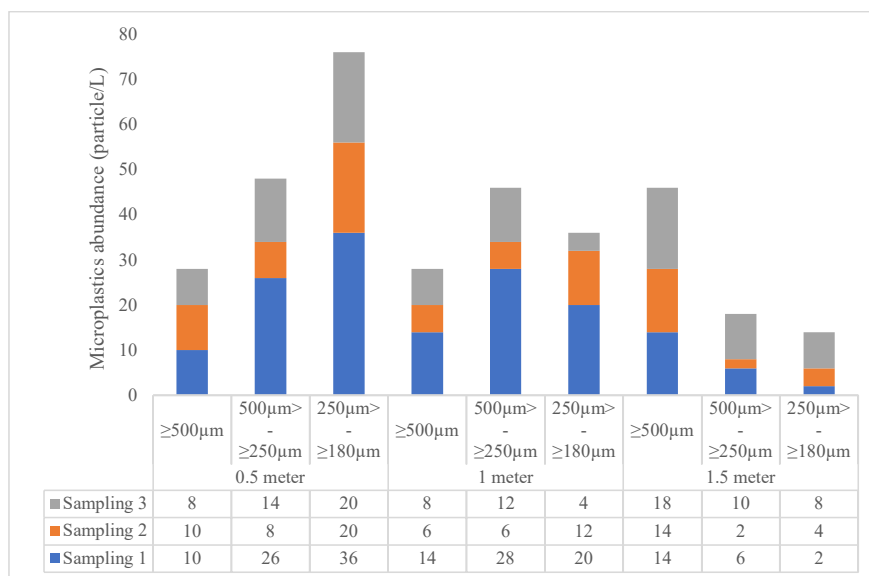


Fig. 1: The Abundance of microplastics refers to The Size and Depth of the River.

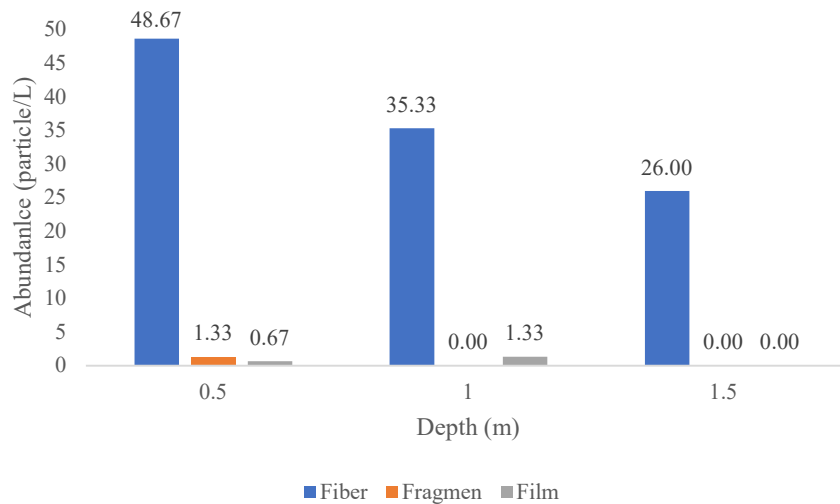


Fig. 2: The abundance of microplastics refers to the type and depth of the river.

Microplastic sampling occurred in August during the rainy season, which typically increases the runoff of microplastics from land into rivers, either directly or through drainage systems. Lighter particles tend to float on the water's surface, while heavier ones either settle in sediments or remain on land (Nizzetto et al. 2016). The Mahakam River, bordered by urban areas, faces a higher risk of microplastic contamination. This observation aligns with previous studies that indicate urbanization, characterized by high population density and activity, significantly impacts the presence and abundance of microplastics in aquatic environments. (Horton et al. 2017, Sari et al. 2021). Similar findings were reported in research conducted on Indian rivers, which explored various types of anthropogenic influences in both urban and rural contexts (Lechthaler et al. 2021).

Microplastic Abundance by Types

Microscopic analysis revealed the presence of three types of microplastics in water samples from the Mahakam River, as illustrated in Fig. 2. The fiber types resemble lines and

threads, while the film types have irregular shapes, are thin, and transparent. In contrast, the fragment types are solid, have jagged edges, and appear as pieces of larger plastics (McCormick et al. 2016). The fiber types were the most common in the sample for all the sizes and depths. Among these, fiber types were the most prevalent across all sample sizes and depths. Microplastic fiber types were identified in every sample across all size categories: $\geq 500 \mu\text{m}$, $500 \mu\text{m} > x \geq 250 \mu\text{m}$, and $250 \mu\text{m} > x \geq 180 \mu\text{m}$. They were most frequently found in samples taken from a depth of 0.5 meters, with an average concentration of 48.67 particles per liter. The lowest abundance of microplastic fiber was at a depth of 1.5m, namely 26 particles/L (Fig. 2). The presence of this type of microplastic fiber will decrease with increasing depth according to the abundance found. Meanwhile, fragments were only found at a depth of 0.5m, and films at depths of 0.5m and 1m in small quantities.

The fiber types of microplastics are thin and long, which makes them resemble synthetic fibers (Ayuningtyas 2019). Most of them are observed to have originated from

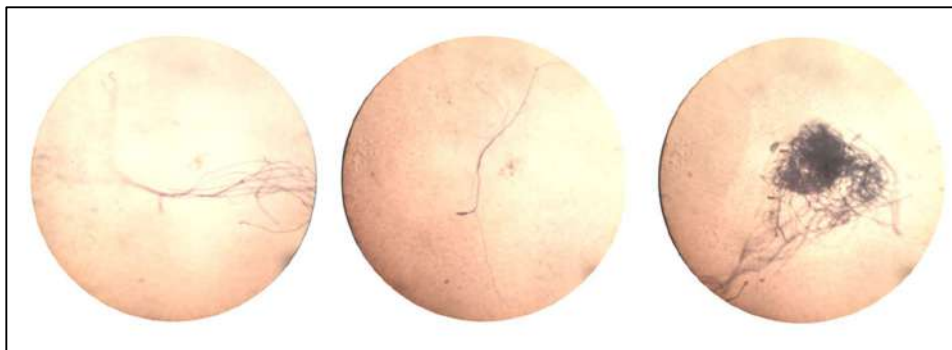


Fig. 3: The type of fiber microplastics.

domestic wastes such as water used in washing clothes and household appliances, dumped into rivers. The fiber type of microscopics is presented in Fig. 3.

Researchers also showed that fiber is the dominant type of microplastic (Malla-Pradhan et al. 2023), approximately 45% at all levels of the water column (Lenaker et al. 2019). This was also recorded in research conducted at several locations in Indonesia that fiber was the dominant microplastic compared to other types (Alam & Rachmawati, 2020, Sutanahaji et al. 2021, Tuhumury & Pellaupessy 2021, Yudhantari et al. 2019, Firdaus et al. 2020, Alam et al. 2019a, Kapo et al. 2020, Yona et al. 2022, Nurwahyunani & Rakhmawati 2022). The high amount of fiber found may have come from the degradation of clothing (textile synthetics) due to the washing process (Wagner & Lambert 2018, Bellasi et al. 2020, Lambert et al. 2014). Moreover, this was generally dominated by polyester and nylon (Horton et al. 2017). Moreover, in Indonesia, mainly through drainage systems where not all domestic sewage wastewater has been treated at the WWTP, it flows directly into the river (Alam & Rachmawati 2020). Thus, it is possible to find many fiber microplastics in rivers. This is related to research (Piskula & Astel 2022) in which the number of existing microplastics in river water is higher in the downstream part of the WWTP.

The films are transparent and thin microplastics (Azizah et al. 2020). They were observed to be present

at 0.5 and 1-meter depth, which is also classified as the water surface area because they have low density. Their sizes were discovered to range between 250 μm and 180 μm and generally include plastic bags or other thin plastic materials. The microscopic observation of the film-type of microplastics is presented in Fig. 4.

The fragments are opaque, thick, and hard microplastics that typically originate from larger plastic waste, such as bottles and pipes (Ayuningtyas 2019) [16]. They were only detected in the 180 μm size category at a depth of 0.5 meters, which corresponds to the water's surface (Fig. 5). This is because plastics on surface land are often exposed to sunlight and high temperatures, accelerating the fragmentation process. In contrast, fragments made from denser polymers are likely to remain in the soil and migrate to deeper layers, while those from lighter polymers can be carried by wind or water to the water's surface. Fragmentation can also occur in aquatic environments due to UV exposure from sunlight and wave action, a process that is similarly applicable to film-type microplastics (Horton et al. 2017). A study by Seyfi et al. (2021) found that fragment and film microplastics were present in over 70% of beach sand. Furthermore, the aging process of microplastics enhances their ability to adsorb metals by 20 to 60%. When bacterial cultures are present, this aging process can increase the adsorption capacity for various metals (Pb, Cd, Cu, Cr, Zn, Ni) to around 60-80%.



Fig. 4: Film microplastics.

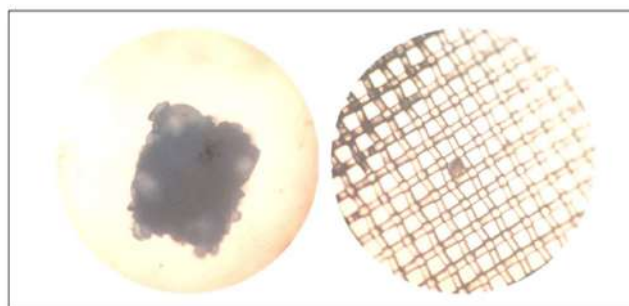


Fig. 5: The Type of Fragmented microplastic.

Effect of The Depth of the River Column

Based on these data, it shows that the microplastics found in the surface area (at a depth of 0.5m) are dominated by small microplastics ($250\mu\text{m} > x \geq 180\mu\text{m}$) with an average of 25.33 particles/L (the regression statistics show an r^2 of 0.9626, p -value 0.068). Whereas in the deeper parts (at a depth of 1.5m), large microplastics ($> 500\mu\text{m}$) are dominant in smaller amounts, namely an average of 15,33 particles/L. The abundance value of microplastics in small sizes will decrease as the depth of the river increases. Meanwhile, the size of microplastic particles was found to be larger with increasing depth, as shown in the graph in Fig. 6, left ordinate. Based on depth, the highest total average microplastic was found in the depth of the water surface area at 0.5m, namely 50.67 particles/l, and the lowest abundance at a depth of 1.5m was 26 particles/l. Microplastic abundance will decrease with increasing sampling depth (the regression statistics show an r^2 of 0.9939, p -value 0.021), as shown in Fig. 6, right ordinate.

The findings indicated that the river's surface layer at a 0.5-meter depth contained the highest abundance of microplastic particles, with sizes as small as $250\mu\text{m} > x \geq 180\mu\text{m}$ (the regression statistics show an r^2 of 0.9727, p -value 0.068), which is associated with their tendency to have smaller densities. Previous studies have shown that the distribution of microplastics in water is influenced by density, with lower-density particles remaining at the

surface. In contrast, higher-density people tend to settle in sediments (Horton et al. 2017). A similar pattern was noted by Kankanige (Kankanige & Babel 2021) that microplastic fractions with a small size ($65\text{--}53\mu\text{m}$) were prevalent in both untreated and WTP-treated water. Previous research also showed that the density of the polymer influenced its presence on the surface, subsurface (middle surface), and bottom of the water (Lestari et al. 2020, Lenaker et al. 2019). Additionally, factors such as channel depth, stream velocity, and particle type play a role in microplastic distribution throughout the water column (Lenaker et al. 2019).

Microplastics can reach the surface water through several routes, with the migration from land observed to the primary and most frequent human habits such as dumping of garbage and recycling process, physical forces such as weather conditions including wind, rain, and flood, as well as environmental and topographic conditions (Eerkes-Medrano et al. 2015, Horton et al. 2017, Kaliszewicz et al. 2020). The primary source of coastal waste was waste disposed of directly by humans at that location or carried by the river flow. So, more waste is found in coastal areas close to the estuary (Łabuz 2021). Another factor is river water, a means of transportation, and agricultural activities that lead to rivers (Rogowska et al. 2021). The Mahakam River is a river that has the primary function of coal pontoon transportation, transportation of goods, and ecotourism (Susanto & Kiswanto, 2020, Tambunan 2014). Even the heavy traffic of coal pontoon transportation has affected

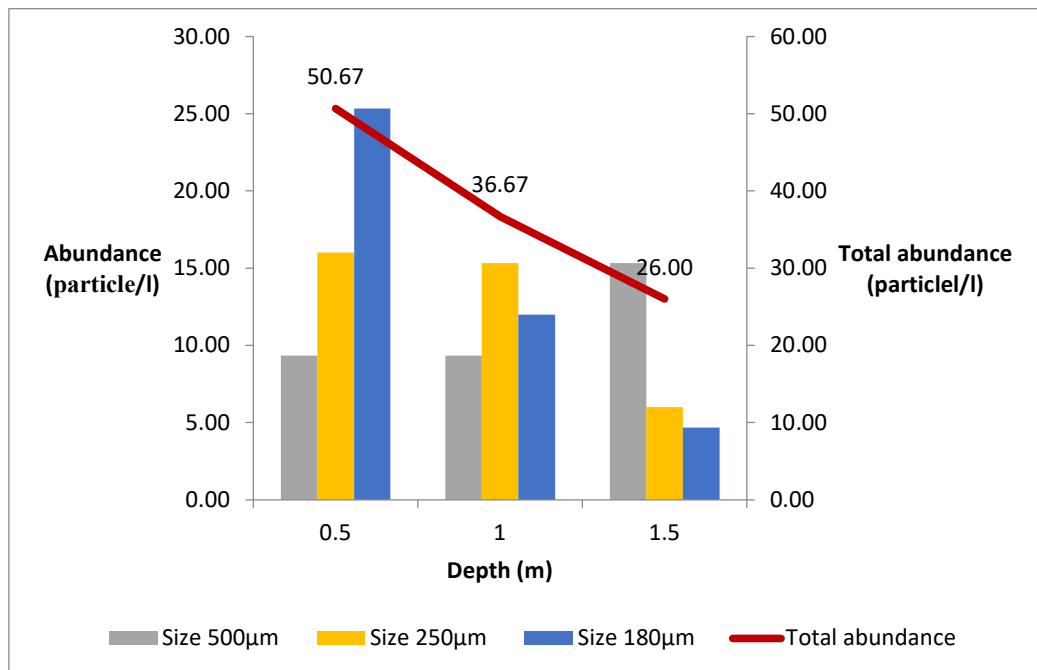


Fig. 6: Effect of the depth of the River on the abundance and size of microplastics.

the native animal of the Mahakam River, namely the Pesut Mahakam, which has moved its natural habitat location (Noor et al. 2013). The report confirmed this regarding garbage, which showed that some communities around the Mahakam River dump garbage into the river (Dewi 2016), thereby causing silting, evidence of waste accumulation (Tambunan 2014). Moreover, one of the river's tributaries was found to have poor water quality due to the industrial and household activities conducted around its location, which leads to the direct discharge of domestic wastes into the river water (Pramaningsih et al. 2017). The data further supported this, showing that the Mahakam River is moderately to severely

polluted (Kemen & Kehutanan 2021). Meanwhile, it is recommended that the research on microplastics should also be limited to large rivers such as the Mahakam River and extended to small rivers or tributaries. This is necessary because small rivers in urban areas have been reported to have higher concentrations of microplastics because they act as dumpsites (Dikareva & Simon 2019).

Microplastics Polymers Analysis

The Identification of the polymer microplastic type using FT-IR produces a wavelength (Fig. 7) that compares and matches it with the wavelength of the type of reference microplastic.

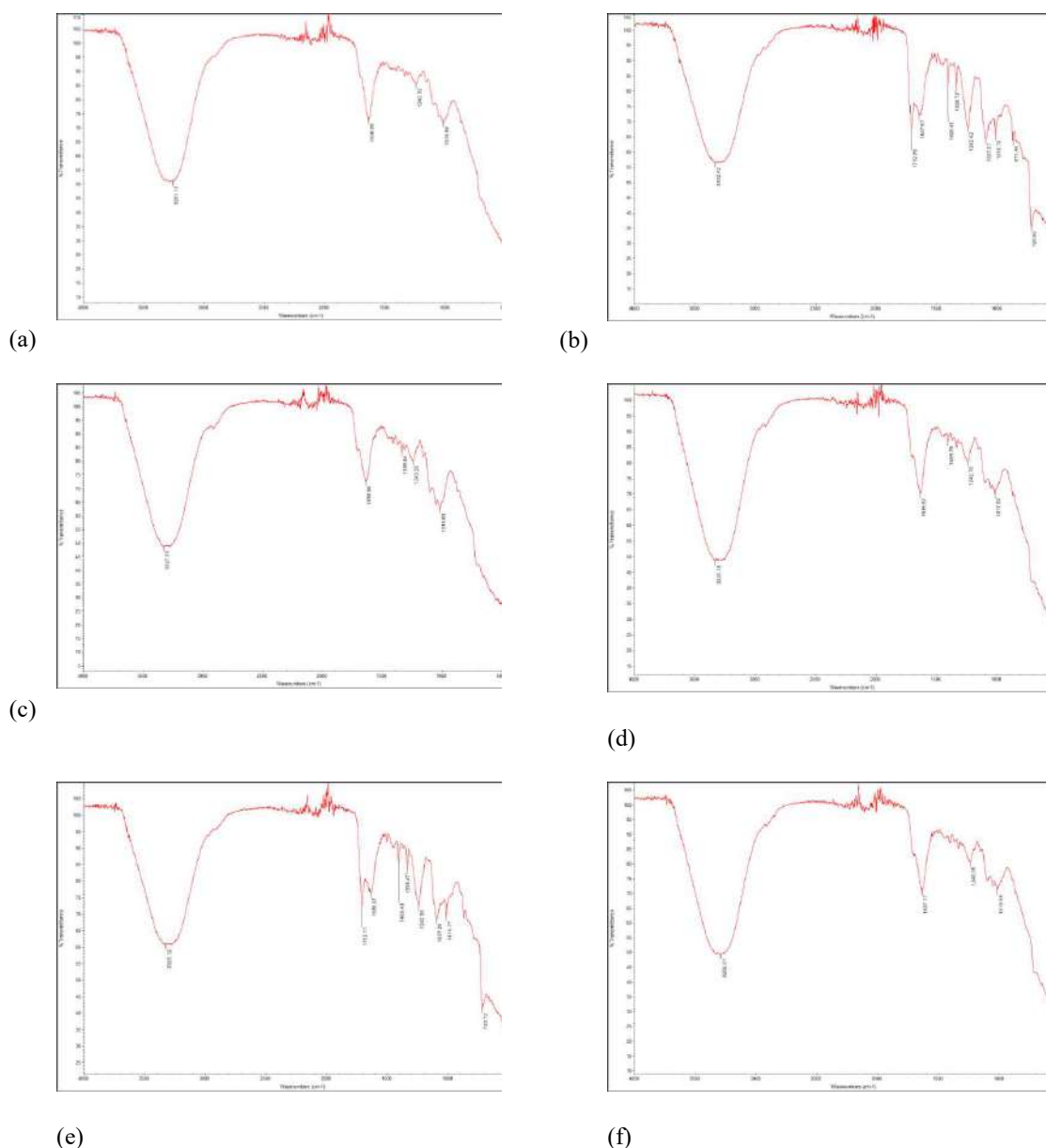


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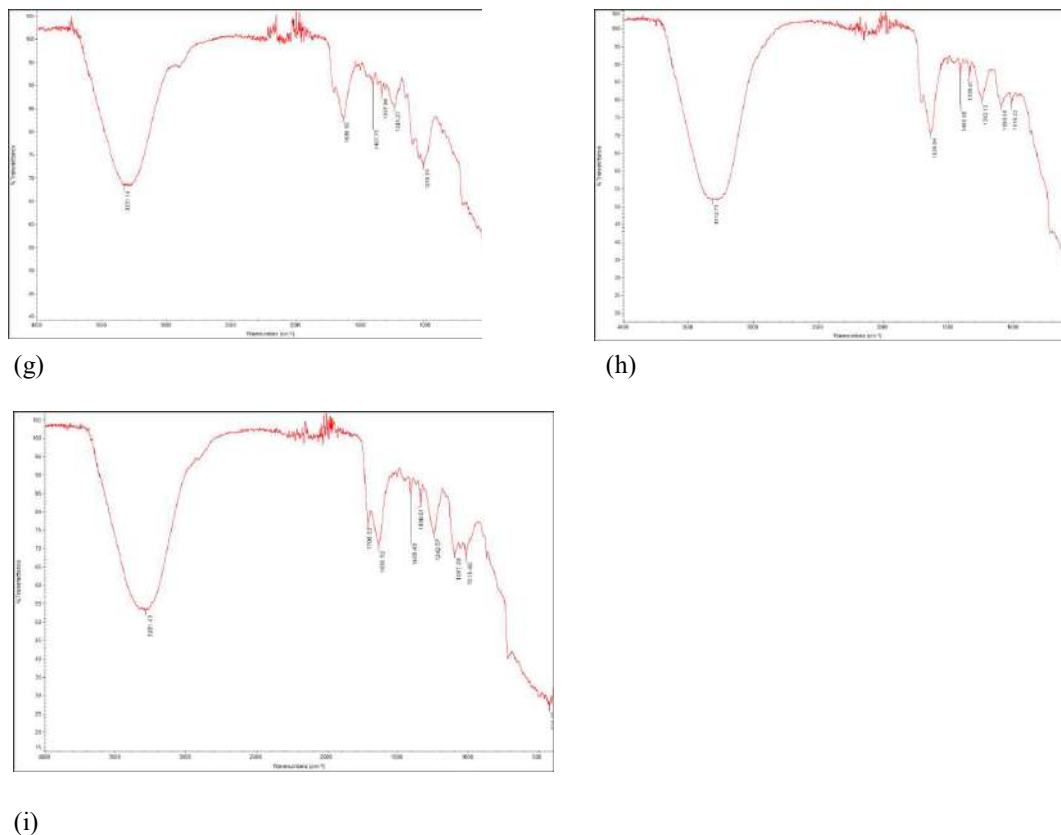


Fig. 7: FTIR Spectra Results: (a) Sample in 0.5 m depth, size 180 μm (b). Sample in 0.5 m depth, size 250 μm (c). Sample in 0.5 m depth, size 500 μm (d). Sample in 1 m depth, size 180 μm (e). Sample in 1 m depth, size 250 μm (f). Sample in 1 m depth, size 500 μm (g). Sample in 1.5 m depth, size 180 μm (h). Sample in 1.5 m depth, size 250 μm (i). Sample in 1.5 m depth, size 500 μm .

Table 2: Microplastic Polymers Based on FT-IR Results.

Sample	Polymers found
0.5 m depth, size 180 - 250 μm	Polyethylenimine Poly (1, 1-dimethyl-3, 5-dimethylene piperidinium chloride)
0.5 m depth, size 250 - 500 μm Poly (1, 4-butylene terephthalate)	Poly (ethylene terephthalate)
0.5 m depth, size >500 μm	Polyester
1 m depth, size 180 - 250 μm	Polyethylenimine Polyethylenimine Poly (1, 1-dimethyl-3, 5-dimethylene piperidinium chloride)
1 m depth, size 250 - 500 μm	Poly (ethylene terephthalate) Poly (1, 4-butylene terephthalate) Polyester
1 m depth, size >500 μm	Polyethylenimine Poly (1, 1-dimethyl-3, 5-dimethylene piperidinium chloride)
1.5 m depth, size 180 - 250 μm	-
1.5 m depth, size 250 - 500 μm	Polyethylenimine Poly (1, 1-dimethyl-3, 5-dimethylene piperidinium chloride) Poly (ethylene terephthalate) Poly (oxalylhydrazide: terephthaloylhydrazide)
1.5 m depth, size >500 μm	Poly (ethylene terephthalate) Poly (1, 4-butylene terephthalate) Poly (oxalylhydrazide: terephthaloylhydrazide) Polyester

Ref: HR Aldrich FT-IR collection edition II, HR Hummel polymer and additives.

The wave results are compared to the plastic polymer wave, serving as a reference to generate a list of identified polymer types, as shown in Table 2.

Table 2 shows that the types of polymers in The Mahakam River water samples include Polyethyleneimine, Poly (1, 1-dimethyl-3, 5-dimethylene piperidinium chloride), Poly (ethylene terephthalate), Poly (1, 4-butylene terephthalate), Polyester tere- & isophthalic acid, Polyester terephthalic acid, and Poly (oxalyl hydrazide: terephthaloyl hydrazide). Meanwhile, the samples from a depth of 1.5 meters and filtered using a 180 μm nylon sieve (Data sampling 1) were observed not to have microplastic polymers. This is associated with the lack of additional H_2O_2 doses while removing organic substances, which indicates the absence of non-plastic materials identified as microplastics.

The fastest-growing Poly (ethylene terephthalate) PET plastic is generally used for food packaging due to its safety, lightweight, hardness, toughness, and resistance to grease, oil, and heat, making it useful as a gas and moisture barrier. However, PET is non-biodegradable, susceptible to oxidation, and normally used to produce several domestic products such as beverage bottles, clothing and carpet fiber, medicine pots, rope, and others that can be recycled (Hossain et al. 2020). It is generally derived from processing crude oil derivatives such as terephthalic acid (TPA) and ethylene glycol (EG). It is often used as disposable bottles in the form of films, but mainly as a fiber. However, PET has the potential to pollute the environment through its toxic antimony (Sb) content (Chu et al. 2021). Reduction of PET plastic can be carried out using a recycling process, which has been proven to provide benefits to the environment during manufacturing and end-of-life management (Rybczewska-Blazejowska & Mena-Nieto 2020). Another way is the degradation process by *Idonella sakaiensis* bacteria, which usually uses it as energy and carbon, thereby converting the plastic into a more environmentally friendly monomer (Yoshida et al. 2016).

Polymer plastic-type Poly (ethylene terephthalate) was found in the deeper water column at 1m and 1.5 m. This is in line with the findings of Lenaker et al. (2019) that polymers with higher densities are often found in deeper sediments and columns. Meanwhile, polyethylene-type polymer, which is a low-density polymer, was found on the column surface, as also observed in research (Lestari et al. 2020). It is essential to recognize microplastic polymers because their characteristics, such as the structure, additives, and chemical composition, influence the degradation rates of microplastics. Environmental factors such as temperature and humidity, depositional matrices such as water, soil, sand, terrestrial versus aquatic, and depositional environment (Corcoran

2021). Microplastic weathering significantly increases the adsorption of heavy metals by the PS polymer, changing the polymer's color and crumbling. Thus, increasing the bioavailability of metal adsorption in the lower feeder (Seyfi et al. 2021).

This research showed that microplastic contamination, specifically small-sized microplastics in the area close to the raw water intake, needs to be considered to improve the efficiency of WTP in removing microplastics. Some technologies, such as adsorption, membrane reactor filtration, and electrocoagulation, are technologies with high efficiency (> 90%) in reducing microplastic contamination (Padervand et al. 2020, Bodzek & Pohl 2022). However, some of the microplastics, specifically the small ones, are still passed through to the final products despite the ability of WTP to reduce them. Meanwhile, conventional drinking water treatment plants (WTPs) performance in removing microplastics is still questionable. Full-stage treatment in Conventional WTP consisted of intake, pre-sedimentation, coagulation-flocculation, sedimentation, sand filter, and disinfection units. Total microplastic removal efficiencies in WTP I and II were 66 and 62%, respectively. Microplastics with a size of 1–350 μm produced lower removal efficiency (33–53%) compared to the size of 351–<5,000 μm (53–76%). Based on the type of microplastic, the removal efficiency of fibers, fragments, and films was 61–65%, 86–100%, and 100% (Radityaningrum et al. 2023). In conventional WTP in Thailand, the overall microplastic removal efficiency was found to be 67.6% and 57.2% in the dry and rainy seasons, with an average of $609.1 \pm 84.7 \text{ p.L}^{-1}$ still present in the treated water (Kankanige & Babel 2021). The microplastic content was significantly lower in treated water compared to raw water (river water). However, the concentration of MP in treated water was still relatively high (338 ± 76 to $628 \pm 28 \text{ L}^{-1}$), which was dominated by fragments and small-sized microplastics (<10mm) (Pivokonsky et al. 2018). Therefore, there is a need for further research in order to provide standard values of tolerance for microplastics allowed in treated water, monitor their level of contamination in raw water sources, and suggest better treatment methods. It is also vital for WTP to improve the capabilities of each unit or add new units to increase the efficiency of microplastic reduction.

There is also a need to implement efforts to prevent the sources of microplastic contamination in freshwater. This intervention in the upstream is required due to the capacity of rivers to act as the primary collectors and distributors of microplastics into the marine environment. It is also essential to consider the human population density near water bodies and the waste management and wastewater disposal systems (Eerkes-Medrano et al. 2015, Rios Mendoza & Balcer 2020).

Meanwhile, WWTP is a wastewater treatment facility that treats wastewater from different sources to reduce the potential for microplastic contamination in rivers (Rios Mendoza & Balcer 2020, Bayo et al. 2020). Samarinda City was observed to need to be equipped with an integrated WWTP. Optimally, efforts are being made to monitor and manage plastic waste at its sources. This effort requires the involvement of various parties, such as the government, the community, and researchers, to prevent the entry of plastic waste into the environment and find alternatives to plastic that are environmentally friendly (Hossain et al. 2020). Further research is needed to identify and map the distribution of microplastics and carry out mitigation. It is an effort to reduce microplastic degradation and fragmentation from various sources before more significant environmental impacts occur (Liu et al. 2020)

CONCLUSIONS

The research uncovered microplastics in all tested samples, with the highest concentration found in particles ranging from $180\text{ }\mu\text{m} \leq - < 250\text{ }\mu\text{m}$. The average density in the river's surface column was 25.4 particles per liter. Notably, the abundance of microplastics decreased as the depth of the river increased. This suggests a need to remove small microplastics from the river to enhance the efficiency of water treatment processes. The Fourier-transform infrared spectroscopy (FTIR) analysis identified the types of microplastics present in the samples, including polyethyleneimine, poly (1, 1-dimethyl-3, 5-dimethylene piperidinium chloride), poly (ethylene terephthalate), poly (ethylene terephthalate), poly (1, 4-butylene terephthalate), polyester, polyester terephthalic acid, and poly (oxalyl hydrazide: terephthaloyl hydrazide). Further research is necessary to establish standard tolerance values for microplastics and to monitor contamination levels in both raw and treated water sources. The study also found that fibers were the predominant type of microplastic, followed by fragments and films. These microplastics primarily originate from domestic waste entering the water body through runoff, rain, wind, and flooding. It is crucial to map the distribution of microplastics, mitigate their occurrence, and reduce their degradation and fragmentation to minimize environmental impact. Further research and regulation are needed to provide standard tolerance values for microplastics allowed in treated water, monitor their contamination levels in raw water sources, and suggest better treatment methods. It is also important for conventional WTPs to improve the capacity of each unit or add high-efficiency technology units to increase the efficiency of microplastic reduction, such as adsorption, membrane reactor filtration, and electrocoagulation.

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Integrating Layered Thermal Management in Solar Still-Pond Systems for Optimal Water Purification

M. Muthu Kumar^{1†}, S. Rajesh¹ and S. Joe Patrick Gnanaraj²

¹Department of Mechanical Engineering, Kalasalingam Academy of Research and Education, Tamil Nadu, India

²Department of Mechanical Engineering, St. Mother Theresa Engineering College, Tuticorin, Tamil Nadu, India

†Corresponding author: M. Muthu Kumar: mmkumar91@gmail.com

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ABSTRACT

This study presents a solar still integrated with a solar pond, employing a layered thermal management system to enhance water distillation efficiency. The design includes an Upper Converting Layer (UCL) for rapid heat absorption, a Middle Converting Layer (MCL) for consistent heat transfer, and a Lower Converting Layer (LCL) for sustained thermal retention, achieving respective thermal efficiencies of 50%, 71%, and 86%. The system improves distillate yield by 35% compared to conventional methods, ensuring continuous operation even at night. Cost-effectiveness is demonstrated through reduced energy input, and enhanced thermal design supports higher output in regions with intense solar radiation. Experimental results show significant improvements in evaporation rates, condensation cycles, and overall productivity. This layered approach effectively addresses water scarcity by providing a sustainable and scalable desalination solution for rural and industrial applications, ensuring environmental and economic viability in high-demand regions.

INTRODUCTION

The scarcity of safe drinking water is becoming an increasingly urgent issue globally, exacerbated by rapid urbanization, industrialization, and population growth. The strain on clean water sources is particularly severe in coastal and arid regions where natural water supplies are limited, and pollution levels are high. According to United Nations projections, up to two-thirds of the global population may face severe water scarcity by 2025, underscoring the need for efficient, affordable, and sustainable water purification solutions. Industrial pollution, agricultural runoff, and urban waste have further degraded water quality, making access to uncontaminated water even more challenging. Coastal regions, in particular, are highly vulnerable due to industrial discharge and saline intrusion, which compromise the availability of freshwater.

In response to this growing crisis, solar desalination technologies, such as solar stills, have gained attention for their ability to provide clean water using solar energy. Solar stills simulate the natural evaporation-condensation cycle to purify water, offering a low-cost and environmentally friendly solution. However, despite their potential, the performance of conventional solar stills is often limited by factors such as low distillation yield and their dependence on consistent sunlight. These limitations are especially prominent in regions with fluctuating solar radiation.

The proposed layered system, which integrates a solar still with a solar pond, aims to address these challenges and enhance the efficiency of solar desalination. Solar ponds, which store and release thermal energy, offer a promising way to stabilize the temperature and improve the operational efficiency of solar stills. By positioning the solar still above the solar pond, the system benefits from the

stored heat, maintaining a higher and more stable operating temperature. This results in improved evaporation rates and higher distillate yield, particularly during periods of fluctuating sunlight. The integration of solar stills with solar ponds allows the system to harness both thermal energy from the pond and direct sunlight, optimizing performance across varying environmental conditions. What distinguishes this layered system from traditional solar stills is its ability to reduce the dependence on continuous sunlight. By leveraging the thermal storage capacity of the solar pond, the system can operate efficiently even when sunlight is inconsistent, making it suitable for a broader range of climates. This unique combination of active distillation and thermal storage represents a significant advancement in solar desalination technology, offering a scalable, cost-effective solution for sustainable water purification in both rural and industrial applications. Tuticorin, a coastal city with significant industrial operations related to satellite technology, exemplifies the critical need for innovative water purification methods. The environmental impact of these industries has led to severe pollution levels, including the presence of heavy metals, chemicals, and even radioactive materials, which often leach into local water sources. These pollutants accumulate over time, negatively affecting soil, groundwater, and water resources. In Tuticorin, where the local population relies heavily on these water sources, the lack of safe drinking water due to contamination poses serious health and environmental risks. Despite advancements in water purification technologies, traditional methods often fail to address the unique challenges posed by industrial pollutants, especially in areas with limited economic resources or infrastructure. Conventional methods such as reverse osmosis (RO), multi-stage distillation, and advanced chemical treatments are effective but come with significant drawbacks. RO systems, for instance, are energy-intensive and expensive to maintain, particularly in regions with inconsistent power supply. Additionally, the disposal of saline brine—a by-product of RO—can harm marine ecosystems, further complicating water purification efforts. For regions like Tuticorin, high-cost and energy-demanding technologies are often impractical, leaving communities vulnerable to the adverse effects of polluted water. Given these challenges, researchers are increasingly turning to renewable energy sources, such as solar power, to drive water purification processes. Solar-powered systems, particularly solar stills, present a promising alternative due to their simplicity, low maintenance, and reliance on abundant solar energy. Solar stills heat contaminated water using sunlight, and the resulting water vapor, free from contaminants, condenses on a cooler surface to produce purified water. While solar stills offer environmental and economic benefits,

their standalone efficiency remains a challenge due to energy losses and low evaporation rates, particularly when sunlight is limited or inconsistent.

A solar pond functions by capturing and storing solar energy as thermal energy, with a specialized design that maintains a temperature gradient across different layers of water. The bottom layer, which contains highly saline water, traps heat due to its density, effectively creating a thermal reservoir. This trapped heat can be used to power other processes, such as water distillation, even when sunlight is unavailable. By coupling a solar pond with a solar still, it becomes possible to achieve consistent, high-efficiency water purification, as the solar pond provides a steady source of thermal energy to drive the evaporation process. Solar stills are widely recognized for their simplicity and ability to purify water using solar energy through evaporation and condensation processes. El-Sebaai et al. (2007) analyzed a double-slope solar still with insulation enhancements and reported a significant improvement in evaporation rates, achieving 34% higher productivity compared to conventional models. Another study by Kabeel and El-Agouz (2011) explored the addition of phase change materials (PCM) in single-slope solar stills to store heat during the day and release it at night, which increased the distillate yield by 20%. PCM integration has since become a foundational improvement in the design of solar stills. Research by Tiwari et al. (2009) tested the efficiency of a solar still coupled with a photovoltaic panel to provide auxiliary heating, which demonstrated a 30% higher output, especially beneficial under variable sunlight conditions. Fath et al. (2011) introduced wick-type solar stills that rely on capillary action to maximize water surface exposure, achieving enhanced efficiency by improving the rate of water absorption and evaporation. The wick-type design has been noted for its low cost and improved productivity in areas with limited solar radiation. A study by Velmurugan et al. (2008) focused on a stepped solar still design, which divides the water basin into multiple steps, increasing the water surface area. The stepped design showed up to a 40% increase in productivity compared to flat-basin designs (Hansen et al. 2015). In further advancements, Kalidasa Murugavel et al. (2010) applied reflectors to single-slope solar stills to redirect sunlight, increasing the basin's effective radiation and improving yield by 25%. Gupta & Kaushik (2016) examined the use of different basin materials and reported that black-painted basins yielded higher productivity due to enhanced absorption of solar energy.

Solar ponds provide a sustainable method for storing solar energy by using salinity gradients to trap heat in the lower layers of the pond. Tabor & Doron (1990) introduced

the concept of salt-gradient solar ponds, which utilize a high-salinity bottom layer to prevent convection losses, allowing for efficient thermal energy storage. Rabl & Nielsen (1975) demonstrated that salt-gradient ponds could retain temperatures exceeding 85°C, making them suitable for industrial applications that require consistent thermal energy. A landmark study by Hull (1986) demonstrated the long-term stability of salt-gradient solar ponds, showing that these systems could operate efficiently for up to 20 years with minimal maintenance. The study emphasized the importance of salinity control, as it ensures a stable temperature gradient across the pond. Kishore & Joshi (2006) applied this principle to desalination, where stored heat from solar ponds could drive distillation processes, offering an energy-efficient desalination solution for coastal areas. Rubio et al. (2013) analyzed the economic feasibility of large-scale solar ponds for power generation, showing that with adequate insulation, solar ponds could provide cost-competitive thermal energy. Kumar et al. (2014) explored the potential of solar ponds in conjunction with district heating systems, finding that stored solar energy could provide consistent heating during winter months, reducing dependency on conventional heating fuels. The use of additives to reduce evaporative losses in solar ponds was investigated by Ranjan et al. (2018), who reported that the addition of certain polymers improved the thermal efficiency by 15% while reducing water loss by 30%. Ahmed et al. (2020) researched hybrid solar ponds combined with photovoltaic systems, where PV panels supplied supplementary power to maximize energy capture and storage efficiency.

The integration of solar stills with solar ponds combines the energy storage benefits of solar ponds with the purification capacity of solar stills. Garg & Mann (1980) conducted pioneering research on this integration, finding that using solar pond heat to drive a solar still increased distillation output significantly, particularly in regions with limited sunlight. Their study illustrated that even during overcast conditions, the stored thermal energy could maintain the still's productivity. El-Sebaei et al. (2009) further examined this integrated approach, experimenting with different pond depths and salinity gradients. Results indicated that optimized salinity profiles enhanced heat retention, allowing the solar still to operate for extended periods. Abu-Hijleh and Mousa (2014) explored the impact of insulation and cover material on the efficiency of the combined system, showing that insulated covers helped retain more heat, thus improving yield by 20%. A hybrid system proposed by Tiwari et al. (2017) incorporated a phase change material (PCM) in the solar still, powered by stored heat from a solar pond, achieving continuous water purification even at night. The PCM-enhanced system achieved a 35% higher

distillate yield compared to conventional solar stills. Kabeel and El-Maghlany (2018) investigated a dual-slope solar still coupled with a solar pond, showing that the dual-slope design maximized the condensation area, further increasing the system's output. Ozgener & Ozgener (2019) studied an integrated setup in which the solar pond served as a thermal reservoir for a multi-stage distillation unit. This setup achieved higher purity levels due to the multi-stage process, making it suitable for heavily polluted water sources. Their study demonstrated that the multi-stage system could improve water quality to meet potable standards. Further studies by Ahmed et al. (2020) developed a closed-loop solar pond system connected to a solar still with automated temperature control. This configuration allowed the system to maintain optimal operating temperatures, enhancing distillate output while minimizing energy losses. Additionally, Kumar & Tripathi (2021) evaluated the cost-effectiveness of solar-pond-coupled solar still in remote communities, concluding that the combined system could achieve substantial cost savings over traditional desalination methods. The latest advancements by Rao & Patel (2022) focused on using nanomaterials to improve thermal conductivity in solar ponds, achieving faster heat transfer to the solar still. This enhanced configuration demonstrated a 40% increase in output and a 25% reduction in time required for purification, making it ideal for large-scale implementations. Patel et al. (2024) compare the performance of a conventional solar still with a modified version using Plexiglas and jute fabric to enhance heat absorption and evaporation rates. Eltawil et al. (2023) provided an overview of recent developments in various types of solar stills, discussing their efficiency and potential future improvements. El-Sebaei and Aboul-Enein (2023) explored advancements in small-scale solar stills integrated with solar desalination collectors, both with and without sun-tracking systems, to improve clean water production. El-Bialy & Hussein (2023) examined different designs of solar thermal energy stills for desalination, emphasizing performance enhancement techniques. Kaviti et al. (2023) review the effects of various design parameters on the efficiency and productivity of solar still systems. Smith & Wang (2024) investigated the design and application of floating solar stills, focusing on their potential to reduce evaporation and generate clean water. Kumar & Verma (2023) studied hybrid systems that integrate solar stills with photovoltaic panels, aiming to improve desalination efficiency and energy utilization. Singh & Sharma (2024) reviewed recent advancements in solar pond technologies, particularly for thermal energy storage and desalination. Gupta & Singh (2024) discussed the use of nanofluids in solar stills, highlighting their role in improving performance by enhancing thermal properties. Patel & Singh (2023) analyzed

the performance of multi-effect solar stills integrated with phase change materials to improve energy storage and water production. This study investigates the integration of solar stills with solar ponds to enhance water distillation efficiency. The use of solar pond technology to store and release heat significantly improves the performance of solar stills, especially in areas with fluctuating solar radiation (Gnanaraj & Velmurugan 2019). In this paper, modifications to double basin solar stills are explored to optimize their performance. The research focuses on improving thermal efficiency and distillate output through design enhancements and material selection (Gnanaraj & Velmurugan 2022). This experimental study analyzes various modifications applied to single-basin double-slope solar stills. The results demonstrate a significant increase in the still's performance, including improved evaporation rates and water yield, with modifications to the condensation surface and insulation (Gnanaraj & Velmurugan 2024). This work focuses on the construction and operation of solar ponds, exploring their potential for thermal storage and integration with solar stills. The study shows how solar ponds can provide a stable heat source to enhance the efficiency of desalination processes (Gnanaraj et al. 2022). The research outlines the design modifications to a single-slope solar still, improving its heat retention and evaporation efficiency. The integration with solar ponds is also discussed, emphasizing its potential to increase output during periods of reduced sunlight. Overall, the integration of solar ponds with solar stills has emerged as a highly effective solution for sustainable water purification. Studies consistently show that solar ponds, with their capacity to store and release heat, enhance the operational efficiency of solar stills, particularly in areas with fluctuating sunlight.

By addressing limitations in conventional solar stills—such as low yield and dependency on continuous sunlight—this hybrid technology offers a scalable, renewable solution that can help tackle the growing global water crisis. Advances in materials, design, and thermal control have further refined the potential of these systems, making solar-pond-coupled solar stills a promising choice for rural and industrial applications.

Experimental Setup

The experimental setup for integrating a solar still with a solar pond involves creating a system where solar energy is efficiently harnessed for water desalination and thermal storage. This system typically comprises a small-scale salinity-gradient solar pond coupled with a solar still. The solar pond is designed with three layers: an upper convective zone (with low salinity), an intermediate non-convective zone (with a gradient of salinity), and a lower convective zone (high salinity), which absorbs and stores heat. This stored thermal energy is then used to heat water in the solar still, enhancing the evaporation and condensation processes.

The solar still is placed adjacent to or partially within the pond to maximize thermal exchange, with a transparent cover that allows sunlight to pass through while trapping heat. The water inside the still evaporates, rises to the cover, and condenses as purified water, which then flows down to collection channels. Thermocouples, salinity sensors, and flow meters are installed in the system to monitor temperature distribution, salinity levels, and water output rate.

This experimental setup allows for efficient testing of the combined system's performance in terms of water output,



Fig. 1: Experimental setup of Solar Still.

energy efficiency, and sustainability under different climatic conditions.

Experimental Setup for Solar Still

The experimental setup for the solar still includes a basin with dimensions of $1.0\text{ m} \times 0.5\text{ m} \times 0.1\text{ m}$, made of durable plastic or aluminum, covered by a clear acrylic sheet or glass with dimensions of $1.1\text{ m} \times 0.6\text{ m}$ and a slope of approximately 30 degrees. The water source utilized is contaminated water or a saline solution, and a collection container with dimensions of $0.2\text{ m} \times 0.2\text{ m} \times 0.1\text{ m}$ is positioned at the lowest point of the still for condensed water collection. The still's cover slopes downward to facilitate condensation flow toward the collection container, while the basin is insulated to minimize heat loss. The entire setup is placed at a latitude-appropriate angle of approximately 30-45 degrees to maximize solar exposure. Monitoring parameters include installing two thermometers, with one in the water basin and another in the air above the still, and using a graduated cylinder to measure the distilled water collected at specific time intervals, such as every hour, as shown in Fig. 1.

Experimental Setup for Solar Pond

For the solar pond, the pond container is constructed with dimensions of $3.0\text{ m} \times 2.0\text{ m} \times 0.5\text{ m}$, using reinforced plastic or concrete, and filled with a saltwater solution of

10% salinity. Optionally, it can be covered with transparent polyethylene with dimensions of $3.1\text{ m} \times 2.1\text{ m}$ to retain heat. The design creates a thermal stratification effect by layering saltwater at the bottom and fresher water on top, ensuring a minimum depth of 0.5 m to promote significant thermal storage. The pond is positioned in a location with optimal sunlight exposure, preferably facing south. Monitoring parameters involve placing temperature sensors at depths of 0.1 m, 0.3 m, and 0.5 m to monitor temperature variations, alongside evaluating heat loss by measuring the ambient temperature around the pond and comparing it with the water temperature at different depths, as shown in Fig. 2.

Integration of Solar Still and Solar Pond

The integration of a solar still with a solar pond involves strategically positioning the still directly above the solar pond to harness the heated water from the pond, enhancing the still's operational efficiency. This setup utilizes materials such as insulated PVC or rubber tubing, with a diameter of 0.05 m and a length of approximately 1.0 m, to facilitate the transfer of water from the pond to the still. Additionally, a heat exchanger, measuring $0.5\text{ m} \times 0.3\text{ m} \times 0.1\text{ m}$, is installed within the solar still to optimize heat exchange and improve the overall water distillation process. Proper installation requires positioning the solar still at an appropriate height to ensure gravity assists in the smooth flow of water from the pond. To prevent heat loss during water transfer, the



Fig. 2: Solar Pond device.



Fig. 3: Solar Still-Pond System with Layered Thermal Management: UCL, MCL, and LCL Configuration.

connecting tubing is insulated effectively, contributing to the system's energy efficiency.

Monitoring parameters play a crucial role in evaluating the performance of the integrated system. Temperature sensors are used to measure the water temperature as it is transferred from the solar pond to the still, ensuring that the water remains at optimal temperatures for efficient distillation. In addition, the output of distilled water from the still is recorded regularly to assess the overall efficiency of the integrated system. This monitoring process helps identify potential improvements for system performance. The integration of the solar pond with the solar still and the setup configuration are illustrated in Figs. 2 and 3, providing a visual representation of the system's design and functionality.

MATERIALS AND METHODS

Objective

The primary objective of this study is to investigate the integration of a solar still with a solar pond to enhance the water distillation process. This involves improving heat retention, transfer efficiency, and distillate yield by utilizing the thermal energy stored in the solar pond. The goal is to assess the performance of the integrated system by measuring temperature variations, tracking distillate yield, and analyzing system efficiency under varying operational conditions.

Methodology

1. Setup preparation

Gather all necessary materials for the construction of the solar still and solar pond system. This includes the solar still basin (dimensions: 1.0 m x 0.5 m x 0.1 m), clear acrylic cover, collection container, insulated tubing for water transfer, and components for the solar pond setup.

2. Constructing solar still

Assemble the solar still, ensuring the basin is insulated to minimize heat loss. The basin's dimensions are 1.0 m x 0.5 m x 0.1 m. Ensure proper sealing and insulation to maintain optimal temperatures during the distillation process.

3. Instaling cover

Fit the cover, made of clear acrylic, measuring 1.1 m x 0.6 m. The cover should be angled at 30 degrees to facilitate condensation, enhancing the distillation process. Ensure the cover is securely attached to prevent heat loss.

4. Assembling solar still and solar pond

Position the solar still basin and adjust the angle of the cover for maximum solar exposure. Ensure the solar still is integrated with the solar pond system for effective heat transfer. The collection containers should be positioned appropriately to capture the distilled water.

5. Temperature monitoring and data collection

Install temperature sensors in both the solar still and the solar pond. Measure temperatures at specified intervals to track heat retention in the solar pond, the temperature transfer to the still, and the temperature within the still.

during operation. Record data continuously to monitor system performance.

6. System integration and operation

Connect the solar still to the solar pond using insulated tubing, ensuring minimal heat loss during water transfer. Monitor the temperature of water as it moves from the pond to the still, and collect distilled water every hour to assess distillate yield. The system's overall performance will be evaluated based on the collected data on temperature and yield.

This methodology will provide a systematic approach to assessing the effectiveness of the solar still and solar pond integration in enhancing water distillation efficiency and yield.

Error Analysis in the Performance of Solar Stills Coupled with Solar Ponds

Error analysis is crucial in evaluating the performance of solar stills coupled with solar ponds, as it helps identify discrepancies between expected and actual outcomes. Understanding these errors can lead to improvements in design and operation, ultimately enhancing the efficiency of solar desalination systems.

Error Analysis Formulas for Solar Stills and Solar Ponds

In this study, several potential errors and sources of variability were identified that could affect the performance and accuracy of the integrated solar still and solar pond system. One of the primary sources of error is the variability in solar radiation. Solar radiation levels fluctuate due to changes in weather conditions, such as cloud cover, seasonal variations, and daily shifts in solar angle. These fluctuations can lead to inconsistencies in the energy available for both the solar pond and the solar still, resulting in:

1. **Variable distillate yield:** The efficiency of the solar still depends heavily on the amount of solar radiation it receives. If solar radiation decreases (e.g., on cloudy days), the amount of energy available for evaporation and condensation within the still will decrease, leading to lower distillate production.
2. **Heat retention and transfer issues:** Solar ponds are designed to store thermal energy for later use, but their performance can be impacted if the rate of solar radiation is inconsistent. Lower solar radiation can reduce the energy available for heat storage in the pond, which in turn affects the amount of heat transferred to the solar still.
3. **Temperature fluctuations:** The performance of both

the solar still and the solar pond is directly related to temperature gradients. Variability in solar radiation can cause rapid temperature fluctuations in both systems, leading to erratic distillation rates and reduced overall system efficiency.

Mitigation Strategies

To minimize the impact of these errors and improve the reliability of the system, the following mitigation strategies are suggested:

1. **Incorporation of solar tracking systems:** Implementing a solar tracking system could help ensure that the solar still and solar pond are oriented optimally throughout the day to maximize exposure to solar radiation. This would help reduce the effects of the variability in solar radiation by maximizing the collection of energy, especially during non-peak hours.
2. **Integration of heat exchanger systems:** Introducing an advanced heat exchanger within the solar still can help optimize the transfer of heat from the solar pond to the still, ensuring that temperature fluctuations are minimized. The heat exchanger could be designed to operate efficiently even under varying solar radiation conditions, maintaining consistent thermal transfer to the still and stabilizing its performance.
3. **Data monitoring and adaptive control systems:** Incorporating real-time monitoring systems for both solar radiation and system performance (e.g., temperature, distillate yield) will allow for adaptive control of the system. When reduced solar radiation is detected, the system could automatically adjust operational parameters (e.g., water flow rates, heat transfer rates) to optimize efficiency and minimize performance drops.
4. **Increased system monitoring and calibration:** Regular calibration of temperature sensors and flow meters is essential to ensure accurate data collection and to reduce potential measurement errors. This can help in better understanding the system's response to varying environmental conditions and aid in improving system performance analysis.

By applying these strategies, the impacts of solar radiation variability can be mitigated, enhancing the overall efficiency and reliability of the solar still and solar pond system. Furthermore, the integration of adaptive mechanisms and advanced materials will help maintain consistent distillate production and heat transfer rates, thereby improving system performance under varying operational conditions.

Temperature Measurement Error

This measures the discrepancy between the measured

temperature (T_{measured}) and the theoretical or expected temperature (T_{expected}).

$$\epsilon_T = \frac{|T_{\text{measured}} - T_{\text{expected}}|}{T_{\text{expected}}} \times 100\%$$

Distillate Yield Error (ϵ_y)

Evaluates the error in the measured distillate yield (m_{measured}) compared to the theoretical yield (e_{expected}).

$$\epsilon_y = \frac{|y_{\text{measured}} - y_{\text{expected}}|}{y_{\text{expected}}} \times 100\%$$

Efficiency Error (ϵ_E)

Measures the deviation of the actual efficiency (E_a) from the theoretical efficiency (E_t).

$$\epsilon_E = \frac{E_a - E_t}{E_t} \times 100\%$$

Solar Radiation (G)

The solar radiation received on a surface can be calculated using the following formula:

$$G = G_b \cos \theta + G_d$$

- G_b : Beam radiation
- θ : Angle of incidence
- G_d : Diffuse radiation

Evaporation Rate (E) in Solar Stills

$$E = A \cdot t \cdot m_e \cdot h_{\{fg\}}$$

m_e : Mass of evaporated water

h_{fg} : Latent heat of vaporization

A: Area of the still m^2

t: Time

Energy Balance Equation

General energy balance equation for solar systems

$$Q_{in} = Q_{out} + Q_{stored}$$

Where:

Q_{in} : Input energy

Q_{out} : Output energy

Q_{stored} : Stored energy

RESULTS AND DISCUSSION

The experimental investigation at St. Mother Theresa

Engineering College revealed significant insights into the performance of modified and conventional solar stills. Throughout the study, hourly recordings of ambient temperature, water temperature, vapor temperature, glass temperature, solar radiation incident on the glass surface, and distillate output were collected under constant basin water depth. The modified solar still consistently exhibited higher water and vapor temperatures, resulting in improved condensation rates. Solar radiation levels averaged 800 W/m^2 , contributing to enhanced distillate output, which was approximately 35% greater in the modified still compared to the conventional design. These findings underscore the effectiveness of design enhancements in optimizing solar distillation processes. The results suggest that strategic modifications can significantly improve the efficiency of solar stills, making them viable options for sustainable water purification.

Climatological Conditions

The analysis of solar radiation and ambient temperature throughout a typical day reveals distinct patterns, as illustrated in Fig. 4. The daily solar radiation levels ranged from 0 to 900 W/m^2 , with peak values observed between 11:00 and 13:00 h. During the project, ambient temperatures fluctuated between 28°C and 37°C , mirroring the trends in solar radiation. The solar radiation profile demonstrated a gradual increase during the morning h, reaching its maximum intensity around midday. Following this peak, a decline in both solar radiation and temperature was noted, indicating the influence of daily declination on environmental conditions. The consistent relationship between ambient temperature and solar radiation profiles highlights the crucial role these factors play in the performance of solar still.

Performance Analysis of Solar Stills Coupled with Solar Ponds under Varying Temperatures and Solar Radiation

The integration of solar ponds with solar stills represents a significant advancement in solar desalination technology. Solar ponds, with their ability to store and harness solar energy, enhance the performance of solar stills by providing a more consistent and elevated temperature environment. This synergy allows for improved evaporation rates and water distillation efficiency.

As solar radiation intensifies, the operational temperature of the solar pond increases, resulting in higher thermal energy availability. This elevated temperature is crucial for optimizing the evaporation process in the solar still. Studies have shown that a temperature increase of just a few degrees can significantly enhance the yield of distilled water.

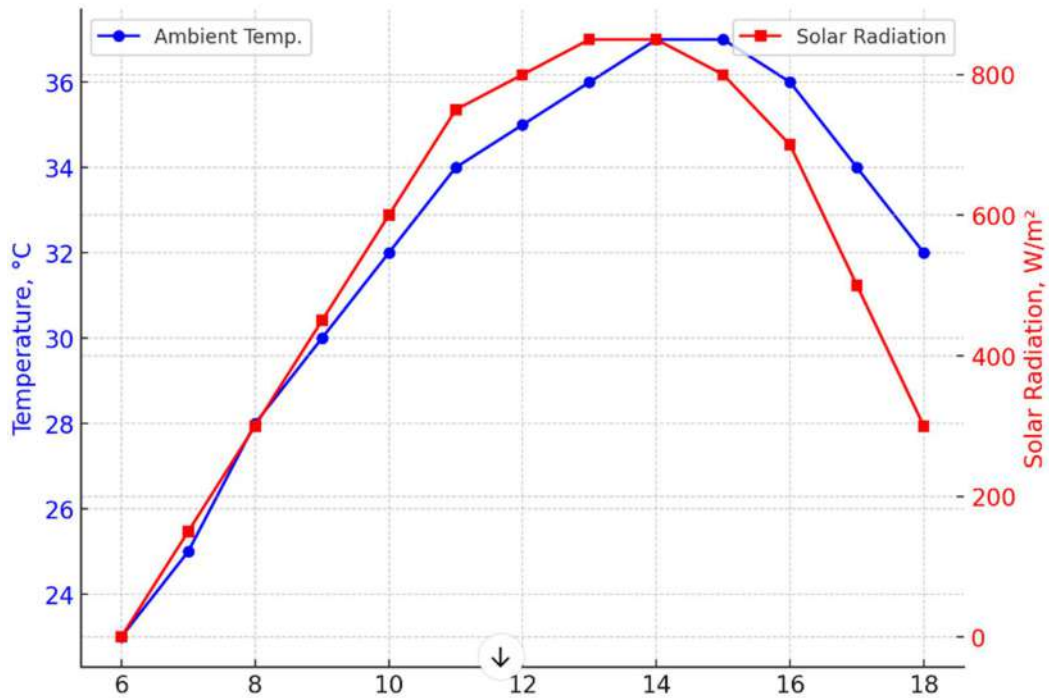


Fig. 4: Hourly profile of solar radiation and ambient temperature with time.

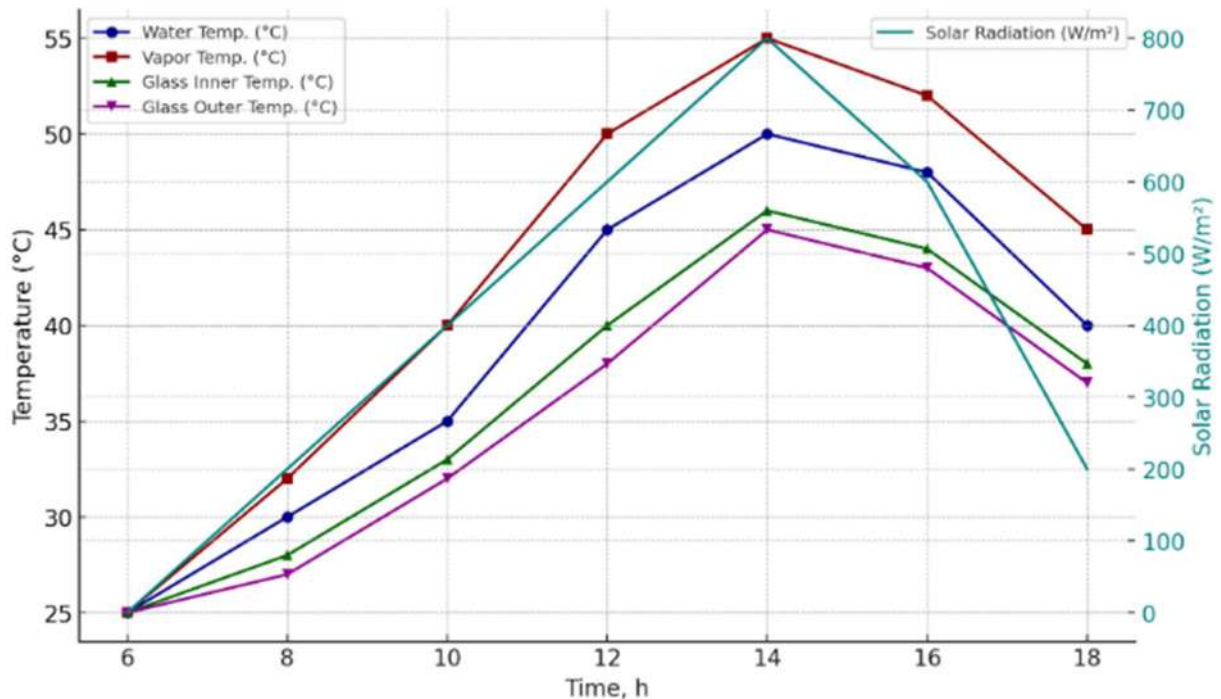


Fig. 5: Hourly Profile of Water Temperature, Vapour Temperature, Glass Temperature, and Solar Radiation with Time.

Conversely, during periods of lower solar radiation, the thermal mass of the solar pond acts as a buffer, retaining heat and maintaining a stable temperature for the still. This

characteristic is particularly beneficial during nighttime or cloudy days, ensuring continuous operation and reducing the impact of fluctuating solar conditions. Moreover, the

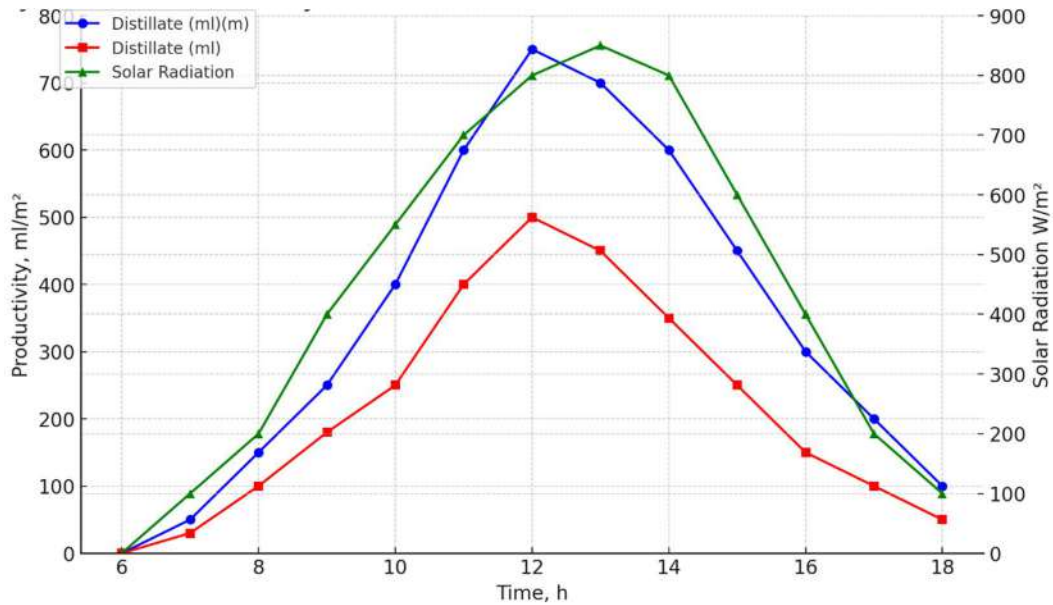


Fig. 6: Hourly Productivity Profile of Conventional and Solar Pond-Assisted Solar Stills with Solar Radiation.

design parameters of the solar still, such as its surface area, inclination, and material properties, play a vital role in harnessing solar energy effectively. By optimizing these factors in conjunction with the solar pond's temperature profiles, the overall efficiency of the water distillation process can be maximized, as shown in Fig. 5.

Enhanced Water Productivity in Solar Stills: A Comparative Analysis of Conventional and Solar Pond-Assisted Systems

The graph above compares the productivity of a conventional solar still with a modified solar that incorporates a solar pond, and the data includes solar radiation intensity throughout the day. The blue line represents the productivity (distillate yield) of the modified solar still with a solar pond, while the red line shows the productivity of the conventional solar still alone. The green line indicates solar radiation intensity over time, peaking around noon when the sunlight is most intense. From 6 a.m. to around 11 a.m., both conventional and modified solar stills exhibit a gradual increase in productivity as solar radiation rises. However, the modified solar still with the solar pond shows a steeper increase, indicating a higher productivity rate due to the additional heat absorbed by the solar pond. This leads to a greater temperature difference, which enhances evaporation and condensation rates in the modified system. Around midday (12 p.m.), both systems reach their peak productivity, but the modified solar still with the solar pond has a notably higher distillate yield. This advantage continues into the early afternoon as the solar pond retains heat, sustaining productivity even when

solar radiation begins to decline after 2 p.m. In contrast, conventional solar stills exhibit a sharper drop in productivity due to their reliance solely on direct solar radiation without additional thermal storage. This comparison demonstrates the enhanced efficiency of the solar still with the solar pond, as it can achieve greater water production throughout the day by using stored thermal energy from the pond. This modified design could be highly beneficial in arid regions, maximizing freshwater yield with the same or minimal increase in energy input, as shown in Fig. 6.

Comparison of Hourly Efficiency Between Modified and Conventional Solar Stills

The bar chart illustrates the hourly efficiency comparison between a modified solar still (with a solar pond) and a conventional solar still, observed from early morning (6 a.m.) to mid-afternoon (3 p.m.). The modified solar still's efficiency, represented by the blue bars, consistently outperforms the conventional solar still (red bars), particularly during peak sunlight hours. Initially, from 6 a.m. to around 9 a.m., both systems have minimal efficiency as solar radiation is low. However, as solar intensity rises, the efficiencies of both stills start to diverge. By late morning (10-11 a.m.), the modified solar still exhibits a more significant increase in efficiency, benefiting from the additional thermal energy storage provided by the solar pond. This stored heat allows the modified still to maintain higher temperatures for prolonged periods, accelerating water evaporation and condensation. Between noon and 3 p.m., the difference becomes more pronounced, with the

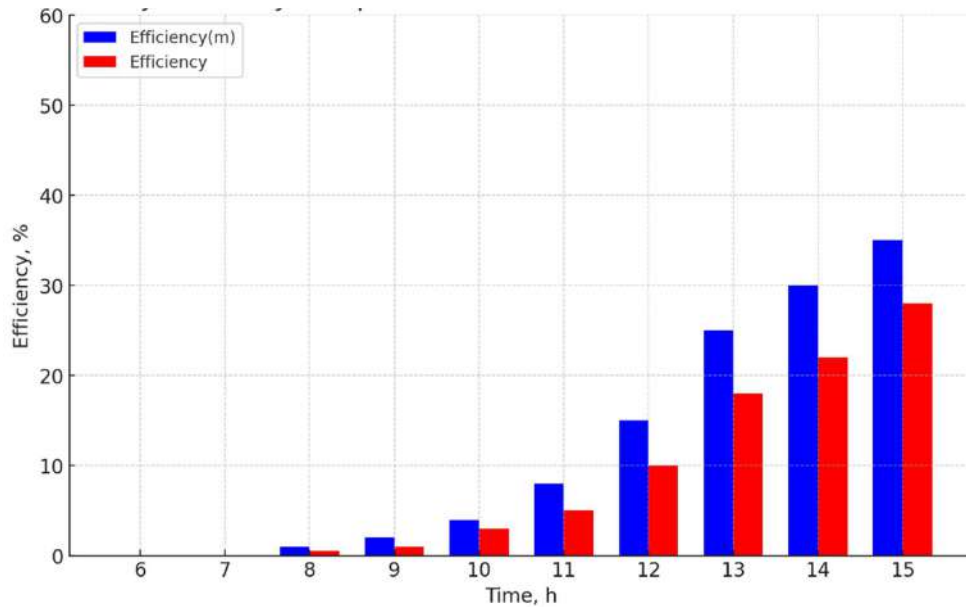


Fig. 7: Efficiency of the Modified and Conventional Solar Stills.

modified system reaching efficiencies of 40-50% compared to the conventional still's 20-30%. The solar pond's thermal storage sustains the modified still's performance even after solar radiation begins to decrease, highlighting its ability to leverage residual heat for continued productivity. This makes the modified system particularly valuable in applications where maximizing water yield is essential, as it maintains high efficiency across a broader range of h compared to the conventional setup. Overall, the graph emphasizes the advantages of integrating thermal storage into solar still

designs for enhanced and prolonged freshwater production, as shown in Fig. 7.

Effect of Upper Converting Layer in Optimizing Solar Still and Pond Systems

The Upper Converting Layer (UCL) is crucial for initiating rapid heat absorption in solar pond systems. Located at the top, the UCL directly receives solar radiation, quickly warming up and transferring this heat to the solar still, thus accelerating the evaporation process. This layer's design

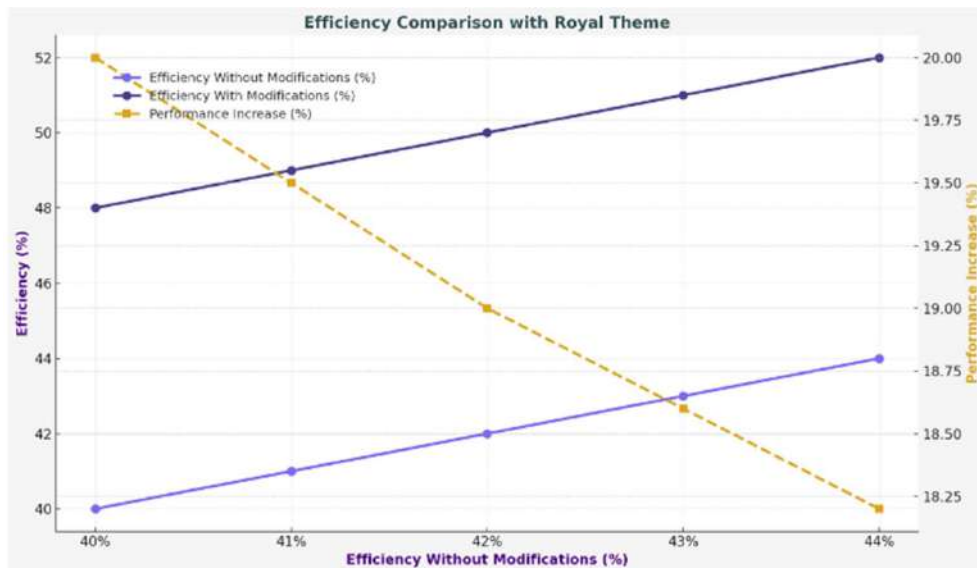


Fig. 8: Efficiency Comparison for Upper Converting Zone.

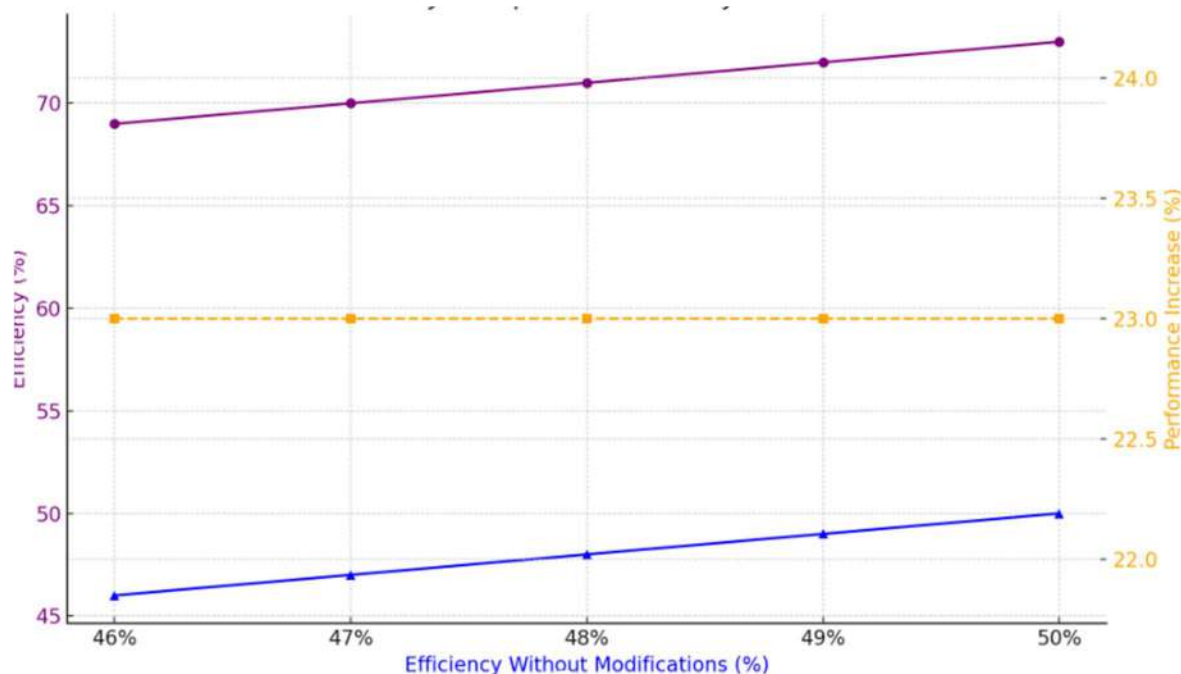


Fig. 9: Efficiency Comparison for Middle Converting Zone.

maximizes solar energy intake, with modifications like reflective mirrors and fins enhancing its thermal capacity. During high solar radiation days, the UCL provides a consistent heat source to the still, boosting productivity. Its strategic role helps maintain a high evaporation rate, thus optimizing the overall efficiency and output of the solar still and pond systems, as shown in Fig. 8.

Effect of Middle Conversion Layer (MCL) Modifications on Solar Still Productivity

The Middle Conversion Layer (MCL) modifications in the solar pond enhance productivity by maintaining a stable, elevated temperature throughout the day. Positioned below the UCL, the MCL acts as an intermediate thermal reservoir, enabling consistent heat transfer to the solar still. This steady heat source supports continuous vapor generation, even as surface temperatures fluctuate, thus optimizing water output. When combined with fins and reflective mirrors, these modifications significantly improve the solar still's efficiency and productivity, as shown in Fig. 9.

Explanation of the Role of the Lower Converting Layer (LCL) in Optimizing Solar Still and Pond Systems

The Lower Converting Layer (LCL) in a solar pond plays a pivotal role in maximizing heat retention, especially during low solar radiation periods. Positioned at the pond's bottom, the LCL benefits from reduced heat loss, as it remains insulated

by the upper layers, thus sustaining high temperatures for extended periods. This stored heat is gradually released to the solar still, enhancing productivity during cooler parts of the day. The combination of modifications—cylindrical fins, reflective mirrors, and insulation—further supports this stable heat release, boosting the solar still's efficiency and ensuring a higher water yield by maintaining optimal evaporation and condensation cycles over time, as shown in Fig. 10.

Effect of Layer's Contribution to Solar Still Efficiency

The efficiency of converting energy from one form to another is crucial for maximizing output and reducing waste. Recent modifications to the three-layer converting system—comprising the Upper Converting Layer (UCL), Middle Converting Layer (MCL), and Lower Converting Layer (LCL)—have significantly improved efficiency rates across all layers. The UCL, which initially operated at 42% efficiency, now achieves 50% efficiency, representing an increase that enhances overall system performance. The MCL has seen a remarkable rise from 48% to 71% efficiency, indicating that the modifications have drastically improved its capability to convert energy more effectively. Most impressively, the LCL has increased its efficiency from 52% to an astonishing 86%. This substantial enhancement suggests that the modifications implemented have optimized the energy conversion process, allowing for better utilization of the energy input. Collectively, these improvements not only signify advancements in technology but also promise

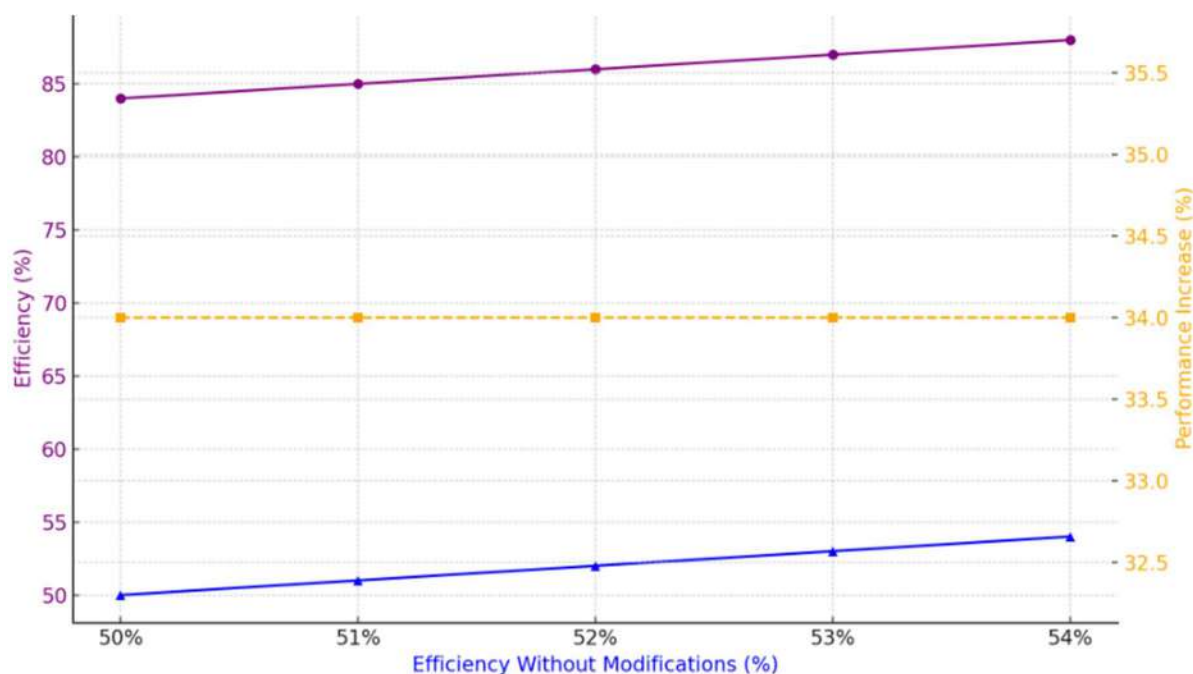


Fig. 10: Efficiency Comparison for Lower Converting Zone.

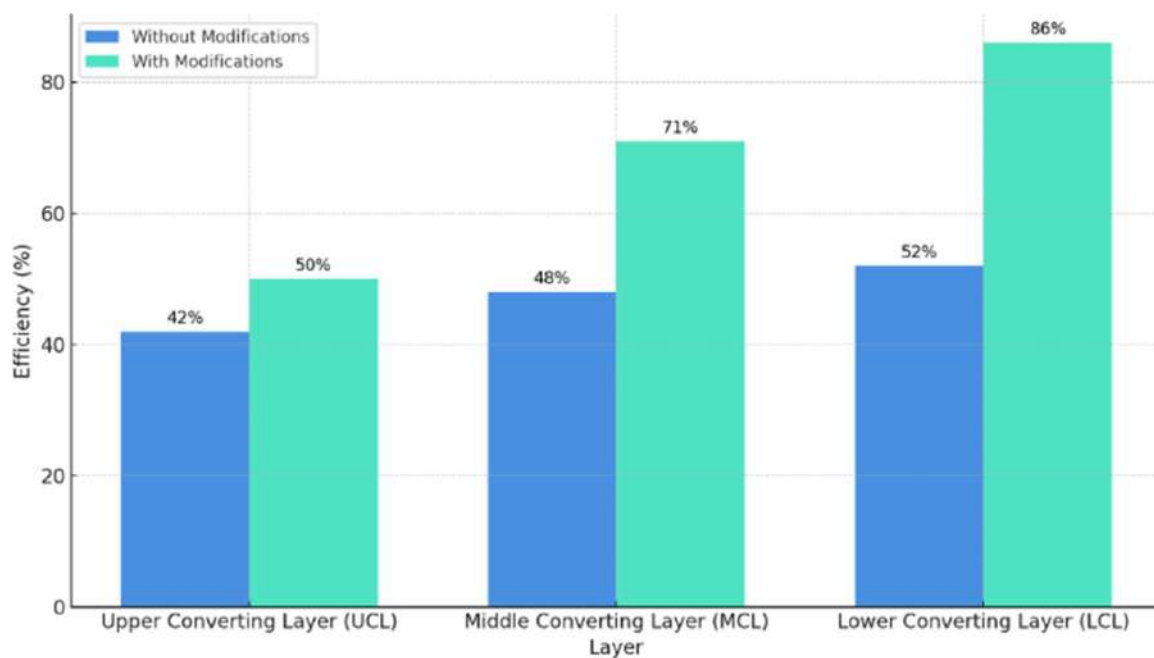


Fig. 11: Efficiency Comparison by Layer with and without Modifications.

greater energy sustainability by reducing energy losses in conversion processes. The transition to higher efficiencies across all layers reflects ongoing research and development efforts aimed at creating more effective energy systems that can meet the growing demands for clean and efficient energy solutions, as shown in Fig. 11.

Comparative Analysis of Distillate Yield Improvements Between the Proposed System and Existing Techniques

The proposed integrated solar still and solar pond system demonstrates a 35% improvement in distillate yield, showcasing significant advancements over existing

Table 1: Performance Comparison of Systems.

Study	Technique Applied	Yield/Performance Improvement [%]	Work's % Improvement	Relative Change [%]
El-Sebaili et al. (2007)	Double-slope with insulation	34	35	2.94%
Kabeel and El-Agouz (2011)	PCM for heat storage	20	35	75.00%
Tiwari et al. (2009)	PV-heated solar still	30	35	16.67%
Velmurugan et al. (2008)	Stepped basin design	40	35	-12.50%
Kalidasa Murugavel et al. (2010)	Reflectors for enhanced heating	25	35	40.00%
Ahmed et al. (2020)	Hybrid solar pond systems	35	35	0.00%
Overall Average Relative Improvement				20.35%

techniques. It outperforms designs utilizing phase change materials by 75% and PV-assisted systems by 16.67% while maintaining parity with hybrid solar pond systems. Although stepped basin designs achieve slightly higher yields, the layered thermal management in the proposed system ensures consistent performance across varying conditions. With an overall average improvement of 20.35% compared to selected studies, this system highlights its efficiency and scalability. These results emphasize its potential as a sustainable solution for water purification in diverse environmental and operational scenarios, as given in Table 1.

CONCLUSIONS

The experimental study on the modified solar still with integrated solar pond systems highlights the advantages of employing layered modifications to optimize freshwater production. Through consistent monitoring, it was observed that modifications to the Upper Converting Layer (UCL), Middle Converting Layer (MCL), and Lower Converting Layer (LCL) notably enhanced the solar still's productivity by maintaining higher and more stable temperatures, which accelerated the evaporation and condensation processes.

- The modified solar still with the layered design (UCL, MCL, LCL) demonstrated a 35% increase in distillate yield compared to conventional solar stills, confirming the effectiveness of these modifications in improving freshwater production. Specific enhancements, such as reflective mirrors and fins in the UCL, boosted heat absorption and transfer efficiency from 42% to 50%. The MCL served as a steady thermal reservoir, maintaining consistent temperature transfer and increasing efficiency from 48% to 71%. The LCL's ability to retain and gradually release heat supported sustained productivity during lower solar radiation periods, achieving an efficiency rise from 52% to 86%, making it especially beneficial for nighttime or cloudy day operations.

- Despite these promising results, several limitations must be addressed for broader scalability. Challenges include the system's dependency on optimal solar radiation, potential material degradation over extended periods, and initial costs associated with constructing layered thermal systems. Furthermore, ensuring consistent performance across diverse climatic conditions remains an area requiring further investigation.
- Future research should focus on enhancing the durability and cost-effectiveness of materials used in the layered configuration. Studies on scaling the system for large-scale desalination applications and integrating advanced thermal storage solutions, such as phase change materials or nanotechnology, could further optimize performance. Investigating automated control systems for dynamic adjustment to varying environmental conditions may also enhance operational efficiency. By addressing these aspects, the technology can be made more robust and adaptable for widespread implementation, offering a sustainable and scalable solution to global water scarcity challenges.

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Flash Flood Susceptibility Mapping Using Geospatial and Analytical Hierarchy Process Modeling - A Study of Wadi Habban Basin, Shabwah, Yemen

Haial Al-kordi¹, Abdulmohsen Al-Amri² and Govinda Raju^{2†}

¹Department of Applied Geology, Kuvempu University, Jnanasahyadri, Shankaraghatta, Shivamogga, Karnataka-577451, India

²Department of Engineering Geology, Shabwah University, Ataq City, Shabwah Governate, Yemen

†Corresponding author: Govindaraju; drgov@yahoo.com

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ABSTRACT

Flash floods are among the most dangerous natural disasters, as they cause widespread damage to property and loss of lives, especially in desert and mountainous areas. This study aims to evaluate the Wadi Habban basin to be exposed to the risk of sudden floods using remote sensing data, geographic information systems (GIS), and the pyramid analysis methodology (AHP). The spatial distribution of hazardous areas has been evaluated through the weight and reclassification of ten main criteria that include geomorphology, elevation, slope, rainfall, drainage density, distance to watercourse, land use and land cover, soil texture, Topographic Wetness Index (TWI), and Stream Power Index (SPI), were integrated into a Geographic Information System (GIS) platform. The analysis classified the basin into five risk categories: 4.3% (very high), 10.2% (high), 29.4% (medium), 42.2% (low), and 13.7% (very low). The results revealed that 14.5% of the basin area is exposed to severe and high floods, which confirms the necessity of protective strategies, such as constructing flood barriers near vulnerable valleys and enhancing infrastructure and drainage systems. These results provide essential insights for disaster preparedness and infrastructure development, serving as a significant reference for policymakers and planners to enhance flood risk management and mitigate susceptibility in analogous settings.

INTRODUCTION

Flooding, also known as inundation, is a significant hydrological disaster that ranks among the most destructive natural hazards globally, leading to substantial annual losses (Penki et al. 2023). This phenomenon can occur rapidly, often within a short period following intense precipitation, and is sometimes accompanied by secondary disasters such as landslides, mudflows, bridge failures, structural damage, and casualties (Hammami et al. 2019). Among the various forms of flooding, flash floods are particularly dangerous. The sudden, high-velocity flows result from intense rainstorms and frequently occur in coastal areas, alluvial fans, and mountainous valleys, inflicting severe damage and disruption to human activities. Population shifts caused by urbanization and unregulated growth have exacerbated the flash flood risk, which now affects basins of all sizes on every continent (Al-Areeq et al. 2023, Alarifi et al. 2022). In terrain conducive to rapid runoff, significant rainfall often triggers catastrophic flash floods (Costache et al. 2020). With over 70% of the global population living in flood-prone areas, the threat posed by flash floods is increasingly alarming (Al-Areeq et al. 2023). Each year, floods claim between 20 and 300 million lives worldwide and cause an estimated USD 60 million in economic losses. The increasing influence the result of climatic alteration, alterations in land use and land cover, and continuous societal

and economic development all suggest that an augmented risk of flooding will become more frequent and severe in the future. This highlights how critical it is to have precise flood assessments, techniques for mitigating damage, early warning systems, and efficient planning (Al-Areeq et al. 2023, Costache et al. 2020). Climate change, combined with urbanization and inadequate infrastructure, exacerbates the situation, with natural disasters, such as floods and droughts, becoming more common each year (Hammami et al. 2019). Developing nations, especially those with large agricultural sectors and limited disaster management capabilities, are particularly vulnerable to these disasters (Breisinger et al. 2012).

Yemen is an example of a disaster-prone country, facing numerous natural hazards annually. As reported by the Emergency Events Database (EM-DAT), more than 100,000 people in Yemen die annually due to natural disasters, with floods contributing significantly to economic and agricultural losses. Several climate models predict that increased precipitation will further intensify the frequency and severity of floods in Yemen (Al-Aizari et al. 2022).

Flooding in Yemen has severe economic repercussions and has considerably deteriorated living conditions. Floods intensify desertification and land degradation, resulting in agricultural losses, livestock fatalities, diminished availability of housing materials, famine, and heightened food insecurity. Yemen's dependence on agriculture and subsistence farming leaves it highly exposed to climate-related issues like flash floods. These events hinder food supply chains, raising the risk of famine (Mcfee 2024). Floodwaters often carry industrial waste and oil residues, worsening pollution and damaging farmland and vegetation (Al-Dailami et al. 2022). Additionally, these conditions increase the likelihood of diseases such as dengue fever, malaria, and cholera (ACAPS 2020, Semenza et al. 2022).

For instance, in October 2008, heavy rainfall led to devastating flooding in Wadi Hadramout. Rapid population growth, urbanization without proper regulation, and insufficient environmental controls have further heightened Yemen's vulnerability to natural hazards (Breisinger et al. 2012). Due to changes in climatic conditions, the likelihood of flooding in the villages situated along the stream banks in the plains areas has increased significantly (Garg & Ananda Babu 2023). The rising frequency and severity of flooding and extreme precipitation events, exacerbated by climate change, are anticipated to intensify health and humanitarian issues in Yemen (Khalil & Thompson 2024).

In Shabwah Governorate, particularly in the Wadi Habban region, frequent floods due to intense rainfall events, combined with geographical and hydrological factors, weak

infrastructure, and limited control measures, significantly increase flood risk. The study area has seen numerous flash floods in the past, such as in the 1996 Oman cyclone, also known as Cyclone 02A (Maathuis et al. 1999), flash floods in Yemen in 2008, and Cyclone Chapala in 2015. In 2020, heavy rainfall severely impacted various governorates, particularly Hadhramaut, Shabwah, and Al Mahrah (UNISDR 2015).

The region's topography, marked by vast desert plains, steep mountains, and rocky landscapes, along with human modifications in flood plains and catchments such as the erection of bridges, roads, and residences, significantly leads to accelerated runoff and the occurrence of flash floods (Garg & Ananda Babu 2023). Natural disasters, being inevitable, require vigilant monitoring and the implementation of risk and vulnerability mitigation strategies, where the indicator creation tool is considered an essential element in flood management. Flood risk assessment is divided into three specific categories: exposure categories, vulnerability categories, and categories of flood hazards (Oyebode & Paul 2023).

The accurate assessment of flood risks in Shabwah Governorate, especially the Wadi Habban basin, represents one of the most important for reducing and managing the risks posed by natural disasters. This study used GIS-based spatial analysis and the Analytical Hierarchy Process (AHP) to identify flood susceptibility zones in the basin, an area without previous studies on flash flood risk assessment. By concentrating on this particular basin, this research fills a critical knowledge gap and provides a methodological framework for addressing flood hazards in arid and semi-arid environments. This approach is important in its combination of AHP and GIS for the Wadi Habban basin, providing a replicable and scalable methodology for flood risk assessment in data-scarce regions. This approach provides a comprehensive means to identify flood-prone areas by analyzing various factors, like rainfall patterns, topography, land use, and drainage networks. Subsequently, flood hazard maps can be developed to inform policy decisions, enhance disaster preparedness, and promote sustainable land management.

The use of multi-criteria decision analysis (MCDA), geographic information systems (GIS), and remote sensing has proven effective in mapping flood-affected areas (Abdo et al. 2024, Corvacho-Ganahín et al. 2023). Remote sensing technology provides essential information about the distribution and behavior of floods. The proposed method was applied for the first time in the Wadi Habban basin. The scientific outcomes of this research resulted in the creation of a flash flood susceptibility map, categorized into five levels: very high, high, moderate, low, and very low susceptibility.

This map can assist planners, engineers, and government officials in developing appropriate strategies to prevent and mitigate future flooding events.

MATERIALS AND METHODS

The methodologies employed in the present study are illustrated in the flowchart (Fig. 2). The fundamental purpose of this work is the identification of flood-prone sites using the GIS environment and analytical hierarchy process modeling (Goumrassa et al. 2021). These techniques were first applied in the Wadi Habban basin.

Study Area

Wadi Habban basin is situated in the southeast part of the Shabwah Governorate of Yemen, as shown in (Fig. 1) between $14^{\circ}10'$ and $14^{\circ}30'$ N latitude and $46^{\circ}50'$ and $47^{\circ}30'$ E longitude, within the 38 UTM grid zone, including an area of approximately 1178.84 square kilometers. The elevation in the research area varies from 514 to 2108 meters above mean sea level (MSL). The study area is situated in an arid region, where floods frequently occur during the monsoon season as a result of heavy rainfall. It is this basin in Yemen that is among the basins impacted by flash floods. Flash floods are a major problem in areas adjacent to a stream during the rainy season. The basin features a variety of terrains,

including valleys, plains, mountains, and plateaus. The basin exhibits dendritic to sub-dendritic drainage patterns, showcasing moderate to high drainage textures. The study area's primary land use is agricultural, while there is some natural vegetation cover in sections of the basin. Residential areas are located on both sides of the valley, which may warn of potential floods. The climate of the region exhibits variability, characterized by hot summers and cold winters, accompanied by significant rainfall. The region's geology comprises sedimentary materials, such as sandstone and limestone, that cover the middle and lower parts of the basin, while the top part of the basin is characterized by volcanic rocks, like granite. As well as the presence of steep and gentle slopes.

Flood Influencing Factors

Fig. 2 describes the research approach used in this work, which uses GIS and remote sensing data to identify locations that are prone to flooding. It also includes determining the "Flood Hazard Index (FHI)" by integrating the Analytic Hierarchy Process (AHP) with Multi-Criteria Decision-Making (MCDM) techniques, thereby forming a comprehensive framework for assessing flood risks. The use of AHP, recognized as one of the most effective methods for evaluating vulnerability to various natural disasters, is

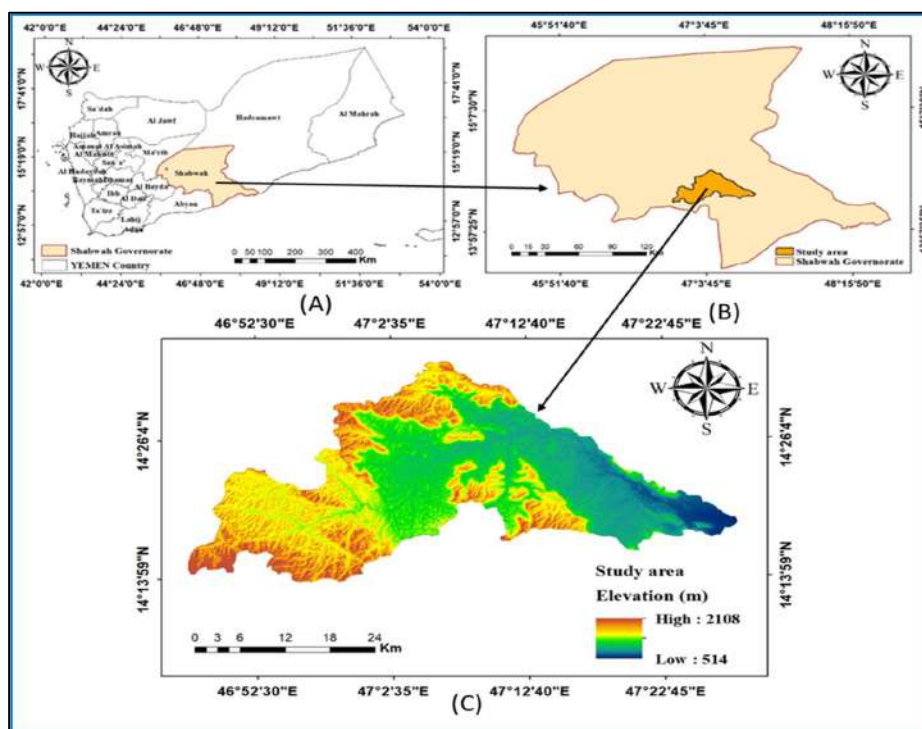


Fig. 1: (A) Yemen Country map, (B) Shabwah Governorate location map, (C) Wadi Habban Basin.

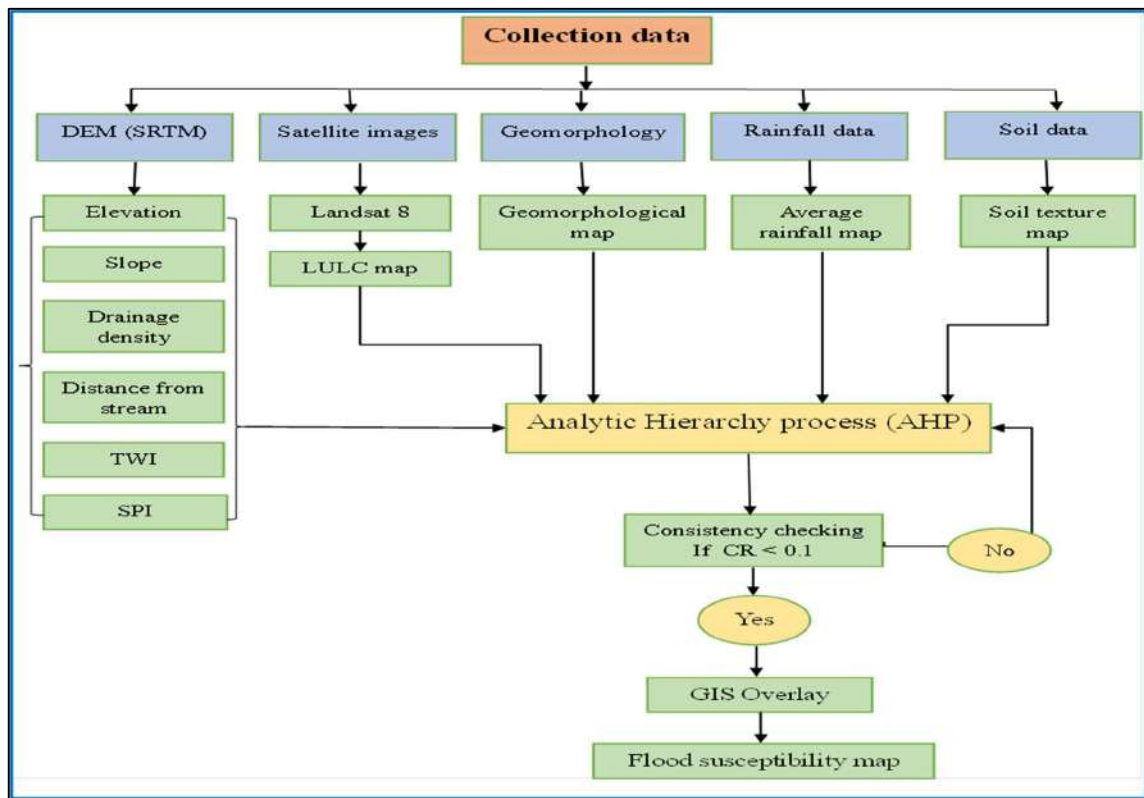


Fig. 2: The flow chart illustrates the methodology for the creation of maps of flood vulnerability maps in the research area.

Table 1: Data sources for flood conditioning factors.

Data Type	Source	Resolution/scale	Format	Purpose
Maps serial number of the map (D38, D39)	Department of Geology and Mineral Exploration in Yemen	1:200000	Shapefile	Geomorphological map
Digital Elevation Model (DEM)	USGS Earth Explorer (SRTM) https://earthexplorer.usgs.gov	30m	TIFF	Extracting: slope, elevation, drainage density, distance to stream, TWI, SPI
Landsat 8 Imagery (05/10/2020)	USGS Earth Explorer https://earthexplorer.usgs.gov	30m	TIFF	Land use/land cover classification
Global Rainfall Data website	Climatic Research Unit (CRU). https://crudata.uea.ac.uk/cru/data/hrg/	0.5°×0.5° (~50 km)	TIFF	Rainfall distribution analysis.
Soil Data	FAO Soil Grids https://www.fao.org/soilsportal/data .	250m	GeoTIFF	Soil texture classification

pivotal to the study. Its strength lies in its ability to consider the relative importance of multiple factors, providing a clear decision-making structure that enables decision-makers to derive information and effective conclusions.

In the study region, both climates, human and natural factors influence the flood flow; various data sources were utilized, such as satellite imagery, a digital elevation model (DEM), climate data as well as spatial and geomorphological maps as data sources as shown in (Table 1), and processing using ArcGIS software, version number (ArcGIS 10.4)

and ERDAS software, version number (ERDAS 2014). Furthermore, the research involved the selection of ten critical parameters essential for pinpointing flood-prone regions, including landforms, elevation, slope, drainage density, distance to the stream, land use/land cover (LULC), rainfall, soil texture, topographic wetness index (TWI), and stream power index (SPI).

The geomorphological map was created from two geomorphological maps supplied by the Department of Geology and Mineral Exploration in Yemen after the

procedure of georeferencing them by ArcGIS software, version number (ArcGIS 10.4). The topographic gradient (slope), elevation values, drainage density, distance to stream, topographic wetness index (TWI), and stream power index (SPI) were extracted from Digital Elevation Model (DEM) data. Utilizing the spatial analysis tools in ArcGIS software, version number (ArcGIS 10.4). We used monthly rainfall data; the annual mean rainfall was computed and obtained from the Global Rainfall data website, covering 20 years (from 2000 to 2020) (CRU TS v4.05 Data Variables, n.d.). Data on land use and land cover (LULC) were acquired from satellite images Landsat 8 from the USGS Earth Explorer site and were processed utilizing supervised classification techniques in ERDAS software, version number (ERDAS 2014).

Created a soil texture from the FAO Digital Soil Map of the World (DSMW) using ArcGIS software, version number (ArcGIS 10.4).

Analytical Hierarchy Process (AHP) Method

Normalization and Factor Weight Evaluation

The method consists of three evaluation stages: constructing hierarchies, establishing priorities, and conducting evaluation using the consistency index (CI) (Saaty 1980). The AHP process encompasses the following sequential steps: (a) identification of influencing factors as components, (b) organization of these factors into a hierarchical structure, (c) allocation of numerical values to assess the relative significance of each factor, (d) development of a comparison matrix, (e) calculation of eigenvectors (final score), and (f) prioritization of alternatives (Makonyo & Zahor 2023). This method is advantageous for flood mapping as it readily identifies inconsistencies in judgment (Mudashiru et al. 2022). The elements have been standardized based on Saaty's 1 to 9 scale of relative importance (Table 2).

Table 2: Shows Saaty's 1-9 scale for AHP (1980).

Level of preference	Preference scale	Inverse
Extremely significance	9	1/9
Very significant to extremely strong	8	1/8
Very strongly Significantly	7	1/7
Strongly significant to very strongly	6	1/6
Strongly significant	5	1/5
Moderately significant to strongly	4	1/4
Moderately significant	3	1/3
Equally significant to moderately	2	1/2
Equally significant	1	1

Pairwise Comparison Matrix (PCM) of Influence Factors

The Pairwise Comparison Matrix (PCM) is organized with factors ranked according to their respective impact on flood occurrence. The diagonal values must exert equal influence (equal to 1). The PCM of the matrix (μ) is computed by Eq. 1, as shown in Table 4.

$$\mu = (a_{ij})_{n \times n} = \begin{bmatrix} a_{11} & a_{12} & a_{1n} \\ a_{21} & a_{22} & a_{2n} \\ a_{n1} & a_{n2} & a_{nn} \end{bmatrix}, \text{ } a_{ii}=1, a_{ij}=1/a_{ji}, \text{ } a_{ij}, a_{ji} \neq 0 \quad \dots(1)$$

Where, a_{ij} is the element at the (i) row and (j) column, ($n \times n$) indicates the size of the square matrix (i.e., it has n rows and n columns), (a_{ii}) this states that all diagonal elements of the matrix are equal to 1, i.e., the importance of an element compared to itself is always equal to 1.

Calculation of the Principal Eigenvalue (λ_w)

The λ_w step helps measure the consistency of assessed weights (Saaty 1980). Appropriate weights are those with λ_w equal to or greater than the number of elements and a consistency ratio (CR) below 10% (0.1) (Saaty 1980). The present study found λ_w to be 10.01, surpassing the number of components (10).

Model Consistency Index (CI) Computation

CI computation in the AHP technique evaluates weight assignment judgment. In (Eq. 2), CI is calculated by dividing λ_w by the number of elements assessed (n).

$$CI = \frac{\lambda_{\max} - n}{n - 1} \quad \dots(2)$$

Whereas, $\lambda_w = 10.01$ $n = 10$ $CI = 0.001$

Calculating Consistency Ratio (CR)

The computation of CR utilizes the Random Index (RI) values established by Thomas Saaty see (Table 3). The RI values provide the index values derived from the number of factors assessed in the model (Saaty 1980). The computation of the CR is conducted as per (Eq.3).

$$CR = \frac{CI}{RI} \quad \dots(3)$$

Whereby, $CI = 0.001$, $RI = 1.49$, Therefore, $CR = 0.0007\%$ (Accepted).

Weighted Overlay Combination (WOC) Analysis

This research included ten factors to accurately assess and define the potential for flood susceptibility, and all parameters are converted to raster format, with the spatial

Table 3: Random inconsistency index (RI).

N	3	4	5	6	7	8	9	10
RI	0.58	0.9	1.12	1.24	1.32	1.41	1.45	1.49

Table 4: Pairwise Comparison Matrix (PCM) of Influence Factors.

Parameters	GE	EL	SL	RF	DD	DS	LULC	ST	TWI	SPI
GE	1.00	1.00	1.00	1.00	1.00	1.00	2.00	3.00	1.00	2.00
EL	1.00	1.00	1.00	2.00	1.00	1.00	2.00	3.00	1.00	2.00
SL	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00	1.00	2.00
RF	1.00	1.00	0.50	1.00	1.00	1.00	1.00	2.00	1.00	2.00
DD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00	1.00	2.00
DS	1.00	1.00	1.00	1.00	1.00	1.00	1.00	3.00	1.00	3.00
LULC	0.50	1.00	0.50	1.00	1.00	1.00	1.00	2.00	0.50	2.00
ST	0.33	0.50	0.33	0.50	0.50	0.33	0.50	1.00	0.33	1.00
TWI	1.00	1.00	1.00	1.00	1.00	1.00	2.00	3.00	1.00	2.00
SPI	0.50	0.50	0.50	0.50	0.50	0.33	0.50	1.00	0.50	1.00

GE Geomorphological, **EL** Elevation, **SL** Slope, **RF** Rainfall, **DD** Drainage density, **DS** distance to stream, **LULC** Land use land cover, **St** Soil texture, **TWI** Topographic wetness index, **SPI** Stream power index.

Table 5: Normalized matrix with weights to consistency ratio (CR) computation.

Parameters	GE	EL	SL	RF	DD	DS	LULC	ST	TWI	SPI	Criteria weight	Ratio
GE	0.015	0.015	0.014	0.010	0.012	0.014	0.015	0.006	0.015	0.006	0.12	0.99
EL	0.015	0.015	0.014	0.020	0.012	0.014	0.015	0.006	0.015	0.006	0.13	0.99
SL	0.015	0.015	0.014	0.010	0.012	0.014	0.008	0.004	0.015	0.006	0.11	1.02
RF	0.015	0.015	0.007	0.010	0.012	0.014	0.008	0.004	0.015	0.006	0.10	1.02
DD	0.015	0.015	0.014	0.010	0.012	0.014	0.008	0.004	0.015	0.006	0.11	1.02
DS	0.015	0.015	0.014	0.010	0.012	0.014	0.008	0.006	0.015	0.008	0.12	0.98
LULC	0.007	0.015	0.007	0.010	0.012	0.014	0.008	0.004	0.007	0.006	0.09	0.99
ST	0.005	0.007	0.005	0.005	0.006	0.005	0.004	0.002	0.005	0.003	0.05	0.99
TWI	0.015	0.015	0.014	0.010	0.012	0.014	0.015	0.006	0.015	0.006	0.12	0.99
SPI	0.007	0.007	0.007	0.005	0.006	0.005	0.004	0.002	0.007	0.003	0.05	1.01

GE Geomorphological, **EL** Elevation, **SL** Slope, **RF** Rainfall, **DD** Drainage density, **DS** distance to stream, **LULC** Land use land cover, **St** Soil texture, **TWI** Topographic wetness index, **SPI** Stream power index.

resolution of each layer modified to a cell size of 30 m x 30 m, then division of each parameter into subclasses and using reclassification tool to that parameters and allocation of weights, as well as compute the flood hazard index based on the weights of the parameters by applying (Eq.4). The obtained data is additionally evaluated utilizing Geographic Information Systems (GIS).

$$FHI = \sum_{i=1}^n Wi \times Ri \quad \dots(4)$$

Where FHI refers to the flood hazard index, n refers to the number of parameters, Wi refers to the weighting factors, and Ri indicates the ratings of the factors.

These layers are then superimposed using ArcGIS, version number (ArcGIS 10.4) weighted overlay of spatial analysis tools, taking into account the effective weight derived from the AHP technique (Table 5). A database has been created for flash floods in the Wadi Habban basin, resulting in the creation of a flash flood susceptibility map categorized into five levels: very high sensitivity, high sensitivity, moderate sensitivity, low sensitivity, and very low sensitivity.

RESULTS AND DISCUSSION

Flood Risk Assessment Parameters

Geomorphological Map

Geomorphological analysis is essential for assessing the

probability of flash flood events. It is crucial for managing water resources and assists in planning and development activities such as flood management and runoff reuse. The research region showcases five significant geomorphic features, as illustrated in Fig. 3a.

Wadi and floodplains: Very high susceptibility (101.38 km², which represents 8.6%), alluvial plains: High/moderate vulnerability (270 km², 23%), Plateaus: Low to moderate susceptibility (132.03 km², 11.2%) and Mountains and hills: Very low susceptibility (651.31 km², 55.25%), as shown in (Table 6).

Table 6: Criteria and subcategories of factors with associated weights.

Flood causative criterion	Classes	Area Km ²	Percentage %	Flood influence	Rating	Classes weight	Criteria weight
Geomorphology	Mountains and hills	651.3	55.25	Very low	1	0.12	12
	Plains	270	23	High	7	0.84	
	Wadi channel, flood plains	101.38	8.6	Very high	9	1.08	
	Proluvial fan	24.16	2.05	Moderate	5	0.60	
	Plateaus	132.03	11.2	Low	3	0.36	
Elevation [m]	< 800	150.89	12.8	Very low	7	0.91	13
	800 - 1200	460.34	39.05	Low	5	0.65	
	1200 - 1400	283.51	24.05	Moderate	3	0.39	
	1400 - 1600	212.54	18.03	High	2	0.26	
	> 1600	71.56	6.07	Very high	1	0.13	
Slope degree	0 - 10	502.78	42.65	Very low	7	0.77	11
	10 - 20	315.93	26.8	Low	5	0.55	
	20 - 30	211.95	17.98	Moderate	3	0.33	
	30 - 40	106.8	9.06	High	2	0.22	
	40 - 75.09	41.38	3.51	Very high	1	0.11	
Rainfall [mm]	< 284	75.68	6.42	Very low	1	0.1	10
	285 - 305	124.49	10.56	Low	2	0.2	
	306 - 322	538.49	45.68	Moderate	3	0.3	
	323 - 346	298.13	25.29	High	4	0.4	
	> 347	142.05	12.05	Very high	5	0.5	
Drainage density [Km.Km ⁻²]	0 - 0.38	223.04	18.92	Very low	1	0.11	11
	0.38 - 0.74	286.1	24.27	Low	2	0.22	
	0.74 - 1.08	300.84	25.52	Moderate	3	0.33	
	1.08 - 1.45	259.94	22.05	High	5	0.55	
	1.45 - 2.29	108.92	9.24	Very high	7	0.77	
Distance from stream [m]	0 - 100	35.36	3	Very high	9	1.08	12
	100 - 200	35.36	3	High	7	0.84	
	200 - 400	64.84	5.5	Moderate	5	0.60	
	400 - 800	117.88	10	Low	3	0.36	
	> 800	925.4	78.5	Very low	1	0.12	
LULC	Vegetation area	20.16	1.71	Very low	1	0.09	9
	Agriculture land	25	2.12	Moderate	5	0.45	
	Built-up area	34.3	2.91	High	7	0.63	
	Barren land	1030.43	87.41	Low	2	0.18	
	Fallow land	26.41	2.24	Moderate	5	0.45	
Soil texture	Waterbody	4.95	0.42	Very high	9	0.81	5
	Flood sands	37.49	3.18	High	7	0.63	
	Sandy loam	87.23	7.4	Very low	1	0.05	
	Loam	783.9	66.5	High	3	0.15	
	Loamy sand	307.7	26.1	Low	2	0.1	
TWI	< 5	259.34	22	Very low	1	0.12	12
	5 - 8	726.87	61.66	Low	3	0.36	
	8 - 11	123.78	10.5	Moderate	5	0.60	
	11 - 14	48.57	4.12	High	7	0.84	
	> 14	20.28	1.72	Very high	9	1.08	
SPI	3.13 - 12.2	40.79	3.46	Very high	9	0.45	5
	0.36 - 3.12	167.75	14.23	High	7	0.35	
	-1.57 - 0.36	360.96	30.62	Moderate	5	0.25	
	-6.26 - -1.58	205	17.39	Low	3	0.15	
	-13.8 - -6.27	449354	34.3	Very low	1	0.05	

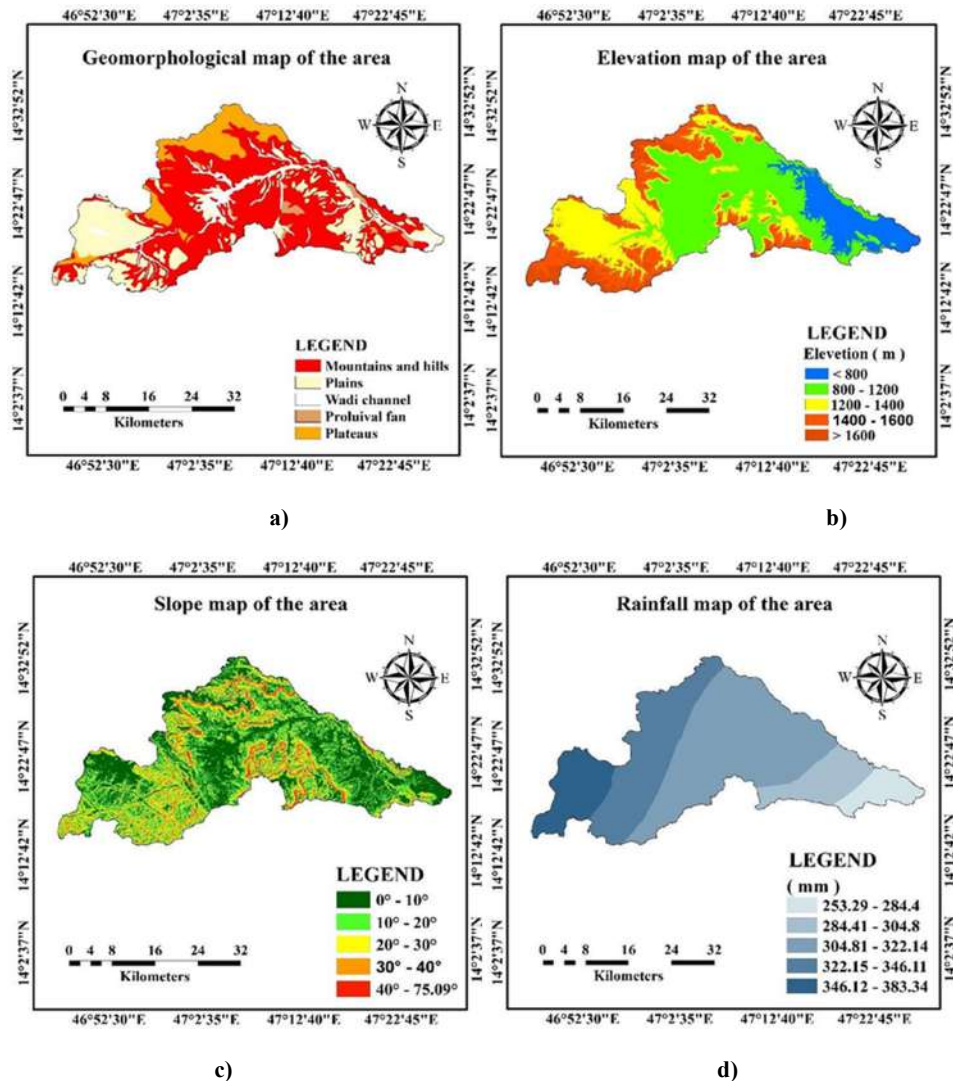


Fig. 3: a) Geomorphological map; b) Elevation map; c) Slope map; d) Rainfall map.

Integrating geomorphological maps with flood susceptibility maps is a powerful approach to improving our understanding and management of flood risks.

Elevation Map

Elevation is an essential variable in flood risk assessment, directly affecting the flow and buildup of floodwaters. The low-lying locations are especially susceptible to flooding during inundation occurrences due to gravitational forces and topographical depressions, frequently serving as focal places for water accumulation and thus experiencing an elevated risk of flooding (Abdo et al. 2024). Previous research has shown that altitude has a significant influence on floods (Tehrany et al. 2019). The created map has five categories, as shown in Table 6.

The lower elevations (<800 m) of sea level are very highly susceptible to floods (150.89 km², 12.8%), while High elevations (>1600 m) suggest that the area is very low in flood sensitivity (71.56km²) shown in Fig. 3b.

Slope Map

The generation and redistribution of flooding are substantially affected by topography, with slope gradient serving as a strong indicator of surface sensitivity to floods (Wałęga et al. 2024). In general, low-slope areas experience greater flooding, as floodwaters can quickly drain into these regions, making them more susceptible to inundation (Alves et al. 2024). The slope map has been classified in a study area into five categories based on the degree of slope (Table 6).

Very low slopes (0 to 10°) are very highly susceptible to floods, indicating a higher risk, where the area (502.78km²)

with a percentage (42.7%) of the basin, however very high slopes indicate very low susceptible of flooding ($>82^\circ$) covering area (41.4km^2) with percentage (3.5%) as shown in (Fig. 3c).

Rainfall Map

The relationship between rainfall and floods has been established by a large body of previous literature (Burn &

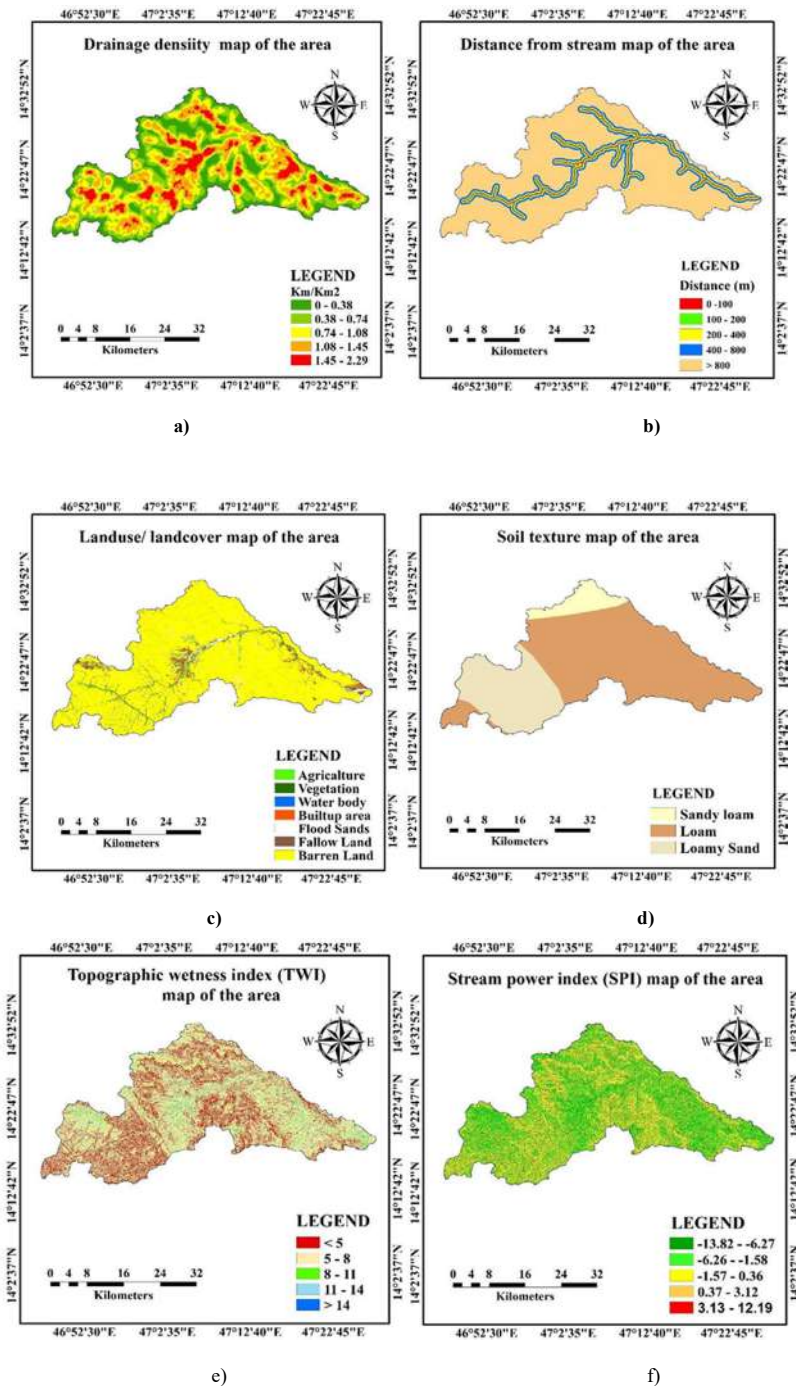


Fig. 4: a) Drainage density map; b) Distance from stream map; c) LULC map; d) Soil texture map; e) TWI map; f) stream power index (SPI) map.

Whitfield 2023). It is impossible to predict the exact degree to which rainfall increases lead to flooding (Jiang et al. 2023). In all environmental environments, precipitation can be considered the primary source of floods (Gao et al. 2023).

A mean annual rainfall map was generated using ArcGIS software (version number (ArcGIS 10.4), and the precipitation scale was classified into five categories, as shown in (Fig. 3d).

The precipitation levels in the study area vary significantly, influencing flood susceptibility. Zones with precipitation exceeding 346 mm, which constitute 33.3% of the area, are categorized as having a very highly susceptible flood region. Furthermore, regions receiving less than 284.4 mm of annual rainfall, accounting for 6.6% of the total area, experience very low precipitation and are consequently less affected by flooding (Table 6).

Drainage Density

Drainage density is a critical factor in flood management, described as the river's length per square kilometer (km/km^2) (Al-Omari et al. 2024). High drainage density refers to low filtration; conversely, high water filtration is indicated by low drainage density (Yang et al. 2022). The drainage density within the research area's topography exhibits significant variation, characterized by elevated densities in mid-slope and low-elevation regions attributable to water convergence and soil infiltration, whereas steeper, higher-altitude zones demonstrate reduced drainage density. The drainage density in the study area is classified into five classes, as illustrated in Fig. 4a.

The high drainage density ($1.45 - 2.29 \text{ km}/\text{km}^2$) represents a very high flood susceptible region covering an area of approximately 108.9 km^2 , which is 9.2% of the total region. Conversely, areas characterized by very low drainage density as mountains, hills, and plateaus, spanning 223 km^2 and accounting for 18.9% of the study area, exhibit low drainage densities (ranging from 0 to 0.38) and are very low in flood susceptibility, as shown in Table 6.

Distances from Stream

The locations nearest the stream source are those most susceptible to and affected by severe flash flooding. The flash flood severity will be highest in those areas when water flow exceeds the buffer zone (Hagos et al. 2022). As a result, the distance from the streams is given significant weight when establishing the flood potential zone.

The study area was divided into five zones based on proximity to streams using the buffer zone tool in ArcGIS software (version 10.4) (Table 6).

The distance from the stream ($<100\text{m}$) is classified as a very high flood risk zone, covering approximately 35.4 km^2 , which is 3% of the all area, while the distance from the stream ($>800\text{m}$) is classified as a very low flood risk zone, covering an area of 925.4 km^2 , accounting for 78.5% of the region as shown in (Fig. 4b).

Land Use Land Cover (LULC)

Land use is an important element in affecting areas susceptible to flooding and plays a crucial role in water percolation, infiltration rate, and groundwater recharge (Xiao et al. 2024). Alterations in land use and land cover (LULC) can profoundly impact hydrological processes. The proliferation of impermeable surfaces in urban environments results in elevated surface runoff, which increases the flow of flood activity there and diminishes infiltration (Nwokeabia & Odinye 2024). With the expansion of the built area, the cover of the wetlands increases, while the cover of the plants diminishes, increasing the flow of water (Arya & Singh 2021, Hagos et al. 2022)

The classification of land use and land cover within the Wadi Habban basin has been divided into seven categories, reflecting their potential impact on flood rates, as illustrated in Fig. 4c.

The study observed that the water bodies, closeness of infrastructure, built-up expansion, and agricultural land near streams significantly increase the flood sensitivity, designating these locations as having a very high flood risk. In contrast, arid environments, such as highlands and hilly areas, are classified as having a very low flood risk (Table 6).

Soil Texture

In actuality, soil features such as structure and texture can significantly affect how permeable it is and how much water storage is crucial in flood mapping (Costache et al. 2020). The three types of soil texture were produced (Table 6).

Loam soil (medium texture), with a high susceptibility to flood, has a cover area of about 783.9 km^2 , while sandy loam (moderate coarse texture), representing a low susceptibility to flood, has a cover area of 87.2 km^2 , as shown in Fig. 4d.

Topographic Wetness Index

The topographic wetness index (TWI) measures the flow accumulation at each point within a drainage basin and the capacity of water to flow down a slope due to gravity (Fig. 4e). This feature refers to the condition of soil moisture (Selvam & Antony Jebamalai 2023), which has influenced runoff generation. The formula for TWI is typically represented as:

$$TWI = \ln \left(\frac{A}{\tan(B)} \right) \quad \dots(5)$$

Where A represents the upstream contributing area, and B is the slope of the terrain.

The potential for high index values to accumulate a significant amount of water is a result of the low slope, and the reverse is also true. Consequently, regions with elevated topographic wetness index (TWI) are at a greater risk of inundation. The topographic wetness index (TWI) map divides the basin into five distinct classes, as presented in Fig. 4e.

Areas with a topographic wetness index (TWI) value (> 14) are classified as having a very high flood risk, covering approximately 20.28 km². In contrast, regions with a topographic wetness index value (< 5) are categorized as having a very low flood risk, covering an area of 259.3 km², as shown in Table 6.

Stream Power Index (SPI)

It is possible to evaluate the potential for river erosion at a particular topographical area using the stream power index (SPI), which describes the relationship between the force of water flow and erosion (Jebur et al. 2014). In the field of research, elevated stream power index values indicate channels and steep gradients where erosion of the stream may occur. The stream becomes able to transport larger amounts of sediments during floods, which can lead to enhanced sediment deposition downstream or in floodplain areas, affecting floodwater flow patterns and increasing flood risk. The classification of stream power index (SPI) into five categories is shown in Table 6.

The negative low stream power index values indicate to high flood risk region. Conversely, the positive high stream power index (SPI) values indicate to low flood risk region, as shown in Fig. 4f.

Flood Hazard Index (FHI)

The reclassify tool in ArcGIS software, version number (ArcGIS 10.4), was employed to reclassify the raster files (Figs. 3,4) in accordance with AHP classifications (Table 6). This process involved assigning weights and ranking each parameter and subclass. The Flood Hazard Susceptibility Index was calculated using the weighted overlay spatial analysis tool in ArcGIS 10.4 software, as outlined below:

$$FHI = \sum_{i=1}^n Wi \times Ri \quad \dots(6)$$

Where n represents the number of parameters, Wi indicates the weight of each parameter, and Ri signifies the factor rating.

According to Elkhachy (2015), the flooding probability rate is assessed by the FHI and can be calculated using the following equation:

$$FHI = 0.12(\text{Geomorphological}) + 0.13(\text{Elevation}) + 0.11(\text{Slope}) + 0.10(\text{Rainfall}) + 0.11(\text{Drainage density}) + 0.12(\text{Distance from stream}) + 0.09(\text{LULC}) + 0.05(\text{Soil texture}) + 0.12(\text{TWI}) + 0.05(\text{SPI}).$$

The Flood Susceptibility Map

The ten maps were reclassified using ArcGIS software, version number (10.4), and then the weighting approach and Analytic Hierarchy Process (AHP) were used to calculate weights. They were combined and superimposed in a GIS environment by spatial analysis tools and then weighted overlay to find zones prone to floods (Mann & Gupta 2023). The final flood map obtained from the AHP approach divided the area into five categories, depending on the potential for flash floods from very high to very low, as shown in Figs. 5 and 6. According to the study area, 13.7% represents very low susceptibility, covering an area of 161.2 km², 42.4% represents low susceptibility, covering 500 km², 29.4% represents moderate susceptibility, covering 346.81 km², 10.2% represents high susceptibility, covering 119.2 km²; and 4.3% represents very high susceptibility, covering 51.2 km², as shown in (Table 7).

Our analysis indicates that zones categorized as having high and very high susceptibility are at significant risk of flash flooding. The research area is characterized by high drainage density and low soil permeability, largely attributed to its topography of gentle slopes and low elevation. Additionally, the dendritic drainage pattern of the basin channels substantial water flow during heavy rainfalls, exacerbated by a high Topographic Wetness Index (TWI) and low Stream Power Index (SPI). Increased human activity in adjacent areas to the Wadi channel exacerbates flood risk, as infrastructure in the valley periphery obstructs natural water flow pathways. These factors align with

Table 7: Classification of food susceptibility and its spatial distribution.

No.	Flood susceptibility classification	Area covered [Sq.km]	Percentage [%]
1	Very low susceptibility	161.2	13.7 %
2	Low susceptibility	500	42.4%
3	Moderate susceptibility	346.81	29.4%
4	High susceptibility	119.8	10.2%
5	Very high susceptibility	51.2	4.3%

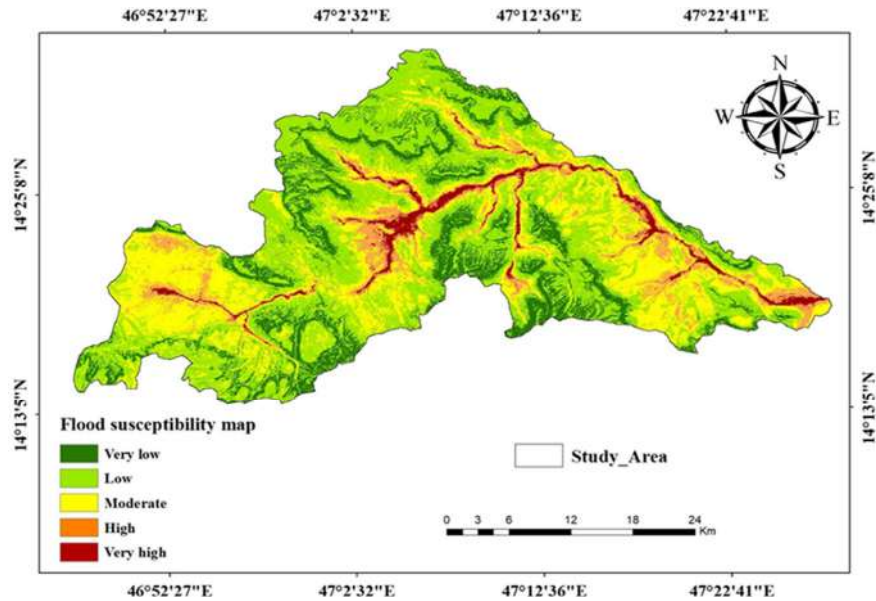


Fig. 5: Flash flood hazard susceptibility map.

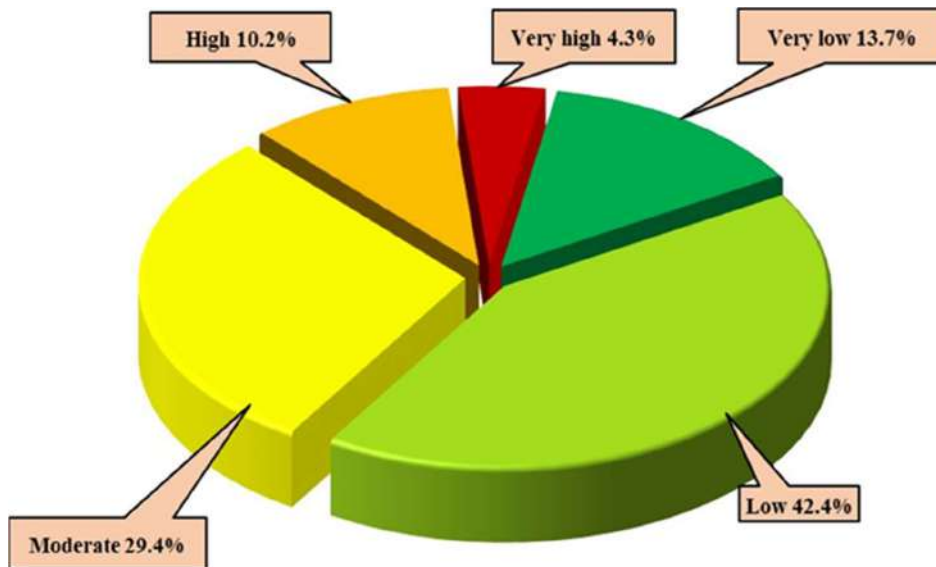


Fig. 6: Distribution of flash flood susceptibility percentages.

the findings of previous studies that emphasized the importance of such indicators in flood mapping using the AHP approach (Shawky & Hassan 2023, Radwan et al. 2019).

Precipitation in the elevated western, northwestern, and southwestern zones of the basin contributes significantly to runoff accumulation. These zones, characterized by steep slopes, rugged terrains, and substantial elevation differences,

generate high runoff volumes that flow downstream into the Wadi Habban Basin, as illustrated in (Fig. 3a, b, and c). The presence of impermeable surfaces in these regions (Fig. 4d) further intensifies flood risks by preventing water infiltration and increasing surface runoff.

This interplay of factors underscores the importance of integrating topographic, hydrological, and anthropogenic parameters in flood susceptibility modeling, consistent with

the approaches recommended in previous regional flood studies (Hagos et al. 2022).

These findings demonstrate the utility of AHP-based flood susceptibility mapping for identifying and mitigating flood-prone areas. In line with previous studies, our results emphasize the necessity of incorporating both natural and human-induced factors to develop effective flood risk management strategies tailored to the unique characteristics of the study region.

CONCLUSIONS

The assessment of flood hazard zones is a crucial element of a flood control strategy; the suggested methodology was implemented in the Wadi Habban basin to map the flash flood, the integration of geographic information systems (GIS) with multi-criteria spatial assessment and the analytic hierarchy process (AHP) has proven to be highly effective in supporting decision-making processes, as this approach takes into account the significant impact of multiple factors contributing to occurrence flooding in the region. Ten distinct input maps have been generated, including geomorphological, elevation, slope, drainage density, distance to the stream, land use/land cover (LULC), rainfall, soil texture, topographic wetness index (TWI), and stream power index (SPI). The research found that topography (elevations, slopes, and valleys), proximity to streams, topographic wetness index (TWI), heavy rainfall, drainage density, and land use are the primary factors contributing significantly to flood occurrence in the region, whereas soil texture and stream power index (SPI) had a lesser influence.

Based on flood sensitivity, which ranges from very low to very high, the flash flood susceptibility maps of the Wadi Habban Basin were classified into five major categories: 13.7% (very low), 42.4% (low), 29.4% (moderate), 10.2% (high), and 4.3% (very high), as shown in (Fig.5), (Table 7). The study concluded that several residential areas near the valley course are at risk of flash floods if the valley cannot absorb the water during heavy rain seasons. These areas include the Al-Said region, Habban, parts of Lahiya, Al-Ghil, Lamatir, and sections of Azzan City.

The final findings enhance our understanding of the relationship between topographic, hydrological, geological, and climatic factors and flash flood conditions. The research demonstrated that the technologies employed, such as remote sensing and geographic information systems (GIS), were reliable and effective. These technologies will help planners identify dangerous areas, protect residents, and improve disaster response after heavy rains.

To reduce flash flood risks in the Wadi Habban basin, authorities and international organizations should use

Geographic Information Systems (GIS) to create detailed flood hazard maps, identifying the most vulnerable areas. These maps can guide key measures such as implementing early warning systems, constructing check dams, and improving drainage infrastructure. Collaboration with international experts can provide the technical and financial support needed for sustainable solutions that also address climate change impacts. Future studies should incorporate real-time hydrological data, examine long-term climatic trends, and utilize machine learning to effective flood risk management in the region. Social and economic data collection will enhance vulnerability assessments, while interactive risk maps and 3D simulations can improve planning. These efforts will provide comprehensive tools for effective flood risk management in the region.

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Aging Aircraft and Emissions: Machine Learning Predictions in Takeoff and Landing Operations

Hala Alrawashdeh¹, Laila A. Al-Khatib^{2†} and Bassam Abed³

¹Communication and Navigation Unit, Civil Aviation Regulatory Commission (CARC), Jordan

²Environmental Engineering Department, Faculty of Engineering, Al-Hussein Bin Talal University, Ma'an, Jordan

³Quality and Management System Unit, Civil Aviation Regulatory Commission (CARC), Jordan

†Corresponding author: Laila A. Al-Khatib, laila@ahu.edu.jo & lailaalkhatib2003@gmail.com

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ABSTRACT

The aviation industry plays a crucial role in global connectivity and transportation. However, its environmental footprint continues to grow alongside the expanding popularity of aviation. By analyzing a decade-long dataset, the novelty of this research lies in delving into the relationship between aircraft age and major aviation emissions, such as hydrocarbons (HC), carbon monoxide (CO), and nitrogen oxides (NO_x), during landing and take-off (LTO) operation using advanced machine learning algorithms. The analysis of this research comprises three horizons. Firstly, an inventory of aircraft emissions was constructed by analyzing aircraft fleet data at Queen Alia International Airport (QAIA) in Jordan. Secondly, the correlation between these emissions and aircraft age was rigorously examined. Finally, predictive models for aircraft age were developed based on pollutant emission features using advanced machine learning algorithms. The findings of the study revealed a discernible impact of aircraft age on emissions, underscoring the importance of considering the aging factor in assessing the environmental implications of aviation. The machine learning models exhibited a capacity to forecast pollutant emissions with a notable degree of accuracy, with a Mean Squared Error (MSE) of about 3.0931. This offers valuable perspectives that can enhance comprehension of aviation's environmental footprint.

INTRODUCTION

The aviation industry plays a pivotal role in global connectivity and transportation, yet its environmental footprint is a subject of increasing concern (Quadros et al. 2020). As aviation continues to burgeon, it becomes imperative to comprehensively grasp the implications of aircraft emissions on environmental issues, such as global warming, and the well-being of affected communities. The impact of aircraft emissions on air quality near airports is an acute concern with potential ramifications for public health. Numerous studies have established a correlation between elevated levels of pollutants near airports and adverse health effects among nearby residents. For example, fine particulate matter, nitrogen oxides, and other pollutants released during aircraft operations can contribute to respiratory issues, cardiovascular problems, and other health concerns (Bendtsen et al. 2021, Jonsdottir et al. 2019, Manisalidis et al. 2020).

Aircraft engine NO_x emissions are mostly produced during takeoff at high temperatures and pressures in the combustor (Bo et al. 2019, Mahashabde et al. 2011). These emissions have been linked to negative health effects, including heightened allergy responses, compromised immune systems, and respiratory systems (Arter et al. 2022). Surface NO_x reacts with hydrocarbons (HC), carbon monoxide (CO), and volatile organic compounds (VOCs) in the presence of heat and sunlight to produce ozone at ground level (Mahashabde et al. 2011). This low-altitude ground-level ozone is a secondary pollutant and a major contributor

to smog. Exposure to smog can cause respiratory problems, damage to crop ecosystems, and several health issues (Brunton et al. 2021, Jonsdottir et al. 2019, Mahashabde et al. 2011, Yim et al. 2015). Furthermore, NO_x serves as the building block for additional oxidized nitrogen compounds, which aid in the production of secondary particulate matter. As a result, NO_x influences the ecosystem and air quality both directly and indirectly.

Another pollutant from aircraft operation is hydrocarbons (HC). Elevated concentrations of hydrocarbons, often originating from anthropogenic sources such as vehicle emissions, industrial activities, and fuel combustion, can contribute to air pollution. In urban areas, high concentrations of hydrocarbons are associated with smog formation and diminished air quality, posing health risks to human populations (Mahashabde et al. 2011). Monitoring and controlling harmful emissions concentrations are essential for mitigating environmental pollution and promoting sustainable practices to minimize adverse effects on both human health and the ecosystem.

As aircraft age, the confluence of changing engine efficiency, evolving technology, and varied maintenance practices may significantly alter the release of pollutants (Behere et al. 2020, Lee 2010). The aging of the engine is believed to be a major factor that impacts emissions. As engine components, particularly the compressor and turbine, deteriorate, the fuel flow needs to increase in order to achieve the desired power settings. This, in turn, influences the emissions. It is estimated that engine aging leads to an increase in fuel consumption of about 4–10% (Patterson et al. 2009, Xu et al. 2020). Only a few studies attempted to correlate aircraft age and emissions. For example, Xu et al. (2020) attempted to explore the effect of engine aging on NO_x emissions in taxi mode. They concluded that there is no significant correlation between aircraft age and emissions. On the contrary, Zaporozhets & Synylo (2017) investigated the effect of engine age on emission indices for CO and NO_x. Their results have demonstrated the dependence of the emission indices on engine age, as there is limited literature investigating the aging of aircraft in the context of environmental sustainability in aviation. This study seeks to investigate the complex relationships between aircraft aging and environmental impact, specifically focusing on aviation emissions.

Leveraging machine learning techniques assumes paramount importance in tackling the complexity inherent in this study problem. Machine learning algorithms possess the capability to sift through vast datasets, identifying nuanced patterns and relationships that might elude traditional analytical methods. In addition, traditional methods can

be limited in their ability to capture the intricate non-linear relationships between emissions and aircraft age, potentially leading to inaccurate predictions. Machine learning algorithms excel at identifying complex patterns and non-linear relationships within data, making them well-suited for the challenging task of predicting aircraft age from emission data. Machine learning involves the exploration of computer algorithms to enable accurate predictions and intelligent responses in specific situations. Fundamentally, it revolves around learning to improve future circumstances based on past knowledge. Machines acquire insights from existing information and experiences, leading to the development of programs that analyze data from diverse sources. These programs select pertinent data and leverage it to predict system behavior in similar or dissimilar scenarios. Machine learning also involves the classification of objects and activities to facilitate decision-making in new input scenarios. The underlying motivation for machine learning lies in the necessity for additional intelligence and learning to address uncertainties (Kulkarni 2012).

Moreover, supervised learning primarily seeks to establish a model that captures the relationship between inputs and their associated outputs within the provided training data. The objective is to enable the prediction of output responses for new data inputs, leveraging the acquired knowledge of the established relationships and mappings between inputs and their target outputs. Supervised training methods fall into two main categories, namely classification and regression, depending on the nature of the machine learning problems being addressed (Rathnayake et al. 2024, Sarkar et al. 2018).

Machine learning has made substantial inroads into the field of aviation research. It has been employed in a multitude of applications. For instance, machine learning models can predict climate change due to CO₂ emissions (Askr et al. 2023, Maruhashi et al. 2022). Kayaalp et al. (2021) utilized the Long-Short Term Memory (LSTM) to predict emissions. Furthermore, Aygun et al. (2023) predicted the emissions indices for NO_x and CO and fuel flow during take-off and climb-out phases using a hybrid model combining a convolutional neural network (CNN) and LSTM (CNN-LSTM). Wan et al. (2022) predicted emissions and noise impact on air quality. Dursun et al. (2022) focused on predicting various emission indexes (EIs), including CO, HC, and NO_x, as well as fuel flow for various commercial aircraft engines during the take-off phase. Two distinct approaches, support vector regression (SVR) and LSTM, were employed for this purpose. Han et al. (2022) utilized machine learning predictive models to investigate the influence of emissions from a civilian airport on the

local air quality of nitrogen dioxide (NO₂). Tian et al. (2019) introduced a framework that categorizes air quality in airports by various supervised learning methods. In addition, machine learning applications in aerodynamics optimization enable the development of more fuel-efficient, stable, and easily controllable aircraft. By training machine learning models on data collected from wind tunnel tests, flight tests, and simulations, engineers can accurately predict and optimize design parameters. These optimized parameters are then used to generate 3D models of the aircraft, which are subsequently rigorously tested in a virtual environment to ensure they meet the desired aerodynamic performance standards (Jiang et al. 2023, Le Clainche et al. 2023, Sabater et al. 2022). Artificial intelligence and machine learning have also been integrated to inform and fortify future aviation safety strategies (Demir et al. 2024). Furthermore, machine learning was utilized in optimizing air traffic routes and schedules to reduce congestion and fuel consumption (Kim et al. 2022).

In the context of this research, machine learning serves as a powerful ally in deciphering the multifaceted interplay between aircraft age and environmental impact. As we navigate the intricate landscape of aviation emissions, the role of machine learning becomes increasingly apparent. The algorithms employed in this research enable the prediction of aircraft age based on pollutant emissions, providing a predictive framework that extends beyond mere observation. This proactive approach to understanding the environmental consequences of aging aircraft is instrumental in developing targeted and effective strategies for mitigating these impacts. By harnessing the power of machine learning, the study aspires not only to understand the current impact of aging aircraft on emissions but also to forecast potential trends, facilitating proactive measures for sustainable aviation practices.

The significance of this research addresses critical issues at the intersection of aviation, environmental sustainability, and public health. The novelty of this research comes from the fact that it not only addresses a pressing environmental problem but also its relevance on the local and international scales alike. When examining the literature in detail, no studies on correlating and predicting aircraft age based on emissions using machine learning have been found to the best of the author's knowledge. Therefore, the present study provides the first attempt to assess the impact of aircraft age on pollutant emissions. Understanding the influence of aircraft age on engine emissions is pivotal for formulating sustainable aviation practices. By identifying the specific environmental implications associated with aging aircraft, this research provides empirical evidence to implement regulations that encourage the adoption of greener technologies and efficient maintenance practices.

The objectives of this study are: (i) develop an inventory of aircraft emissions, including NO_x, CO, and HC, during the landing and take-off (LTO) cycle operations in QAIA, (ii) examine rigorously the correlation between the age of aircraft and these emissions utilizing a decade-long dataset, (iii) develop predictive models for aircraft age based on pollutant emissions by employing advanced machine learning algorithms. This will offer insights into the current impact and facilitate the anticipation of potential trends for the implementation of proactive measures.

MATERIALS AND METHODS

Study Area

The research area was Queen Alia International Airport (QAIA) in Jordan. QAIA is located about thirty kilometers south of Amman, the capital of Jordan. It has a latitude of 31°43'12.59" N and a longitude of 35°59'21.59" E. It is the largest and busiest airport in Jordan, serving more than 8 million passengers annually. The airport serves as a hub for more than 50 carriers and airlines that connect the country to various destinations in the Middle East, Asia, Africa, and Europe.

Data Collection and Processing

The required data for developing an inventory of aviation emissions for QAIA were collected. Initially, the fleet data spanning from 2013 to 2022 of QAIA were obtained from the Jordan Civil Aviation Regulatory Commission and the air transport department. The recorded flight data encompassed details such as aircraft engines, registration, country, types, weight, aircraft movements (ACM), total passengers, and cargo. An examination to ensure the cleanliness, consistency of the data, and detection of any outliers has been carried out. Information about the typical engine combinations on various aircraft types was obtained from the Airfleet Aviation website (Airfleets Aviation 2023). Engine emission indices were obtained from the ICAO Aircraft Engine Emissions Databank (ICAO 2023).

Aircraft Emission Calculation

Aircraft emissions depend on various factors, including the type and number of aircraft, engine type, fuel, duration of each operation phase, power settings, and flight distance (Yang et al. 2018). Usually, research on aircraft emissions and their effects is typically divided into two categories. The first category is related to aircraft pollutant emissions that occur during the LTO phase, which are referred to as local pollutant emissions. The second category is the non-LTO flight phase, which takes place above 915 meters and at cruise

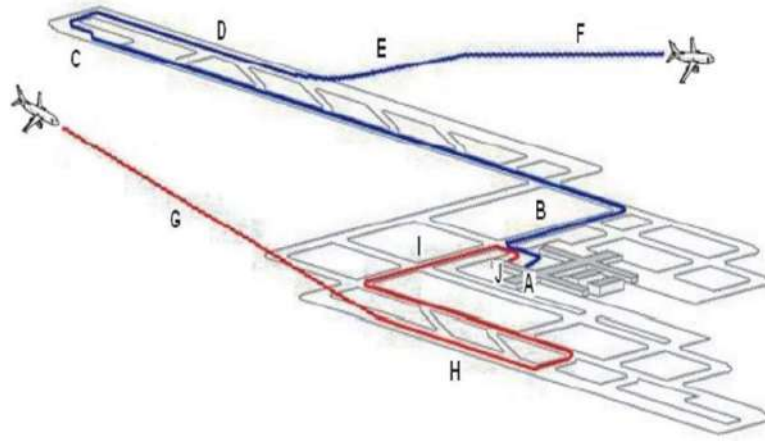


Fig. 1: ICAO standard operational flight landing and take-off (LTO) cycle (ICAO 2016).

level (Bajgai & Shrestha 2023). The focus of the current study was the aircraft emissions' impact on local airport air quality. Therefore, the emissions of aircraft during the LTO phase were only considered. The LTO cycle comprises four phases: approach, taxi/idle, take-off, and climb. A schematic diagram of the LTO cycle operation is shown in Fig. 1. Emissions during a particular phase of the LTO cycle are also proportional to the amount of time spent on each mode, main engine index, and engine fuel flow. In this study, the time in mode and thrust setting on each operation phase are taken from the ICAO standard LTO cycle (Table 1).

The emissions for a specific pollutant i of a particular aircraft type j are calculated by the following equation:

$$Ei_j = \sum(TIM_{jk} \times 60) \times (FF_{jk}) \times (Ei_{ijk}) \times (Ne_j) \quad \dots(1)$$

Where Ei_j = total emissions of pollutant i (e.g., NO_x, CO or HC), in grams produced by aircraft type j for one LTO cycle, Ei_{ijk} = emission index for pollutant i , in grams pollutant per kilogram of fuel (g/kg of fuel), in mode k (e.g., take-off, climb-out, idle and approach) for each engine used on aircraft type j , FF_{jk} = fuel flow for mode k , in kilograms per second (kg.s^{-1}), for each engine used on aircraft type j , TIM_{jk} = time-in-mode for mode k , in minutes, for aircraft

Table 1: Time in mode and thrust setting on each operating phase during the LTO cycle (ICAO 2016).

Operating phase	Time-in-mode [min]	Thrust setting (percentage of rated thrust)
Approach	4.0	30
Taxi/idle	267.0 (in) 19.0 (out)	7
Take-off	0.7	100
Climb	2.2	85

type j , Ne_j = the number of engines used on aircraft type j .

Machine Learning and Artificial Neural Networks

An artificial neural network (ANN) is a model inspired by the human brain's structure and functioning, with nodes and interconnections resembling neurons. In a standard ANN, there are typically input and output layers, with at least one hidden layer in between, as shown in Fig. 2. The network comprises specific link patterns, layer connections, connection weights, and neuron activation functions mapping inputs to outputs. During training, weights are adjusted using the backpropagation algorithm, involving propagation and weight update stages. Input data sample vectors are forwarded through the neural network to generate output values. The produced output vector is compared with the desired output vector. Performance is evaluated using mean square error (MSE), which measures the average squared difference between the actual values (Y_i) and the predicted values ($\hat{Y}_i$) generated by a model and is computed according to the following equation:

$$MSE = \frac{1}{n} \sum_{i=1}^n (Y_i - \hat{Y}_i)^2 \quad \dots(2)$$

The node weights can then be adjusted based on corrections that minimize the error in the entire output for the th data point, given by the following equations:

$$\varepsilon(n) = \frac{1}{2} \sum_{\text{output node } j} e_j^2(n). \quad \dots(3)$$

Using gradient descent, the change in each weight $\Delta w_{ji}(n)$ is

$$\Delta w_{ji}(n) = -\eta \frac{\partial \varepsilon(n)}{\partial v_j(n)} y_i(n) \quad \dots(4)$$

where $y_i(n)$ is the output of the previous neuron i , and η is the learning rate, which is selected to ensure

that the weights quickly converge to a response without oscillations. In the previous expression, $\frac{\partial \varepsilon(n)}{\partial v_j(n)}$ denotes the partial derivative of the error $\varepsilon(n)$ with respect to the weighted sum $v_j(n)$ of the input connections of neuron i .

These stages are repeated through multiple iterations (epochs) until reliable results are obtained. A multilayer perceptron (MLP) is a fully connected feedforward artificial neural network with at least three layers. Backpropagation can train MLPs and even deep neural networks with multiple layers (Sarkar et al. 2018).

The data has been preprocessed to handle any missing values or outliers. Techniques like imputation and standardization have been employed. Missing values in the dataset were addressed using an imputation strategy. Continuous features were filled using the mean value of the respective feature, ensuring the data's central tendency was preserved. Outliers were identified through visual inspection of plots for each numerical feature, and data points that deviated significantly from the general distribution of values were flagged as potential outliers. Consequently, each flagged data point was evaluated in the context of domain knowledge. For instance, unusually high emissions for certain aircraft types were cross-verified against aircraft specifications and operational anomalies. However, outliers deemed to be errors or non-representative of typical conditions were either removed or corrected based on corroborating evidence from the dataset. Finally, after handling outliers, the data distribution was re-evaluated to ensure that adjustments preserved the overall trends and relationships in the dataset. All numerical features were standardized to a mean of 0 and a standard deviation of 1 to ensure that each feature contributed equally to the model training process. These preprocessing steps were implemented systematically using the Sci-Kit Learn library, and the codebase ensures reproducibility by following a documented workflow.

The correlation between aircraft age and various emission characteristics has been assessed to identify which emissions exhibit a significant correlation with age. The neural network architecture has been initially configured and fine-tuned based on the specific features that show meaningful correlations with age. The normalized dataset was divided into two datasets: 80% training and 20% testing. To measure the model's generalization performance, MSE was applied to the test dataset.

The architecture of the MLPRegressor model, consisting of two hidden layers with 100 and 50 neurons, respectively, was chosen after performing hyperparameter tuning experiments. These experiments tested various configurations to balance model complexity and computational efficiency. The first hidden layer with 100 neurons captures complex relationships within the dataset, while the second layer with 50 neurons further refines the feature interactions. This structure ensures that the model effectively handles the nonlinearities inherent in the relationship between emissions and aircraft age. The rectified linear unit (ReLU) was used as the default activation function for Multilayer-Perceptron Regression (MLPRegressor) in scikit-learn. The output layer contains a single variable – predicted age in this case.

RESULTS AND DISCUSSION

LTO Emission Estimation

The LTO emissions in QAIA were analyzed for temporal variations in flights from 2013 to 2022. Fig. 3 shows the number of aircraft movements over the investigated period. There was a gradual increase in the number of flights with time between 2013 to 2019. In 2020, there was a significant drop in the number of aircraft movements due to the impact of the COVID-19 pandemic, which resulted in widespread travel restrictions and reduced air travel. There was a partial recovery in the number of flights in 2021 compared to 2020, but it didn't reach the levels seen in the pre-pandemic years.

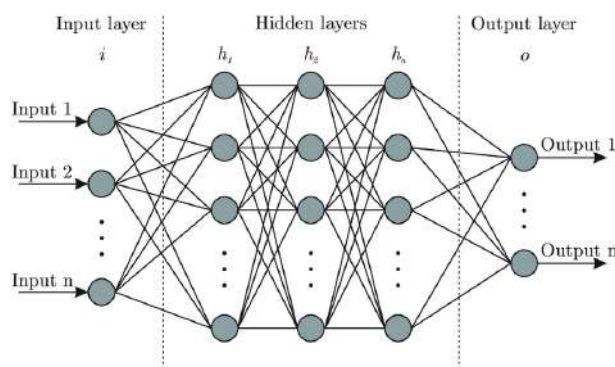


Fig. 2: Architecture of Neural Network.

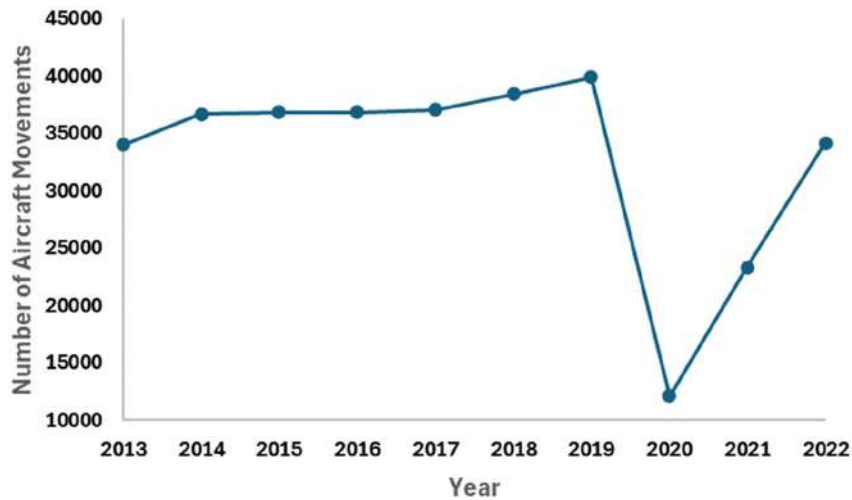


Fig. 3: Total aircraft movements at Queen Alia International Airport (QAIA) from 2013 to 2022.

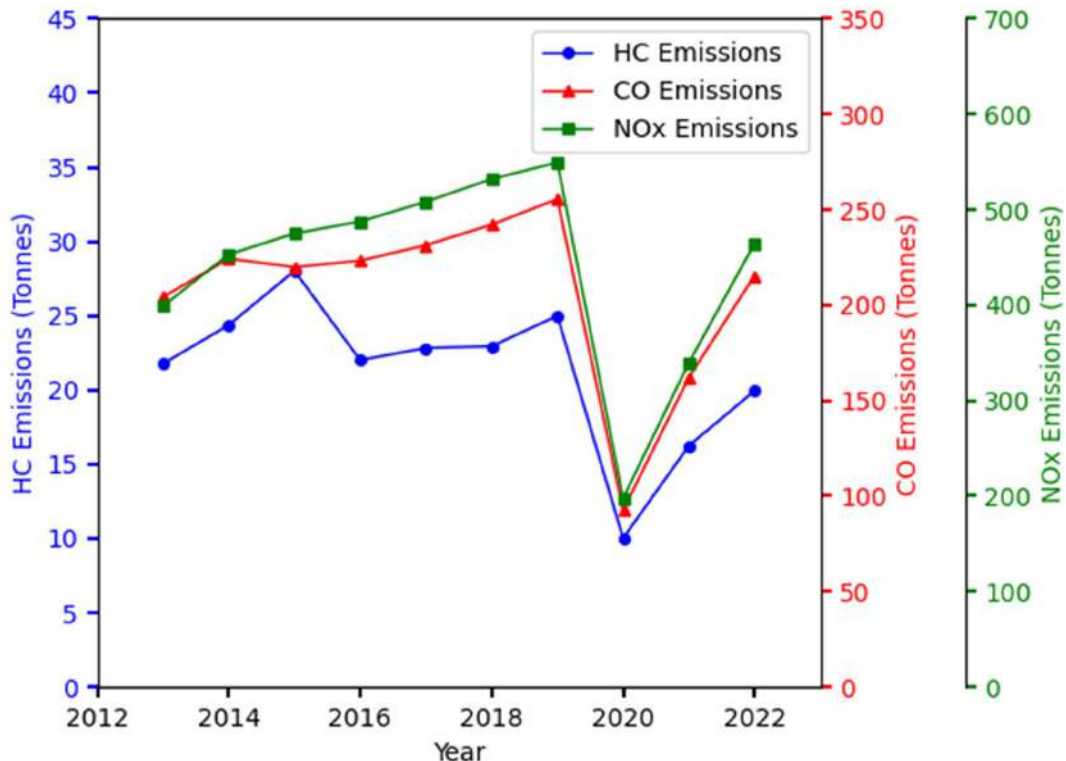


Fig. 4: Estimated total LTO cycle emission in QAIA.

In 2022, there was a notable increase in the number of flights, possibly indicating a continued recovery of air travel.

The estimated LTO emissions followed a similar trend to the number of aircraft movements, as illustrated in Fig. 4. The emissions generally increased up to 2019, where peak emissions of 25, 256, and 549 tonnes were recorded for HC, CO, and NOx, respectively. The average LTO emissions

of HC, CO, and NOx were estimated to be around (21.3 ± 4.8) , (207.1 ± 44.9) , and (440.6 ± 99.9) tonnes per year, respectively. NOx emission has the highest contribution to LTO cycles, followed by CO and HC in QAIA. The significant drop in the three emissions in 2020 is a clear reflection of the impact of the COVID-19 pandemic. This emphasizes the direct relationship between aviation activity

and environmental emissions. The recovery in 2021 and 2022, seen in both the number of flights and pollutant releases, may suggest a return to pre-pandemic levels of air travel and associated environmental impact. This recovery trend raises questions about sustainable practices and the need for continued efforts to reduce emissions.

Emissions Distribution in Different Aircraft Operation Modes

The results revealed that taxi/idle mode has the largest contribution of 35.9% to LTO emissions, while emissions during approach, take-off, and climb phases accounted for

10.8%, 18.5%, and 34.9% of the total emissions, respectively. By analyzing the distribution of each pollutant in different aircraft operation phases of the LTO cycle, including take-off, climb, taxi/idle, and approach, it was found that there were significant discrepancies in the emission rates of various pollutants in each mode. Fig. 5 and Fig. 6 show that HC and CO were significantly emitted in the taxi/idle phase, accounting for 92.3% and 91.6% of the total emissions. Similar trends were observed by other researchers (Bajgai & Shrestha 2023). The emission indices of HC and CO decreased with increasing thrust (Stettler et al. 2011, Yang et al. 2018). So, when the aircraft is on the ground and in

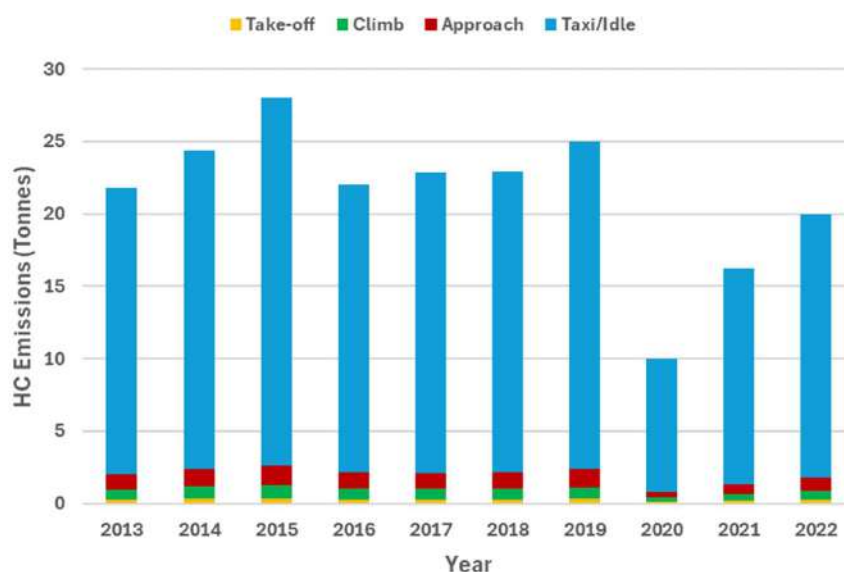


Fig. 5: HC emissions of different LTO operation modes.

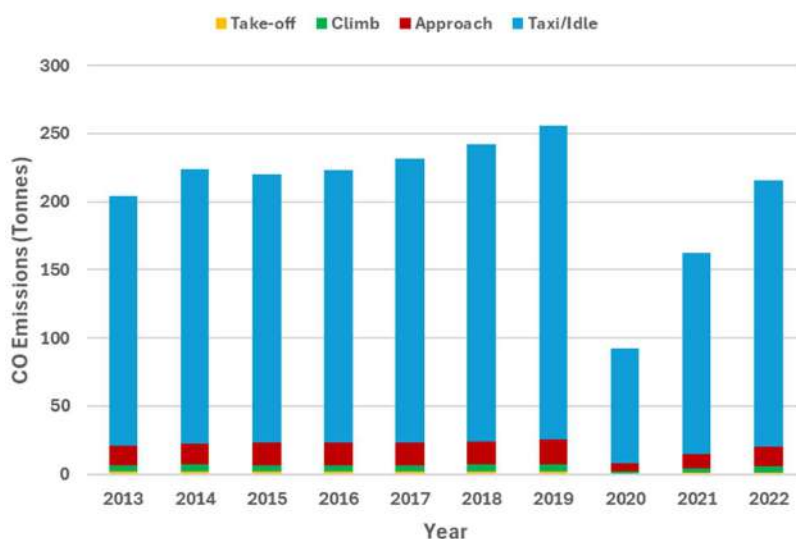


Fig. 6: CO emissions of different LTO operation modes.

taxi/idle mode, it operates at a low power setting where the pressure and temperature are relatively low. This can cause incomplete combustion of fuel and result in increased emissions of CO and HC. The emissions during the taxi/idle phase contribute consistently to the total. Identifying factors influencing emissions during this phase and implementing measures to reduce emissions may have a substantial impact. This can provide insights into changing operational practices, technological advancements, or regulatory influences.

On the other hand, the highest contribution for NO_x was in the climb phase, with 51.6%. While it accounted for 27.6%, 13%, and 7.5% in take-off, approach, and taxi/idle phases, respectively (Fig. 7). The NO_x emissions indices for all aircraft engines are positively correlated to thrust setting. Thus, the highest emission rates of NO_x were in the high thrust operation mode of the aircraft during the phases of climb and take-off. Moreover, NO_x emissions during take-off show an increasing trend over the years, reaching the highest level in the last few years. This suggests a potential need for optimizing engine performance during the take-off phase. Climb NO_x emissions also exhibit a general upward trend, indicating a possible correlation with increased air traffic or changes in flight profiles. Efficient climb-out procedures may be explored to mitigate these emissions. Observing yearly fluctuations in NO_x emissions highlights the dynamic nature of aviation emissions. Identifying the reasons behind these fluctuations can aid in implementing targeted measures for emission reduction. Understanding the environmental impact of NO_x emissions during different

flight phases is crucial for developing strategies to minimize the overall contribution of aviation to air pollution. These strategies include optimizing flight procedures (minimizing ground idling, implementing continuous descent approaches, and optimizing climb profiles), improving infrastructure, encouraging the use of sustainable aviation fuels, investing in research and development of new technologies, and implementing regulatory measures such as emissions trading schemes, performance-based standards, and environmental charges.

Correlation Between Aircraft Age and Pollutant Emissions

In order to investigate the effect of aircraft age on emissions, the aircraft were grouped into five years period. Fig. 8 illustrates how emissions of HC, CO, and NO_x change across different aircraft age groups. The average HC, CO, and NO_x emissions show a notable increase from age 5 to 40, indicating a correlation between age and emissions. The magnitudes of emissions vary significantly between different age groups. Age 40 appears to be a notable peak for all three types of emissions. This may indicate that the engine deteriorates with age, besides other factors related to this age group, which contribute to higher emissions. Linear fit analysis of each pollutant emissions with aircraft age shows a positive correlation with a coefficient of determination (R^2) equal to 0.5183, 0.4767, and 0.6078 for HC, CO, and NO_x, respectively. A similar positive correlation was obtained by other researchers when they correlated engine age with

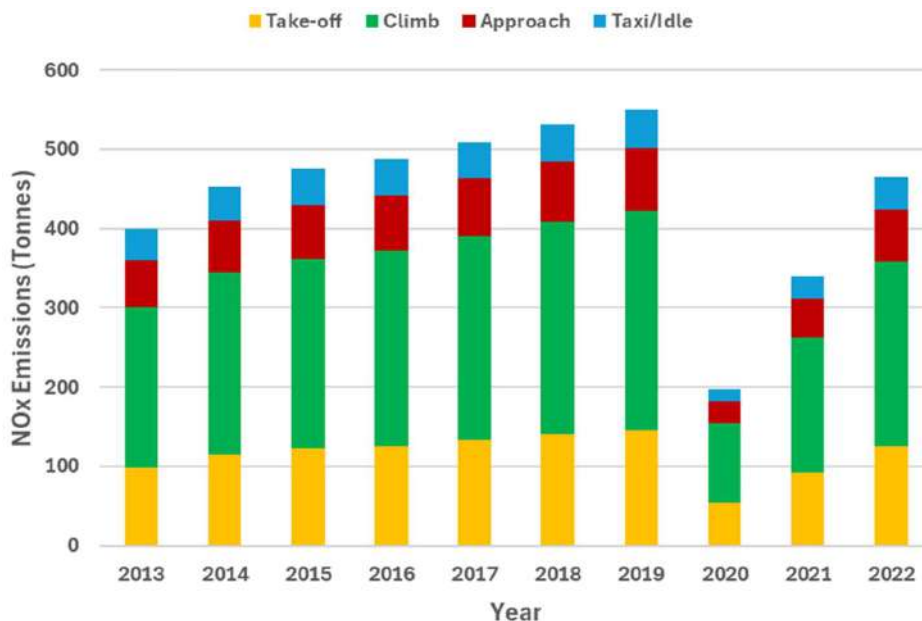


Fig. 7: NO emissions of different LTO operation modes.

emission indices in the idle phase only for CO and NO_x (Zaporozhets & Synylo 2017). Conversely, trends were observed by Xu et al. (2020), who stated that there was no significant correlation detected between the age of the aircraft and NO_x emissions in taxi mode. Also, emissions are influenced by other factors in addition to age, including aircraft type, engine efficiency, maintenance practices, and operational methods, among others. These additional elements contribute to the complexity of the study.

Machine Learning Predictions for Age

In this section, we embarked on a detailed exploration of a regression task employing the MLPreprocessor model. The foundation of this analysis lies in a dataset containing pertinent features, with the aim of predicting the variable 'age'. To perform a detailed analysis of the results based on the provided features, correlation coefficients were calculated between the 'age' variable and various features in our dataset, as shown in Table 2. These coefficients provide insights into the linear relationships between age and various emissions characteristics. Table 2 shows that the correlation coefficients between age and different features span the range of 0.30 to about 0.55. This indicates that some variables have a negative inverse relationship, such as NO_x EI with a correlation coefficient of 0.30, and the others have positive, like NO_x Dp/Foo characteristic (which is the mass of NO_x emitted during the reference landing and take-off cycle, divided by the rated output of the engine). While some variables have strong linear relationships, others have weak correlations. This gives an insight into this correlation.

Table 2: Correlation coefficients between the 'age' variable and various features in our dataset.

Features	Correlation Coefficient
HC T/O [g] (takeoff), HC C/O [g] (climb out)	0.30
HC App [g] (approach), HC Idle [g], HC TOT (total in g)	0.22
CO T/O [g] takeoff	0.36
CO C/O [g] climb out	0.32
HC Dp/Foo Characteristic [g.kN ⁻¹]	0.16
HC Dp/Foo Characteristic (% of Reg limit)	0.18
HC LTO Total mass [g]	0.22
CO Dp/Foo Characteristic [g.kN ⁻¹]	0.24
CO Dp/Foo Characteristic (% of Reg limit)	0.26
NO _x Dp/Foo Characteristic [g.kN ⁻¹]	0.24
NO _x Dp/Foo Characteristic (% of original standard)	0.52
NO _x Dp/Foo Characteristic (% of CAEP/2 standard)	0.52
NO _x Dp/Foo Characteristic (% of CAEP/4 standard)	0.55
NO _x Dp/Foo Characteristic (% of CAEP/6 standard)	0.55
NO _x Dp/Foo Characteristic (% of CAEP/8 standard)	0.55
NO _x EI T/O [g.kg ⁻¹]	-0.17
NO _x EI App [g.kg ⁻¹]	-0.12
NO _x EI Idle [g.kg ⁻¹]	-0.30
NO _x TO, NO _x APP, NO _x TOT, NO _x IDLE, NO _x LTO Total mass	<-0.01

Positive correlations indicate that as the age of the aircraft increases, the corresponding feature also tends to increase, and vice versa for negative correlations.

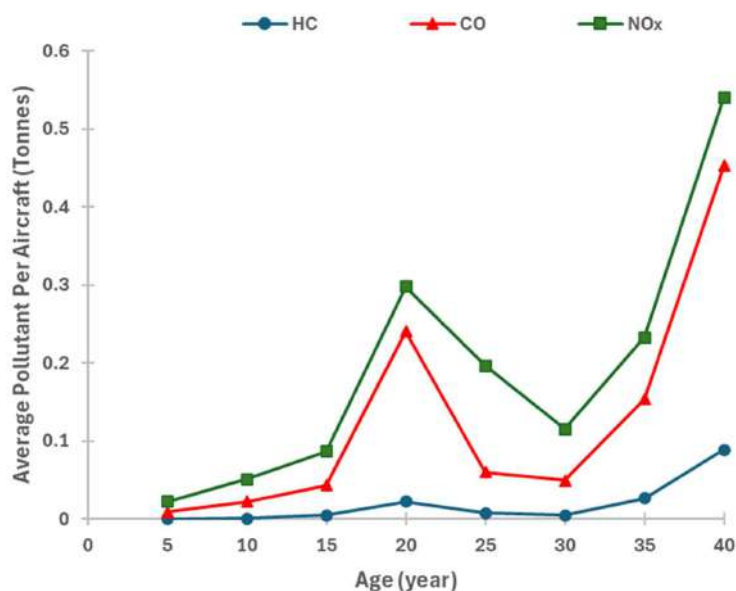


Fig. 8: The 5-year age group with emissions of HC, CO, and NO_x.

The predicted age values can be visualized against the actual age values in a scatter plot to realize how well the model is capturing the relationships. Fig. 9 shows a scatter plot where the horizontal axis represents the actual age values, and the vertical axis represents the predicted age values. Points closer to the diagonal line indicate accurate predictions, while deviations from the line suggest errors in the predictions. The findings showed an MSE value of 3.0931. In this case, since the target variable is age, the estimated MSE is considered reasonable based on the specific context and requirements of our study. More details about model performance are shown in the residuals' plot (Fig. 10). It highlights the areas where the model was able to capture the underlying patterns in the data.

The analysis of emissions across different aircraft types revealed a nuanced relationship with age. While there were

general trends of increasing emissions with age, the patterns were diverse and specific to each aircraft model. Peaks and drops in emission levels within certain age groups suggest that age was indeed a factor influencing emissions. Evidence indicates that older aircraft generally exhibited higher emissions, aligning with industry expectations. However, this impact was not uniform across all aircraft types, emphasizing the uniqueness of each model. This variable has not been thoroughly examined in this study. Further investigation is required.

CONCLUSIONS

The rise in aircraft flights and the subsequent increase in emissions have raised global concerns. This research is crucial because it addresses vital challenges at the nexus of

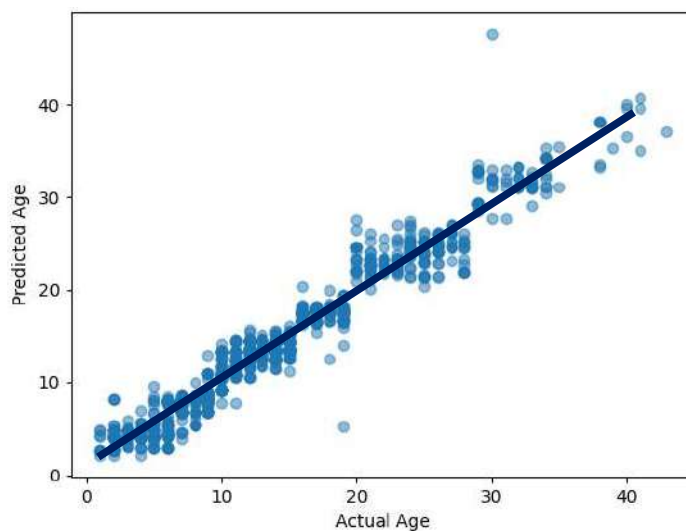


Fig. 9: The predicted age values against the actual age values in a scatter plot.

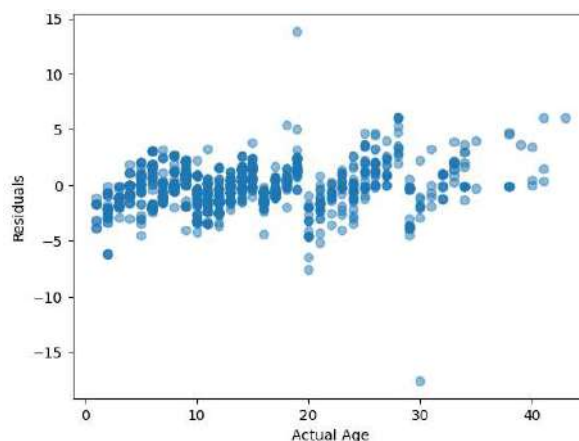


Fig. 10: The residuals plot between the actual and predicted age values.

aviation, environmental sustainability, and public health. This study provided significant contributions at both the local and international scales. Firstly, for the first time, the overall emission of the LTO cycle in QAIA in Jordan was estimated in the period from 2013 to 2022. Secondly, it assessed the relationship between aircraft age and major engine emissions, such as HC, CO, and NOx. Such investigations were not covered fully in the open literature. Thirdly and most importantly, upon detailed examination of the literature, any studies correlating aircraft age with emissions features using machine learning have not been seen to the best of the author's knowledge. So, the novelty of this paper is the use of these advanced machine learning tools to uncover the complex relationship between different emission features and aircraft age. As understanding how aircraft age influences engine emissions is crucial for developing sustainable aviation operations, this research makes significant contributions by identifying the specific emission features associated with aging aircraft. This can be translated into actionable insights for the development of targeted strategies aimed at minimizing the aviation environmental footprint. This may include implementing age-based maintenance programs, investing in fuel-efficient aircraft by replacing older aircraft with newer and more environmentally friendly models, investigating and adopting advanced technologies like biofuels, electric propulsion, and hydrogen-powered aircraft to reduce emissions across their fleet, incentivizing sustainable aviation practices, and promoting the use of sustainable ground operations.

Future research activities include expanding the investigation to assess the emissions from other pollutants, such as CO₂ and particulate matter, and to apply this methodology to other airports in the region. Finally, challenges encountered in this research include variability in the quality of the available records, some aircraft types having a limited number of flights, preventing a thorough investigation, and difficulties in obtaining measured emissions. In addition, a limitation of the used dataset lies in the potential variability of engine types and maintenance practices across the aircraft fleet. Differences in engine models, their age, and maintenance histories can significantly influence emission profiles.

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Green Synthesis of Semiconductor Nanocomposite (O-ZnO) Using Onion Peels Extract for Degradation of Organic Materials

Nabaa A. Al-Wahab[†]  and Rasha A. Al-Husseiny

Water Resources Management Engineering Department, College of Engineering, Al Qasim Green University, Babylon, 51013, Iraq

[†]Corresponding author: Nabaa A. Al-Wahab; nabaaabedalwahab@wrec.uoqasim.edu.iq

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ABSTRACT

The overuse of pharmaceuticals in recent years and the subsequent discharge of pharmaceutical waste, liquid waste, and harmful organic pollutants into the aquatic environment are important issues that should be seriously addressed. Therefore, this work presented an economical, environmentally sustainable, and simple method for producing zinc oxide nanocomposite using onion peel extract (OPE) and using this nanocomposite (O-ZnO) for organic pollutant removal as cefixime (Cfx) from aqueous solution using an advanced oxidation process. The synthesized materials in this study were characterized using analytical techniques, including (Brunauer–Emmett–Teller (BET), X-ray diffraction (XRD), field emission scanning electron microscopes (FESEM), and Fourier transform infrared spectroscopy (FTIR). The analysis showed that the surface area of onion peel extract (OPE), ZnONPs without OPE, and ZnO with OPE (O-ZnO) were 4.22, 30.1, and 49.3 m²/g, respectively. The findings indicated that the cefixime elimination efficiency of the O-ZnO nanocomposite attained 94%. Under the best operational conditions of pH 4, the dosage of O-ZnO (0.4 mg/L), Cfx concentration was (10 mg/L) with an optimum contact time of 120 min. The kinetic degradation rate of (Cfx) adhered to the pseudo-first-order equation with an R² value of 0.9682, giving a constant degradation rate of 0.031/min.

INTRODUCTION

The daily activities of humans, factories, and other sources have recently generated large volumes of wastewater. These sources can produce compounds that are hazardous to the environment and human health, as well as materials that are not biodegradable (Ajiwokewu 2024). Furthermore, significant harm to sustainable development is caused by the rapidly growing amounts of wastewater containing harmful organic contaminants, which are not biodegradable even in drinking water and are very poisonous (Jabbar et al. 2023a). Emerging contaminants, such as antibiotics, have been found in drinking water, sewage effluent, surface water, and even reclaimed land (Moosavi et al. 2019). Cefixime (Cfx) is one of the most commonly used antibiotics. Cefixime can be purchased in white or nearly white powder. Cefixime does not dissolve well in water (Abdullah et al. 2022). Additionally, the presence of these substances in water supplies, even at low concentrations, strengthens their resistance to bacteria (Widyasari et al. 2022). To eliminate organic pollutants, several instead of a number of technologies have been used, such as sedimentation, adsorption, solvent extraction, chemical precipitation, conventional biological processes, and electrochemical processes. Unfortunately, these technologies are not cost-effective, the processes are slow, and they need to be controlled in terms of pressure and temperature (Haroon et al. 2023). (AOP) has proven effective, affordable, dependable, and promising for eliminating pollutants from aquatic environments (Hidayah et al. 2023). Among the different AOP techniques, homogeneous and heterogeneous photocatalysis

have shown promise in degrading a range of pollutants by employing different catalysts, which use different kinds of catalysts (Al-Husseiny & Ebrahim 2021). AOP procedures provide low-cost and ecologically friendly treatment options (Al-Husseiny & Ebrahim 2023).

Nano-semiconductor photocatalysts like TiO_2 , ZnO , Fe_2O_3 , and ZnS have drawn a lot of attention and study due to their great efficiency, non-toxicity, low energy consumption, and efficiency in treating sewage (Al-Husseiny & Ebrahim 2022a,b, Xu & Xu 2020). (ZnO) It is classified as a wide-band gap semiconductor and is a member of the II-VI semiconductor group. ZnO catalysts facilitate the photodegradation of organic compounds through exposure to light. When ZnO is exposed to light with energy higher than its bandgap, electrons in the valence band (VB) are stimulated to move to the conduction band (CB), resulting in the creation of photogenerated electrons in the CB and photogenerated holes in the VB. Biological synthesis is a highly environmentally friendly approach for synthesizing ZnONPs (Nagore et al. 2021, Roy et al. 2022, Raina et al. 2020). The novelty of this study was the synthesis of O-ZnO by using and utilizing low-cost waste materials to get rid of the problem of solid waste resulting from daily household use or any sources containing quantities of such onion peels. The onion is known to include a variety of phytochemicals, flavonoids, and enzymes that aid in the production of nanoparticles and to achieve sustainability, be environmentally friendly, and inexpensive. The natural compounds in onion peel extract promote the generation of hydroxyl radicals ($\bullet\text{OH}$) and superoxide anions ($\text{O}_2^{\bullet-}$), which contribute to the breakdown of organic pollutants. These compounds also support the adsorption and catalysis of organic materials, accelerating the degradation process. This work aimed to synthesize nanocomposite O-ZnO utilizing a simple and environmentally friendly method. The reducing and capping agent employed in this process is an extract derived from onion peel waste. The ZnO nanostructures were tested using several spectroscopic and microscopy tools. The photocatalytic effectiveness of ZnO nanostructures was examined when exposed to UV photo-irradiation to degrade

organic antibiotic contaminants, specifically cefixime. The synthesized materials were examined using X-ray diffraction, field-emission scanning electron microscopy, Brunauer-Emmett-Teller, Fourier-Transform infrared spectroscopy, and Barret-Joyner-Halenda techniques. Cefixime removal's kinetic model was studied under some of the conditions, such as contact times, pH, O-ZnO dose, and initial cefixime concentrations.

Photocatalysis Process

Photocatalysis is a significant component of advanced oxidation processes (AOPs). These are commonly employed for the elimination of stubborn organic components from industrial and municipal effluent (Thongam & Chaturvedi 2021). Semiconductor photocatalysis is triggered by the generation of electron-hole pairs following the stimulation of the material's bandgap. When a photocatalyst is exposed to light with energy equivalent to or higher than the band-gap energy, the electrons in the valence band can be stimulated to move to the conduction band, resulting in the creation of a positive hole in the valence band. A photocatalyst, such as zinc oxide (ZnO). The excited electron-hole pairs can undergo recombination, resulting in the dissipation of the input energy as heat, without any chemical alteration. Nevertheless, if the electrons (and holes) go to the surface of the semiconductor without undergoing recombination, they can engage in a range of oxidation and reduction reactions with adsorbed substances like water, oxygen, and other organic or inorganic species. The oxidation and reduction reactions serve as the fundamental processes in photocatalytic water/air remediation and photocatalytic hydrogen production, respectively. Fig. 1 shows a Schematic representation of the photocatalytic mechanism of the ZnO photocatalyst.

Comparison with Other Technologies

Advanced water treatment technologies have been created to remove pharmaceuticals effectively. Table 1 provides a comprehensive overview of the advantages and disadvantages of various wastewater treatment systems.

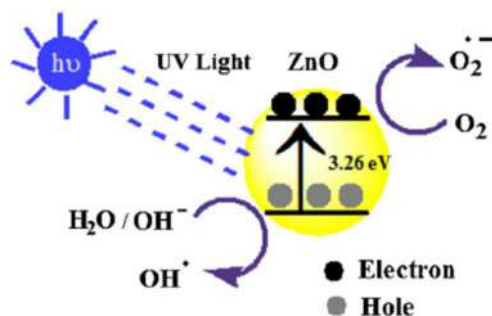


Fig. 1: Schematic representation of the photocatalytic mechanism of ZnO photocatalyst.

Table 1: Advantages and Disadvantages of different Wastewater Treatment Technologies.

Treatment Technology	Advantage	Disadvantage	References
Coagulation (physical)	• Simplicity and profitability. • Separates many types of particles of water.	• It requires the use of chemical products. • Toxic compounds are transferred to the solid phase	(Xu et al. 2021)
Membrane Bioreactor Technique (biological)	• Membrane filtration ensures water quality is treated regardless of the decantability of the sludge. • High quality and high levels of water disinfection-treated	• High cost of implementation and exploitation. • Accumulation of toxic substances in the bioreactor. • Membrane fouling.	(Phoon et al. 2020)
Activated Sludge (biological)	• It is a process that is used in the treatment of industrial wastewater. • It is a process that is used in the treatment of industrial wastewater.	• High costs of design, construction, operation, and maintenance. • High costs of design, construction, operation, and maintenance. • Sludge storage problems, residuals.	(Henstra et al. 2007, Couto et al. 2010, Huang et al. 2018)
Biological Treatment Process (biological)	• The construction and process operation of these reactors are comparatively simple. • An acceptable and steady pH can be maintained without the addition of chemicals	• Pathogens and nutrients are partially removed, and hence, post-treatment is needed • The process start-up takes a long time due to the low progress rate of microbes to become active.	(Cecconet et al. 2017, Couto et al. 2019, Dogan et al. 2020) .
Constructed Wetlands Process (physical)	• Energy consumption is quite low. • Operational and maintenance costs are low	• A large land area and a longer setting time are required. • Many pharmaceuticals cannot be removed completely	(Verlicchi & Zambello 2014) .

The typical approach described in Table 1 has significant disadvantages, including partial elimination, large energy requirements, and the production of hazardous compounds (Al Qarni et al. 2019). Various techniques have been employed to eliminate toxins from polluted water sources. Advanced oxidation processes (AOP) have developed as a potential, effective, affordable, and dependable method for removing contaminants from aquatic environments. Homogeneous and heterogeneous photocatalysis, employing diverse catalysts, has become a feasible approach for breaking down a wide range of contaminants among the many AOP approaches. AOP procedures are classified as cost-effective and ecologically friendly treatment options.

MATERIALS AND METHODS

Materials

Various materials were utilized in the experiments, and all chemicals used were of analytical grade. Table 2 lists all the materials used in this work.

Onion Peels Extract Preparation (OPE)

The onion peels waste obtained from the market for vegetables was thoroughly rinsed several times using twice as much pure water to remove any accompanying microscopic bits of dust. In addition, the item was air-dried at room temperature for two days until complete moisture evaporation occurred. Subsequently, 1 g of the onion peels was introduced into 100 mL of distilled water and subjected to extraction

through boiling for 30 minutes. The onion extract was isolated through filtration and utilized to manufacture zinc oxide nanoparticles. Fig. 2 represents the steps of preparing onion peel extract.

Synthesis of Nano-Composite (O-ZnO)

A magnetic stirrer was used to prepare and hold approximately 50 mL of a 0.2 M solution of zinc acetate ($\text{Zn}(\text{CH}_3\text{CO}_2)_2$). During the experiment, the acidity level of the solution was monitored. Kept at 12 by utilizing a 0.2M NaOH solution since the parameters were specified and tuned, and the optimal pH value was determined to be at pH 12 for synthesis. The solution above was value of speed and temperature stirred while approximately 10 mL of onion peel extract solution was included in a decrease by drop (Al-Husseiny & Ebrahim 2022a). Once the incubation duration validated the (O-ZnO) synthesis, the solution was left in stirring conditions

Table 2: Sources of materials and chemical formula of starting materials utilized.

Material Name	Chemical Formulas	Supplier
Cefixime	$\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_7\text{S}_2$	The general company for the drugs industry (Iraq)
Sodium hydroxide	NaOH	Thomas Baker, India,
Hydrochloric acid	HCl	Thomas Baker, India,
Distilled water	H_2O	Lab
Zinc Acetate	$\text{Zn}(\text{CH}_3\text{CO}_2)_2$	Thomas Baker, India,

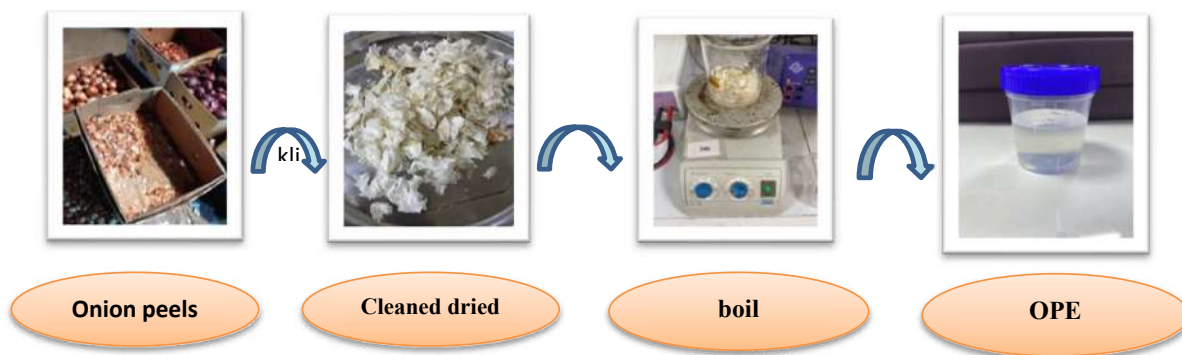


Fig. 2: Steps of preparation of onion peel extract.

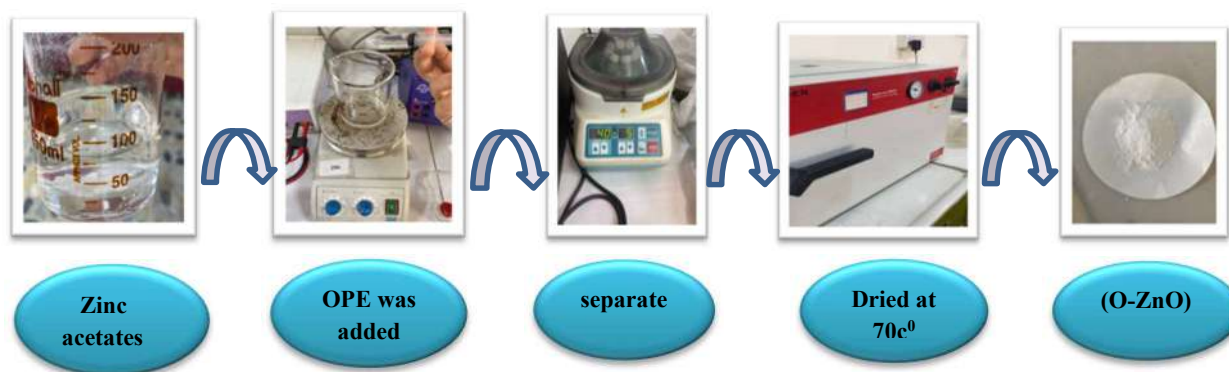


Fig. 3: Steps of synthesis of nano-composite (O-ZnO).

for two hours. To distinguish the solution from the residue, the mixture was centrifuged for five minutes at 6000 rpm. While the residue was kept, the solution was decanted. The residue was repeatedly cleaned with D.W. to get rid of any contaminants that might have attached themselves to its surface. After obtaining the residue, it was dried entirely at 70°C in an oven. Fig. 3 represents the steps of the synthesis of (O-ZnO).

Comparison Between Zinc Commercial and Nanocomposite (O-ZnO)

In this study, zinc manufactured from onion peel extract was compared with commercial zinc. Fig. 4 compares these materials to choose the optimal removal efficiency for (Cfx). It was noted that the percentage of pollutant removal by (O-ZnO) was 94%, while the pollutant removal by commercial zinc was 49%. This indicates the importance of using peels in the synthesis of zinc; the natural compounds in onion peel extract promote the generation of hydroxyl radicals ($\bullet\text{OH}$) and superoxide anions ($\text{O}_2\bullet^-$), which contribute to the breakdown of organic pollutants. These compounds also support the adsorption and catalysis of organic materials, accelerating the degradation process as nanocomposite (O-ZnO) works as a new and inexpensive

absorbent material to remove organic pollutants such as (Cfx) due to the higher surface area of the nanocomposite compared to ZnO commercial.

Photocatalytic Degradation Reaction

The photocatalytic degradation of cefixime under UV light was examined utilizing green-synthesized ZnO nanoparticles derived from onion peel extract and zinc acetate solution in a batch photocatalytic reactor. Initially, 0.150 g of OPE/ ZnO was incorporated into 100 mL of cefixime solution at a starting concentration of 10 mg.L^{-1} . The solution was thereafter subjected to magnetic stirring in the absence of light for 30 minutes to achieve adsorption equilibrium. Subsequently, the suspensions were exposed to UV light, as illustrated in Fig. 5. Following predetermined illumination durations, 5 mL was extracted and centrifuged at 6000 rpm to quantify cefixime concentration. The cefixime concentration was evaluated using UV-vis spectroscopy (Hitachi U2900), with a maximum absorption wavelength of 280 nm.

Parameters Affecting the Photocatalytic Process

In this study, the experiments were conducted using changeable parameters in a batch system, as shown in Table 3.

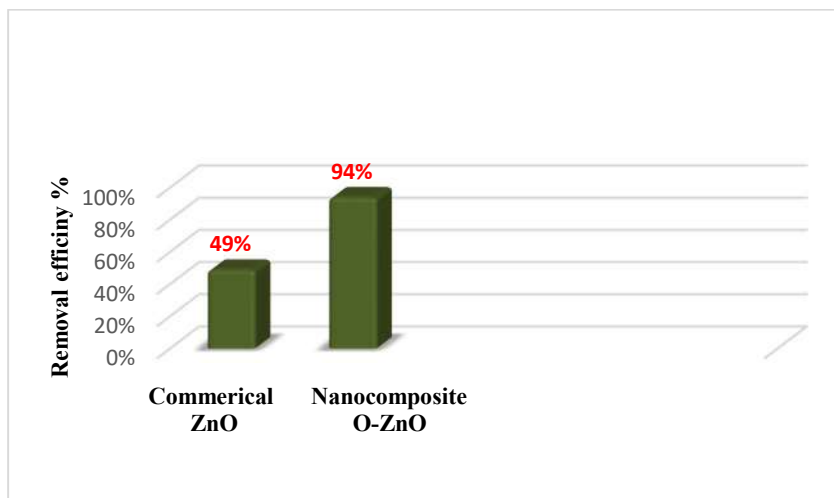


Fig. 4: Comparison between zinc commercial ZnO and (O-ZnO) nanocomposite.

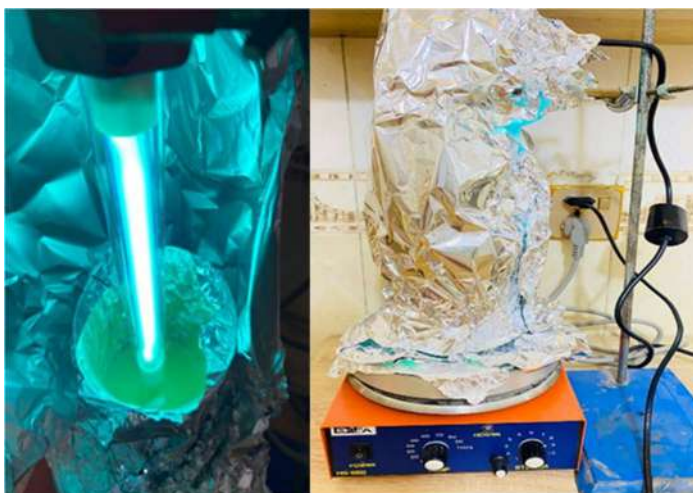


Fig. 5: Batch oxidation system.

All experiments were performed at room temperature using a magnetic stirrer. An external magnet was employed to isolate the adsorbent from the solution. A UV spectrophotometer was employed to measure optical properties. The removal effectiveness of Cfx, denoted as R (%), was computed using the eq. 1 (Al-Husseiny & Ebrahim 2022a, b).

$$\text{Degradation efficiency \%} = \frac{C_0 - C_t}{C_0} * 100\% \quad \dots(1)$$

Where C_0 represents the initial concentration, while C_t is the cefixime concentration at a certain reaction time.

RESULTS AND DISCUSSION

Results of Characterization

BET analysis: The Brunauer–Emmett–Teller (BET) Analytical techniques were employed to ascertain the surface areas of the adsorbents, and the Barrett-Joyner-Halenda (BJH) Analytical methods were utilised to determine the properties of the pore volumes. And pore sizes of nanomaterials used in this study are presented in Table 4. The analysis showed

Table 3: Experimental parameters examined in a batch system.

Parameters Range	Ranges
pH	2-10
Dosage mg.L^{-1}	0.1-0.6
Initial concentration (ppm [mg.L^{-1}])	10-70
Contact time [min]	30-180

that the expanse of the surface of onion peel extract (OPE), ZnONPs without OPE, and (O-ZnO) were 4.22, 30.1, and 49.3 $\text{m}^2\cdot\text{g}^{-1}$, respectively. (O-ZnO) Before removal exhibited the most surface area was exhibited from OPE and ZnO without OPE. The pore volumes were measured (0.015594, 0.094318, and 0.1284 $\text{cm}^3\cdot\text{g}^{-1}$ for OPE, ZnO, and (O-ZnO) before removal, respectively, and (O-ZnO) before removal exhibited the greatest pore volume. The pore sizes of OPE, ZnO without OPE, and (O-ZnO) before removal were (10.61, 6.95, and 4.65 nm), respectively. Increasing BET, the surface area of (O-ZnO) before removal, a higher surface area of (O-ZnO). The effectiveness of this adsorbent in removing (Cfx) has been demonstrated through several experiments, highlighting the impact of its elevated pore volume and mean pore diameter of (O-ZnO) before removal compared to OPE, ZnO Without OPE material, It improves the process of adsorption (Cfx) and The accumulation of the substance on the adsorbent is affected by the BET surface area, pore volume, and mean pore diameter, as these are primary parameters affecting adsorption.

XRD (X-Ray diffraction) analysis: X-ray diffraction is an effective method for determining the composition of crystalline materials. (XRD) yields information about the sample's crystalline phase, orientation, and network. The crystallinity, analysis of phase, and Particle size were examined using the X-ray diffraction (XRD) technique. Fig.

(6a to 6d) presents the crystalline structure of the synthesized materials (OPE, ZnO without (O-ZnO), (O-ZnO) before removal of Cfx, and (O-ZnO) after removal of Cfx). X-ray diffraction peaks for OPE at ($2\theta=14.6^\circ$ and 32.5°) are shown in Fig. 6a using a certain code for reference of (96-201-2670) and intensity of (111 and -114). The X-ray diffraction (XRD) peaks corresponding to the produced zinc oxide (ZnO) nanoparticles are displayed in Fig. 6c. XRD pattern of (O-ZnO) shows in fig.(6-b,c)the prominent diffraction peaks observed at the specified diffraction angles (2θ): (32.4° , 34.2° , 36.5° , 47.8° , 57.6° , 63° , 68.14° , 69.25° , 72.35° and 77°) with reference code of (96-220-4398) and intensity of (220, 31-1, 22-3, 051, 13-8, 15-7, 149, 158, and 43-8), respectively. The observations validated the crystalline structure of the nanoparticles as Wurtzite (O-ZnO). Furthermore, the presence of distinct and sharp diffraction peaks suggests a high level of crystallinity in the nanoparticles. The lack of additional diffraction peaks resulting from contaminants and intermediary components demonstrated the exceptional purity of the produced ZnO nanoparticles. Comparable crystalline size dimensions by (Abbes et al. 2022, Ramesh et al. 2021, Bekele et al. 2021, Harouni et al. 2019). Utilizing XRD data, it was determined that the peaks are broad, indicating that the crystallites possess sizes within a specific range, and the diameter (D) was computed using Scherrer's formula.

Table 4: OPE, ZnO without OPE, (O-ZnO) before removal, and (O-ZnO) after removal.

Characteristics	OPE	ZnO Without OPE	(O-ZnO) before removal	(O-ZnO) after removal
Specific surface area [$\text{m}^2\cdot\text{g}^{-1}$]	4,2217	30,098	49,277	15,391
Total pore volume ($\text{cm}^3\cdot\text{g}^{-1}$)	0,015594	0,094318	0,1284	0,1047
Mesoporous and microporous volume [$\text{cm}^3\cdot\text{g}^{-1}$]	0,9699	6,9151	11,322	3,5361
BJH volume of pores [$\text{cm}^3\cdot\text{g}^{-1}$]	0,014591	0,091405	0,1227	0,1003
BJH surface area of pores [$\text{m}^2\cdot\text{g}^{-1}$]	1,4296	14,724	19,668	17,682
BJH average pore diameter [nm]	10,61	6,95	4,65	10,61

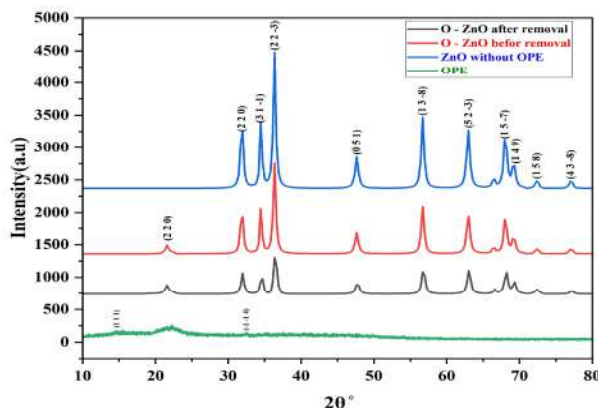


Fig. 6: The X-ray diffraction pattern depicts the structure of (a)OPE, (b) ZnO without OPE, (c) (O-ZnO) before removal Cfx, and (d) (O-ZnO) after removal Cfx.

$$D = K\lambda/\beta \cos\theta \quad \dots(2)$$

Where the symbol “K” represents the Debye Scherrer constant, “ λ ” denotes the X-ray wavelength, “ β ” represents the breadth of the half maximum peak, and “ θ ” signifies the diffraction angle (Drummer et al. 2021). X-ray diffraction data measure the estimated crystalline size of (O-ZnO). The measurement was taken at around 22 nanometers.

Fourier-transform infrared spectroscopy (FTIR) of synthesized materials: Fig. 7 depicts the standard diagram of Fourier Transform Infrared Spectroscopy (FTIR) analysis of Zinc Oxide Nanoparticles (ZnONPs) that were manufactured from onion waste (O-ZnO). The peaks seen at (427.91 and 503.42 cm^{-1}) are ascribed to the ZnO bond and molecular stretching, suggesting the creation of the particles. The band observed at 1036.94 cm^{-1} in the nanoparticles from the peel is attributed to the presence of an amide group. On the other hand, the sharp and deep band observed at 1632.01 cm^{-1} is attributed to the stretching of the C = O bond and the existence of a water molecule (–OH) in the nanoparticle. A little signal at 2988.16 cm^{-1} indicates the presence of ambient carbon dioxide in the sample. The presence of hydroxyl molecules in the produced ZnONPs is indicated by a wideband at 3426.3 cm^{-1} . O-ZnO after the removal of cefixime is displayed in Fig. 7. Vigorous stretching and the (O-ZnO) link are responsible for the peaks at (435.71 and 498.89135 cm^{-1}), showing how the particles form. The band at (1031.04 cm^{-1}) results from the existence of an amide group in the peel nanoparticles, but a deep and sharp band at (1629.71 cm^{-1}) is caused by the presence of a water molecule (–OH) owing to C = O tiny peak (2923.5 cm^{-1}) (Jabbar et al. 2023b). The presence of hydroxyl molecules in the produced (O-ZnO) is determined by a wideband at (3429.05 cm^{-1}). Comparable Additional findings were acquired by Modi and Fulekar (2020) and Modi et al. (2022).

Field-emission scanning electron microscopy (FE-SEM): FESEM analysis of the synthesized ZnONPs was carried out to determine the morphology of synthesized ZnO nanoparticles. For the FESEM studies, The shape and morphological features of the produced materials were examined using (FE-SEM), as shown in Fig.(8 a-c); the OPE surface was consistent and showed that they have leaf-like structures with a size of 39.94 nm, as seen in Fig.(8a). Several tiny cavities and holes were found. That indicated that OPE can be an excellent surface to synthesize ZnO nanoparticles. Fig. 8b shows the synthesized (O-ZnO). All the particles are spheroidal and submicron in size; however, they have merged to form a macroscopic structure that exceeds 100 nm in dimensions. At the same time, the Photocatalytic activity caused the fusion of some nanoparticles. In actuality, the morphological attributes of the (O-ZnO) surface provide an advantage by increasing the available surface area for adsorption. Comparing Fig. 8c and the FESEM image after adsorption of (Cfx) in Fig. 8c, it can be shown that during (Cfx) adsorption, (O-ZnO) morphological qualities were considerably altered. Because the pore surfaces were saturated with (Cfx) molecules, the surface of (O-ZnO) becomes brighter and smoother, and some previously separated agglomerates coalesce. Similar results were also reported by (Jan et al. 2021, Modi et al. 2022, Bekele et al. 2021, Xu & Xu 2020).

Batch Experiments

The impact of pH: The initial pH of the solution is a crucial factor in photocatalytic processes, influencing the degradation and adsorption capacity of target organic compounds by modifying the photocatalyst's surface electrical charge properties and dictating the ionization state of the catalyst surface. The pH value affects the adsorption and dissociation capacity of chemicals, the charge distribution on the catalyst

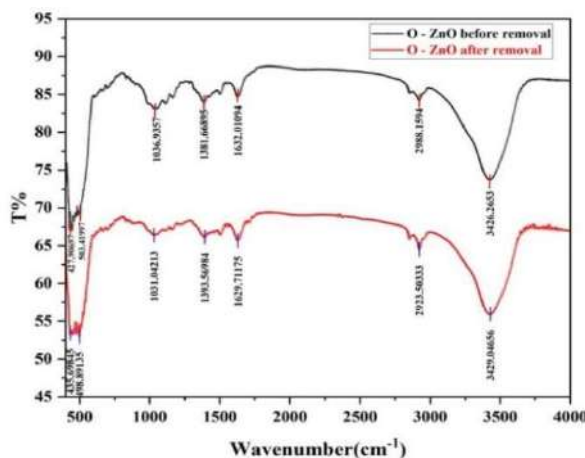


Fig. 7: FTIR spectra of (a) O-ZnO before removal and (b) O-ZnO after removal.

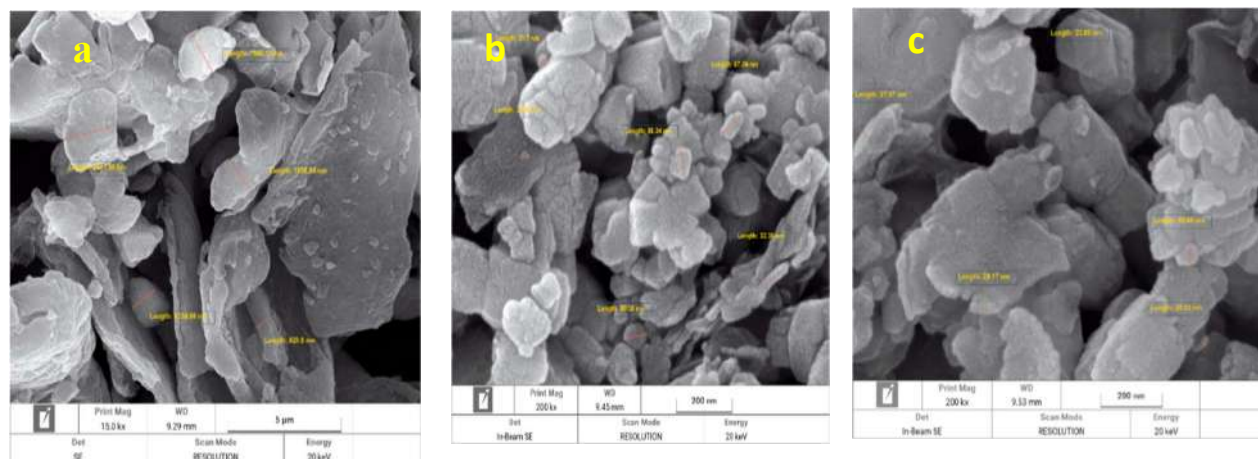


Fig. 8: FE-SEM images for (a) OPE, (b) (O-ZnO) before removal of Cfx, and (c) (O-ZnO) after removal of Cfx.

surface, and the oxidation potential of the catalyst's valence band. The pH of the solution varies with the degradation of pollutants; the pH impact was studied by shifting pH from an acid medium (pH=2) to a basic medium (pH=10) as well as a neutral medium (pH=6). Fig.9 Hence, the degradation of cefixime was studied under various pHs (Fig.9). From the results obtained, it was evident that the degradation catalyst, i.e., (O-ZnO), yielded a maximum degradation efficiency at pH = 4 (94%) and a similar degradation efficiency at pH=3 (90%). When the pH was increased from 3 to 5, the degradation efficiency was increased; however, when the pH was reduced from 7 to 10, it showed a relatively low effect on the degradation efficiency of cefixime. The variation in the degradation efficiency with varying pH could be attributed to the electrostatic interactions between (O-ZnO) charged particles and the contaminants. The pH of the solution had a significant role in determining the degree of dissociation of the pollutant. This point was related to the pKa value of the pollutant, which was considered to be 3 for cefixime. The acid-alkaline properties of the (O-ZnO) surface can have significant effects on its photocatalytic function.

The impact of dose: The effect of catalyst dose on the photocatalytic degradation of cefixime using O-ZnO nanocomposite has been studied with nanocomposite concentrations varying from 0.1 to 0.6 g.100 mL⁻¹ (Fig.10). Results indicated that initially, with the increase in nanocomposite concentration, the process efficiency of Cfx removal increased. The maximum removal was at the nanocomposite concentration of 0.4 g.100 mL⁻¹. Still, with the increase of nanocomposite concentration to 0.6 g/100 mL of the nanocomposite, the degradation rate of contaminants exhibited a nearly linear trajectory, maybe attributable to the self-competitive interactions of the nanocomposite inside the solution. As the concentration became 0.4 g.100 mL⁻¹,

the number of active sites on the nanocomposite surface, the generation of hydroxyl radicals, and the subsequent removal of Cfx escalated, contingent upon the catalyst surface sites and the intensity of UV radiation. Therefore, the catalyst concentration of 0.4g/100 L was chosen as the optimal Concentration for the next experiments in the study of photocatalytic Cfx removal utilizing O-ZnO nanocomposite (Derakhshani et al. 2023).

The impact of initial concentration: Following the identification of the optimal solution pH, the findings from the analysis of antibiotic concentration effects are illustrated in Fig. 11. To enhance the elimination of cefixime by (O-ZnO), four additional doses of Cfx were tested alongside the standard concentration of 10 mg/L to identify the optimal concentration for degradation (El-Nahhas et al. 1992). The experiment results (Fig. 11) indicate that an increase in the initial Cfx concentration to 10 mg/L enhances degradation. Nevertheless, once the concentration above 20 mg.L⁻¹, the degradation efficiency diminished, exhibiting a reduction of over 10%. The reduction may result from the influence of Cfx and intermediate metabolites on the photocatalytic reaction during degradation, including the adsorption of UV light. The greatest degradation occurred at a concentration of 10 mg.L⁻¹; the primary nanocomposite at the ideal pH demonstrated satisfactory effectiveness in removing Cfx within the 10 mg.L⁻¹ concentration range.

The impact of contact time: Contact time was an important factor that played a vital role in ensuring maximum efficiency of the photodegradation of the contaminant. To ascertain the optimum contact time for our catalyst, i.e., (O-ZnO), the degradation percentage for the contaminant was studied. A sample was collected at different time intervals in order to study the degradation percentage (Fig. 12). From the results obtained, we concluded that the removal trend for cefixime

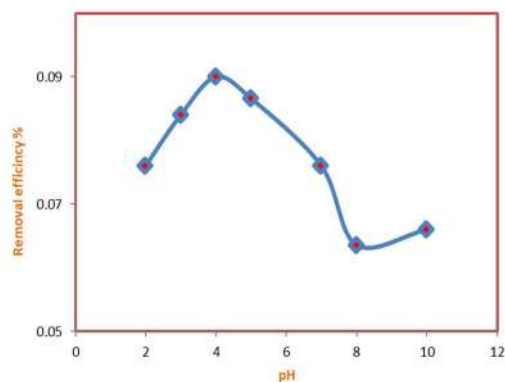


Fig. 9: Effect of pH on the degradation of (Cfx).

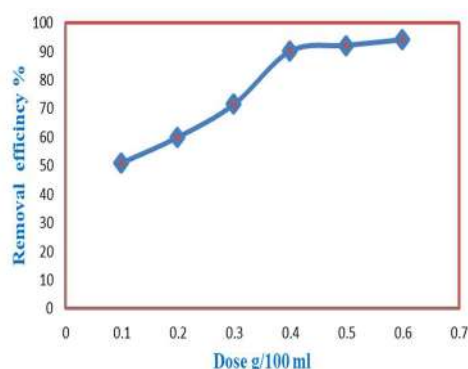


Fig. 10: Effect of dose on the degradation of (Cfx).

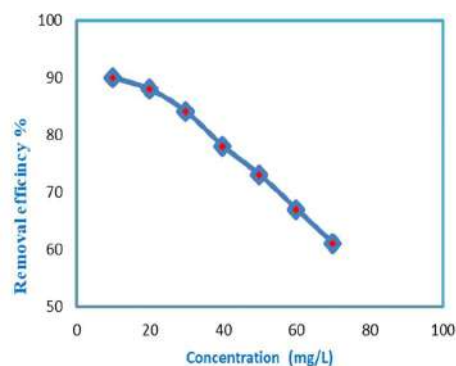


Fig. 11: Effect of Concentration on the degradation of (Cfx).

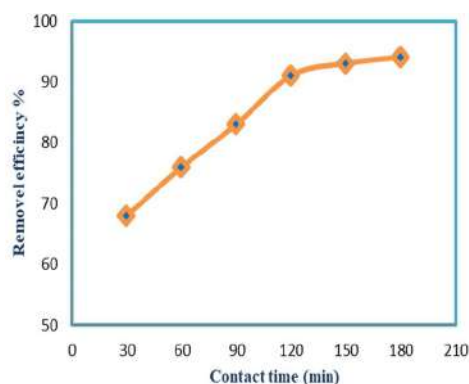


Fig. 12: Effect of time on the degradation of (Cfx).

by the O-ZnO nanocomposite increases at 30 min and then gradually increases. The removal trend increased at 120 min, where the removal rate reached its peak and began to follow a straight line at minute 180. It was then observed that no further degradation took place. Therefore, the best time for the removal of cefixime by the O-ZnO nanocomposite, based on the following experiments, is 120 minutes. Hence, it can be said that initially, cefixime was oxidized rapidly by the generated radicals; however, with time, due to the formation of intermediates, the radicals generated were consumed, and the degradation efficiency was reduced (Ahmed & Kareem 2024a).

Kinetic Studies

The kinetics of the photocatalytic reaction for Cfx degradation were examined based on the optimal conditions established in the preceding section (Ahmed & Kareem 2024b). The kinetic models of the pseudo-first-order reaction (Equation 1) and pseudo-second-order reaction (Equation 2) were used for studying 10 mg/l of Cfx residues removal from aqueous solution at pH =4 and O-ZnO 0.4 mg/l; their results are shown in Table 5. The results revealed that the pseudo-kinematics of the first order showed the best correlation coefficient

($R^2 = 0.9682$) shown in Fig. (13-a and Fig. (13-b). The final summarised version of this model is:

$$\ln \frac{C_0}{C} = Kt \quad \dots(3)$$

$$\frac{1}{C} - \frac{1}{C_0} = Kt \quad \dots(4)$$

Where k is the reaction's rate constant, C and C_0 represent the Cfx concentrations (mg/L) following exposure time t and the initial Cfx concentration (mg/L), respectively, and t is the exposure duration (min).

CONCLUSIONS

Zinc oxide synthesis nanocomposite (O-ZnO) was performed. Onion peels extract was carried out for green synthesizing methods, while photocatalytic was selected from (AOP synthesis methods). ZnO without (OPE) was used as a salt precursor. In contrast, onion peel extracts were

Table 5: Reaction rate constants in heterogeneous photocatalysts.

First order		Second order	
K[min^{-1}]	R ²	K[min^{-1}]	R ²
0.0099	0.9682	0.0175	0.9186

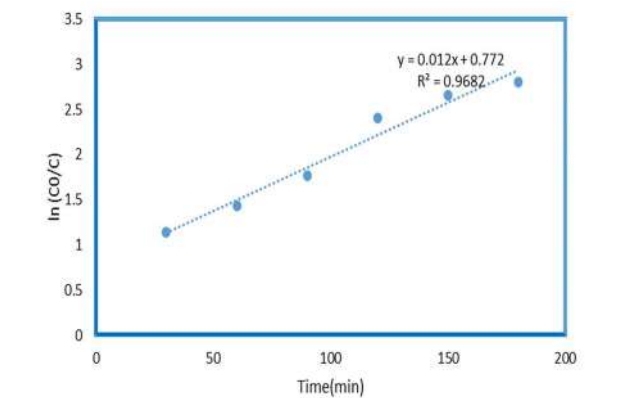


Fig. 13a: Kinetic study (first_order) of Cfx degradation based on optimum conditions.

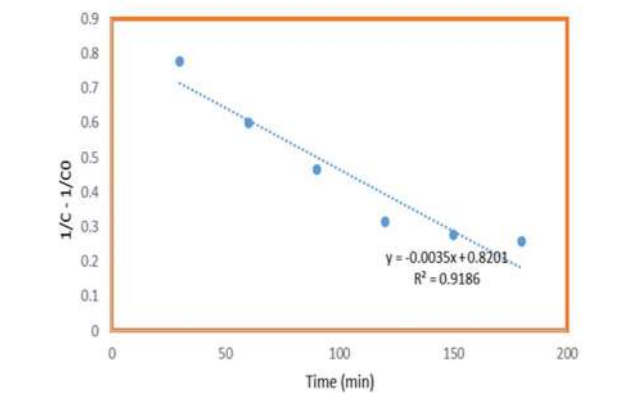


Fig. 13b: Kinetic study(second_order) of Cfx degradation based on optimum conditions.

Table 6: A summary shows a comparison between previous studies on the preparation of ZnO for Cfx removal.

Composite	Pollutant	Efficiency [%]	Reference
(ZnO/GO/DES)	Cfx	86%	(Ciğeroğlu et al. 2022)
SWCNT/ZnO/Fe ₃ O ₄	Cfx	94.19%.	(Erim et al. 2022)
ZnO/Fe ₃ O ₄ @MWCNTs	Cfx	90%	(Paidar et al. 2024)
O-ZnO	Cfx	94%	This Study

utilized as agents for the reduction of green synthesizing methods. (O-ZnO) was characterized by various techniques, including Fourier transform infrared (FT-IR), X-ray diffraction (XRD), scanning electron microscopy (SEM), Brunauer–Emmett–Teller (BET), and Barrett-Joyner-Halenda (BJH). The activities of prepared nanocomposite (O-ZnO) were evaluated on the antibiotic cefixime. The crystal dimensions of the synthesized materials were determined using the Scherrer equation (21.7nm) for green synthesis nanocomposite (O-ZnO). Minor clusters were detected. From FESEM results of prepared (O-ZnO), OPE, and (O-ZnO) after removal (Cfx). Prepared (O-ZnO) showed strong properties. These materials’ morphology showed that the nanocomposite’s size varied between 20–80 nm. In conclusion, this study revealed that 94% of the maximum removal efficiency was achieved at 4 pH and a concentration of 10 mg/L O-ZnO, 120 min, respectively. The green synthesis of O-ZnO nanocomposite displayed appealing photocatalytic efficiency in deleting Cefixime residues from aqueous solutions. The results validated the efficiency of O-ZnO as a catalyst in the UV photocatalysis method for removing Cfx from an aqueous solution. Hence, the (O-ZnO) nanocomposite could be used as a semiconductor photocatalyst and a safe, eco-friendly, low-cost, and effective way to remove organic pollutants from an aqueous solution.

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Assessment of Air Pollution Index (API) Using FAHP and Correlations Between PM₁₀ and Sentinel 5P (TROPOMI) AOD of Jharkhand, India

Suvankar Naskar¹, Sanjib Majumder² and Brototi Biswas^{1†}

¹Department of Geography and RM, Mizoram (Central) University, Aizwal, Mizoram, India

²Department of Geography, North Eastern Hill University (NEHU), Shillong, Meghalaya 793022, India

†Corresponding author: Brototi Biswas; brototibiswas@gmail.com

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ABSTRACT

In the World Air Quality Report 2022, India ranked 8th in terms of average PM_{2.5} concentration globally with a value of 53.3 µg/m³, and a number of the 7 most polluted cities are located in India out of 50 top cities. The presence of poor air quality in mining cities has a positive relationship with mining activities, and this scenario persists in different cities of Jharkhand state. In this study, we draw the API (Air Pollution Index) with the help of the fuzzy analytical hierarchy process (FAHP) across the state of Jharkhand by considering parameters such as CO, O₃, NO₂, SO₂, and PM₁₀ and the relationship between Sentinel 5P aerosol optical depth (AOD) and CPCB published ground data of PM₁₀ (i.e., monthly and seasonal) was also explored. The outcome depicts that a high concentration of API is dominant along the north-eastern part of the state due to the intensive mining activity along this part of the state, and the trend of concentration of PM₁₀ in the air is continuously increasing from 2012-2018 as per the GOJ (Government of Jharkhand) report. This study will give insight into the pollution scenario in the mining-dominated state of Jharkhand, and along with that, it will also spread awareness of the impact of mining activities on the atmosphere.

INTRODUCTION

In recent decades, the global environment has undergone rapid changes in the form of climate, flora, fauna, urban morphology, and various anthropological changes (Hase et al. 2021). Several factors have influenced and hastened these global changes, including global warming (Change 2018), deforestation (Ritchie & Roser 2021), unplanned urban growth (Patra et al. 2022), rapid population increase (Ray & Ray 2011), and, most notably, the deterioration of air quality in developing countries (Foster & Kumar 2011). Surprisingly, despite being heavily polluted in the past, most developed countries have already reduced their air pollution levels and are now implementing norms and regulations for developing countries to reduce the emission of air pollutants (Tao & Hou 2023). According to the World Health Organization (WHO), the consolidated impact of household air pollution and ambient air pollution causes approximately 6.7 million premature deaths globally each year (WHO 2022). WHO considered the concentration of PM_{2.5} as the most significant determinant of air pollution (WPR 2023).

According to the World Air Quality Report 2022, India ranked 8th in terms of average PM_{2.5} concentration globally, with a value of 53.3 µg.m⁻³, and Bhiwandi (92.7 µg.m⁻³) was the top-ranked PM_{2.5} concentrated city in India (IQAir 2022). Delhi is ranked as the 4th most polluted city globally out of 50 cities, and a number of the 7 most polluted cities are present in India, according to a 2018 Greenpeace South Asia report (Greenpeace 2018). India ranked 2nd in terms of coal production after China in 2022, and in India, Jharkhand accounts for almost 86500 million metric tons of coal reserves, making it 2nd after Odisha (88000 million metric tons)

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(MoC 2022). Jharkhand state shares a decent percentage of other mineral production in India, such as iron ore (10%), bauxite (8%), kyanite (31%), and copper ore (1%) (MoM Annual Report 2023).

Environmental issues are very common in the mining sector, and the presence of poor air quality has a positive relationship with mining activities because it requires drilling, blasting, mine hot spots, mineral recovery, acid effluents, underground mines, and poor ventilation (Chaulya 2004, Huertas et al. 2012, Asif & Chen 2016, Asif et al. 2018). Health issues due to short-term and long-term exposure to mining activities-induced air pollution are many. It can cause simple breath difficulties to permanently damage the respiratory system, which can lead to death also (Patra et al. 2016, Nayak & Chowdhry 2018, Tian et al. 2019, Manisalidis et al. 2020, Hendryx et al. 2020). Moreover, mining activities and air pollution also affect the environment through acid rain, haze, ground-level ozone concentration, and global climate change (Ghose & Majee 2000, Fugiel et al. 2017, Manisalidis et al. 2020). Other than these factors, the proximity of railways and roads to mining areas induces air pollution (Dubey et al. 2012). Air quality issues related to mining sectors are primarily focused on particulate matter, including $PM_{2.5}$, PM_{10} , and dust deposition (Schwegler 2006, Dong et al. 2009, Moreno et al. 2019). Bui et al. (2019) applied artificial intelligence techniques such as “support vector regression (SVR) and particle swarm optimization (PSO)” to estimate and control PM_{10} concentrations influenced by mining activities to mitigate health risks caused by poor air quality. Bada et al. (2013) have used descriptive statistics, correlation, and Duncan multiple range (DMR) tests to demonstrate significant $PM_{2.5}$ ($0.130 \pm 0.010 \text{ mg.m}^{-3}$) and PM_{10} ($0.231 \pm 0.018 \text{ mg.m}^{-3}$) concentrations on the drilling and crushing site in the vicinity of the quarry, and these suspended particles are inversely correlated with the distance from the quarry site. Aside from these, Prusty attempted to monitor ambient air quality in terms of NO_2 , SO_2 , RPM, SPM, and CO in the Kachchh region of Gujarat, India. (Prusty 2012). In recent years, the application of geospatial technology in the field of air quality monitoring has taken a spike, and nowadays, a majority of researchers are applying this technology and associated techniques to measure the concentration of different indices (i.e., particulate matter, SO_2 , NO_2 , O_3 , and CO) of air quality in mining areas of India as well (Patra et al. 2021). Amar et al. (2021) proposed using a Python data science tool (i.e., pandas, tkinter, and matplotlib) to generate an exception report on the state of air quality over coal mines in Maharashtra, India. Another study conducted by Choudhary & Kumar (2021) used time series analysis methods such as moving average (MA), standard error (SE), and root mean square error (RMSE) to measure

the concentration of AOD at coalfield sites in India. Apart from these, various studies have used different statistical, data science, geospatial, machine learning, and AI techniques such as principal component analysis (PCA) (Pandey et al. 2014), Poisson regression (Nayak & Chowdhury 2018), linear regression (Hendryx et al. 2019), analytical hierarchy process (AHP) (Chen et al. 2007), fuzzy logic (Katushabe et al. 2021), ANN & Random Forest (Kang et al. 2018), dynamic neural network (Zhu et al. 2018), SOM algorithm, and deep learning (Bougoudis et al. 2016) to analyze air quality in the atmosphere on different scales.

The usage of satellite data (i.e., MODIS) and ground data (i.e., AERONET) to measure the AOD always differs (Endale et al. 2024); thus, Soni et al. (2016) conducted a comparative study on these two datasets to overcome the error in the Indo-Gangetic Plains. A long-term (over 20 years) AOD observation over India and the Indian Ocean using AATSR, MODIS, and MERRA-2 data exhibits higher and lower AOD concentrations over the Indo-Gangetic Plains and the Indian Ocean, respectively (Kuttiiputah & Raj 2021). The dust deposition rate varies from the summer season (7.51 to $28.58 \text{ g.m}^{-3}.\text{month}^{-1}$) to the winter season (7.40 to $26.37 \text{ g.m}^{-3}.\text{month}^{-1}$) and varies from iron mine areas to copper mine areas in Singbhum district, India (Mahato & Singh 2020). Saini et al. (2016) attempted to assess the “Environmental Impact Assessment (EIA)” by applying AHP in the Jharia coalfield, Jharkhand, and the results identified air as the most affected parameter in the area. Spatio-temporal variation of air pollutants in the Jharia Coalfield disclosed that the air quality is 1.5 times higher in coal mine fire areas compared to non-mine fire areas (Mondal et al. 2020). Mondal & Singh (2021) investigated the level of air pollution and associated health risks in the Dhanbad coal belt region by taking into account the presence of trace elements (i.e., Fe, Mn, Cr, Cd, Ni, Zn, and Pb), and the results show that Cr, Mn, and Fe pose a higher health risk potentiality.

It is evident from the existing literature that over the years, many studies have been conducted to explore different dimensions of air quality in the Jharkhand state (Kundu & Pal 2018, Chaulya et al. 2019, Halder et al. 2024, Mishra et al. 2024); however, few of the previous studies have calculated the air quality index with the help of an MCDM technique. Additionally, very few studies have been done on AOD in the Jharkhand state while focusing on mining activities (Soni et al. 2015, Ranjan et al. 2020, Bandyopadhyay et al. 2021, Mohammad et al. 2022). This study primarily focused on the assessment of CO , O_3 , NO_2 , SO_2 , PM_{10} , and AOD air quality indicators to determine the variation and trend of particulate matter in the atmosphere over mining areas in Jharkhand state with the help of the Fuzzy-AHP method.

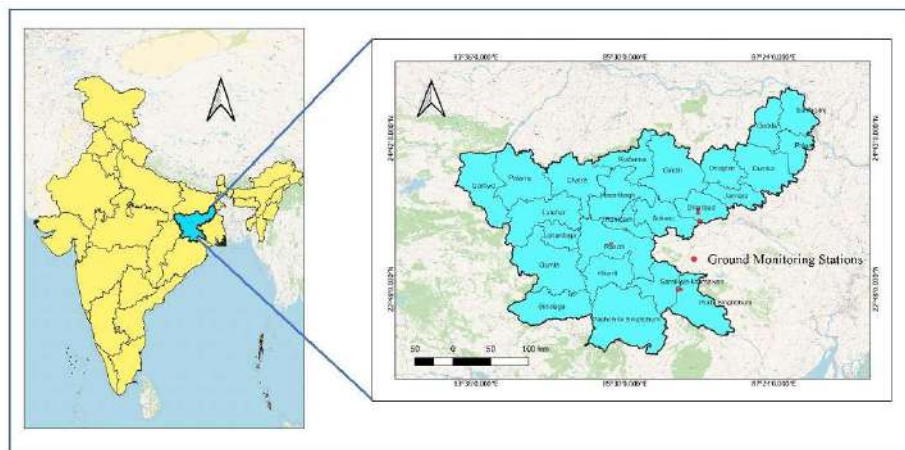


Fig. 1: Study area map.

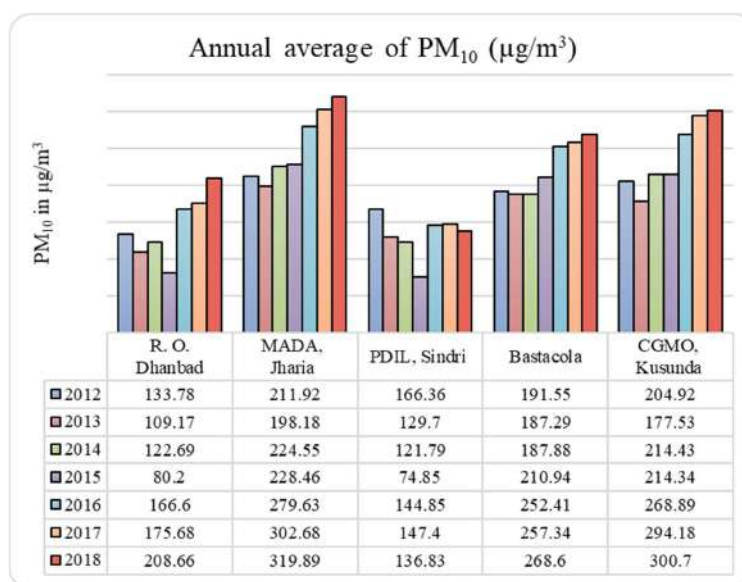
This paper has also pointed out the correlation of Sentinel AOD with ground station observed PM_{10} data and the API of the selected parameter in Jharkhand.

MATERIALS AND METHODS

Study Area

Jharkhand is situated in eastern India and is considered one of the most significant states in India in terms of mineral reserves. Geographically, the GPS coordinates of the Jharkhand state fall between $83^{\circ}20'00''$ E to $87^{\circ}50'00''$ E and $25^{\circ}20'00''$ N to $22^{\circ}05'00''$ N (Fig. 1). Jharkhand is a state that is famous for its dense forest, mineral resources (i.e., coal, iron ore, bauxite, copper, and kyanite), and relief. This is

why Jharkhand is often referred to as the “Land of Minerals and Forests”. Some of the notable rivers that pass through Jharkhand are Damodar, Subarnarekha, Brahmani, Barakar, Sankh, Ajay, Sone and Mayurakhshi. The highest elevation (1376 m) point is located in the southern region (Chota Nagpur Plateau), and on the contrary, the lowest elevation point is located along the banks of rivers (e.g., Damodar River). Jharkhand state covers an area of around 79700 km^2 , which consists of a population of about 32966238, according to the Census of India 2011 (CoI 2011). Another thing that is significant is its major diversity in tribal communities (32) that include Birhor, Korwa, Lohra, Mahli, Santhal, Oraon, Munda, Sauria Paharia, and Ho tribes with a total population of 8645042. This study has been initiated to assess the air

Fig. 2: Annual average of PM_{10} concentration ($\mu\text{g.m}^{-3}$) in Dhanbad district (GoJ 2018).

pollution in Jharkhand state by applying a multi-criteria decision model (MCDM) in order to explore the growing issue of air pollution in the state.

Fig. 2 presents the annual average of PM_{10} concentration ($\mu g.m^{-3}$) in Dhanbad district from 2012 to 2018. Most of the monitoring stations around the Dhanbad district showed a positive trend in $PM_{10}(\mu g.m^{-3})$ concentration in the atmosphere between 2012 and 2018, except for PDIL, Sindri station, as it presents the minimum variation in the concentration of PM_{10} particles.

Data Source

In this study, the extraction of CO , O_3 , NO_2 , SO_2 , PM_{10} , and AOD (aerosol optical depth) data was carried out using satellite data (i.e., Sentinel-5P), and Daily average PM_{10} data was acquired from ground station data available from CPCB of the Indian Government (Table 1). This study was conducted by following the methodological framework illustrated in Fig. 3.

Selected Parameters of Air Pollution

Air pollution is determined based on the concentration of different suspended pollutants in the atmosphere that can adversely affect human health, depending on the

population's vulnerability (e.g., time of exposure and pollution level). WHO considered the concentration of $PM_{2.5}$ as the most significant determinant of air pollution (WPR 2023). Dust particle concentrations are relatively high in Jharkhand mining areas, particularly in coal fields (e.g., Jharia, Dhanbad), iron ore, and bauxite quarry areas due to activities such as crushing, drilling, blasting, and open-cast mining. In this study, the researcher has selected five key elements to assess the air pollution in the Jharkhand state: carbon monoxide (CO), ozone at ground level (O_3), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and smaller than $10 \mu m$ suspended particulate matter (PM_{10}). The Central Pollution Control Board (CPCB) of the Government of India emphasizes calculating air quality (AQ) by incorporating at least three pollutant elements, one of which must be particulate matter (either $PM_{2.5}$ or PM_{10}). However, this study focuses on assessing air pollution (AP) using an alternative method that incorporates FAHP while taking all five pollutants (i.e., CO , O_3 , NO_2 , SO_2 , and PM_{10}) into account as criteria. Data concerning all five parameters of AP were obtained from satellite data and monitoring stations combined. In the AP assessment, all the different parameters have different pollution potentials, and the actual damage (e.g., health risks) caused by the parameters is perfectly known through this process as well.

Table 1: Data used in this study.

Data	Acquisition time	Source	Agency
Political boundary map	August, 2023	https://onlinemaps.surveyofindia.gov.in/Home.aspx	Survey of India
Sentinel-5P	August, 2023	https://code.earthengine.google.com/?scriptPath=Examples:Datasets/COPERNICUS/COPERNICUS_SSP_NRTI_L3_AER_AI	European Union/ESA/Copernicus (Downloaded from Earth Engine)
Daily Avg. PM_{10}	August, 2023	https://cpcb.nic.in/index.php	Central Pollution Control Board (CPCB), Government of India

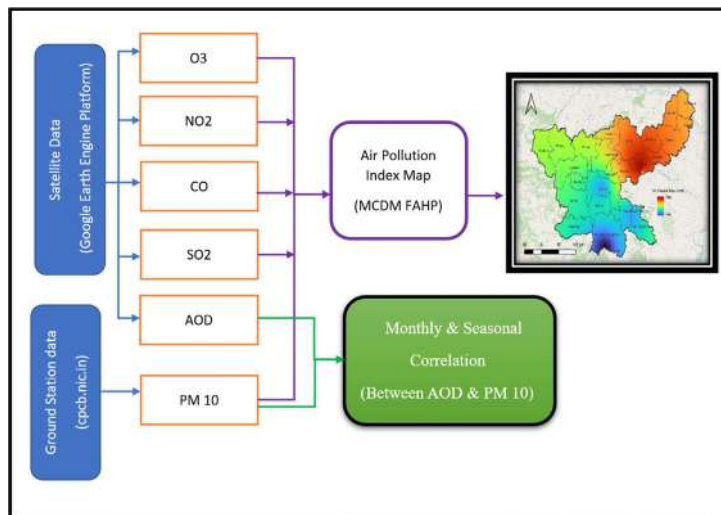


Fig. 3: Methodological framework followed in this study.

The concentration of CO gas added from fuel-burning activities, especially wood, propane, charcoal, etc., burning, and its high concentration leads to the replacement of red blood cells in the body to CO, which can cause serious damage to the tissue and even death. O₃ is a pale blue gas that smells pungent, and its intake in higher concentrations leads to abnormalities in lung function, asthma attack, which sometimes causes death, and respiratory dysfunction such as throat irritation, pain sometimes burning in the chest. Similar health issues are also seen due to taking a high concentration of NO₂, and researchers claimed more than 200 µg/m³ can cause significant health issues (Pandey et al. 2021). SO₂ is emitted especially from industries and irritates the skin, nose, throat, and lungs. Particulate matter (PM) carries a large number of toxic substances, and by inhaling, it directly accumulates in the lungs. The region belongs to coal mining, and open-cast coal mining is practiced in the region, which highly contributes to PM, and thus, the study gives much significance (Temesi et al. 2003).

Fuzzy AHP

Generally, the analytical hierarchy process (AHP) is a multi-criteria decision analysis (MCDA) technique that considers different criteria organized hierarchically. The hierarchical organization of the criteria in this technique is determined by the relative importance of the criteria themselves, and the relative importance is given on the basis of an AHP scale. This AHP scale was first proposed by Saaty, and the scale value is determined through experience and knowledge of the respective field (Saaty 1977). Because real-world problems and scenarios are so complex, AHP techniques fall short due to their simplified scale and less critical approaches (Darko et al. 2019). Thus, the Fuzzy AHP comes into the scenario to overcome those drawbacks and tries to solve real-life problems with complex and critical introspection. In FAHP, instead of only using AHP crisp values, fuzzy crisp values are assigned to each of the criteria to organize them hierarchically. The FAHP is used with the help of a linguistic statement that compares two criteria and is expressed on a Fuzzy Comparison Ratio Scale. In this scale, each of the membership functions is composed of three possible fuzzy

values in a triangular fuzzy number (TFN), namely, smallest (*l*), most appropriate (*m*), and largest (*u*) (Table 2). These values are as follows:

The implementation of F-AHP in this study has passed through various steps, and these steps are mentioned in the following paragraphs.

Step 1: Construction of pairwise comparison matrix (PCM) (Buckley 1985)

In this section, first, all the criteria in the study are allocated linguistic terms in the PCM based on the question of which is the more influential or important of each two criteria, and according to their linguistic terms, TFN and reciprocal TFN are assigned in the PCM, as following matrix $\tilde{C}$ and Table 3 present the application of this matrix $\tilde{C}$.

$$\tilde{C} = \begin{bmatrix} 1 & \tilde{a}_{12} & \dots & \dots & \tilde{a}_{1n} & \tilde{a}_{21} & 1 & \dots & \dots & \tilde{a}_{2n} & \vdots & \dots & 1 \\ & \dots & \vdots & \dots & \dots & \vdots & \tilde{a}_{n1} & \tilde{a}_{n2} & \dots & \dots & 1 & \dots & \dots \\ 1 & \tilde{a}_{12} & \dots & \dots & \tilde{a}_{1n} & \frac{1}{\tilde{a}_{12}} & 1 & \dots & \dots & \tilde{a}_{2n} & \vdots & \dots & 1 \\ & \dots & \vdots & \dots & \dots & \vdots & \frac{1}{\tilde{a}_{1n}} & \frac{1}{\tilde{a}_{2n}} & \dots & \dots & 1 & \dots & \dots \end{bmatrix} \dots (1)$$

Step 2: Calculating the geometric mean of PCM using a geometric mean technique for each criterion (i.e., 5) involved in the study (Hsieh et al. 2004)

There are multiple studies available to obtain FAHP weights from PCM. It includes the geometric mean (GM) method (Buckley 1985), a programming-based linear method (Wang & Chin 2008), a nonlinear method based on programming (Mikhailov 2003), a least square method using logarithms (Wang et al. 2006), extended method (Chang 1996), and synthetic extend method (Murat et al. 2015). However, in this study, the researcher has opted for the GM method to calculate the crisp weights for all the criteria (Table 4).

$$\tilde{r}_i = [\tilde{a}_{i1} \otimes \dots \otimes \tilde{a}_{ij} \otimes \dots \otimes \tilde{a}_{in}]^{\frac{1}{n}} \dots (2)$$

This Where, $\tilde{a}_{ij} = i$ dimension fuzzy comparison value of j criteria, $\tilde{r}_i$ = geometric mean of PCM of criteria for each.

Step 3: Calculating respective crisp weights of each criterion (Table 5) involved in this study (Ayhan 2013)

$$\tilde{w}_i = \tilde{r}_i \otimes [\tilde{r}_1 \oplus \dots \oplus \tilde{r}_i \oplus \dots \oplus \tilde{r}_n]^{-1} \dots (3)$$

Where, $\tilde{w}_i$ fuzzy weight of th criterion.

Step 4: Defuzzification and normalization of crisp weights

After the calculation of respective crisp weights of all the criteria, it is de-fuzzified (Table 6) by a technique called "Centre of area method" and normalized using equation 5 (Chou & Chang 2008).

Table 2: Linguistic Variables of FAHP (Chang 1996, Sun 2010, Tripathi et al. 2021).

Linguistics	TFN	Reciprocal TFN
Absolute/Perfect	(9, 9, 9)	(1/9, 1/9, 1/9)
Very Good	(7, 8, 9)	(1/9, 1/8, 1/7)
Good	(4, 5, 6)	(1/6, 1/5, 1/4)
Appropriate	(3, 4, 5)	(1/5, 1/4, 1/3)
Equal	(1, 1, 1)	(1, 1, 1)

$$M_i = \frac{l_{w_i} + m_{w_i} + u_{w_i}}{3} \quad \dots(4)$$

Where, M_i = de-fuzzified number and l_{w_i} , m_{w_i} , u_{w_i} Refers to the TFN numbers and their respective weights in a fuzzy scale.

$$N_i = \frac{M_i}{\sum_{i=1}^n M_i} \quad \dots(5)$$

Where, N_i = normalized number derived from a defuzzified number.

In the very last step of the process, the de-fuzzified weights of all the selected criteria are used to calculate the air pollution index (API) value. It is computed by multiplying each criterion by its respective weights and finally by the summation of all the values.

$$API = (CO * WCO) + (O_3 * WO_3) + (NO_2 * WNO_2) + (SO_2 * WSO_2) + (PM_{10} * WPM_{10}) \quad \dots(6)$$

There have been numerous studies that have used the FAHP method to solve problems from various knowledge domains and have attempted to assist in risk prediction (Pan 2008, Shaverdi et al. 2014, Mosadeghi et al. 2015, Chou et al. 2019, Liu et al. 2020, Bakir & Atalik 2021, Tripathi et al. 2021, Majumder & Fatma 2024).

RESULTS AND DISCUSSION

Assessment Using the FAHP Model

The fuzzy AHP model is a very compact method for finding a solution to a complex problem using multi-criteria, and it is also applicable for API assessment. In this study, the researcher has applied fuzzy AHP to create an air pollution index by integrating five air pollution elements as criteria to identify air pollution levels across the state of Jharkhand. Fuzzy AHP takes expert opinion, advice, and knowledge into consideration to select the criteria of a complex problem and determine the linguistic terms and their respective TFN values of the criteria based on experience and expertise.

Calculation of Pairwise Comparison Matrix (PCM)

The selection of linguistics and their respective TFN values

are assigned to the criteria based on comparing each two criteria at a time and determining the importance of one criterion over another and their respective reciprocal values. This procedure aided the researcher in identifying the most important parameter (i.e., O_3) as well as the least essential parameter (i.e., SO_2) in the calculation of API (Table 3). The PCM was implanted by following equation 1.

Geometric Mean Calculation Of Fuzzy PCM

After calculating the pairwise comparison matrix (PCM) following equation 1 mentioned in the methodology section, the researcher has calculated the geometric mean using equation 2, which is presented in Table 4. The geometric mean is calculated for each criterion from PCM and arrayed hierarchically according to their fuzzy number. Additionally, the total values and their respective reciprocal values are mentioned.

Table 5 shows the relative fuzzy weights (W_i) of air pollution parameters obtained using equation 3, and the average fuzzy weights (M_i) calculated by adding the triangular fuzzy weights (TFW) and dividing them by three using equation 4 (Table 6). Equation 5 is used to compute the normalized non-fuzzy weights (N_i) of each parameter.

Assessment of Air Pollution Index (API)

Jharkhand has a literacy rate of 66.41%, is ranked 34th in the HDI report of 2019, is ranked 26th in per capita income, is ranked 2nd in the Poverty report of 2021, is ranked 19th in GDP, and is ranked 27th in India's sustainable development index. These facts describe the multifaceted challenges that the state is currently experiencing, and the overexploitation of natural resources adds to the misery of this current scenario. Jharkhand is a state with a large reserve of natural resources; however, over-exploitation of these natural resources (e.g., coal, iron ore, bauxite, kyanite, and copper) has caused plenty of challenges in various dimensions, the most concerning of which include water pollution and air pollution.

The API is estimated in this study using five air pollution determinants (CO , O_3 , NO_2 , SO_2 , and PM_{10}). The distribution and fluctuation of these five air pollutants in the Jharkhand

Table 3: Pair-wise comparisons of air pollution parameters.

Parameter	O_3	NO_2	CO	PM_{10}	SO_2
O_3	(1,1,1)	(3,4,5)	(4,5,6)	(7,8,9)	(9,9,9)
NO_2	(1/5,1/4,1/3)	(1,1,1)	(3,4,5)	(4,5,6)	(7,8,9)
CO	(1/6,1/5,1/4)	(1/5,1/4,1/3)	(1,1,1)	(3,4,5)	(4,5,6)
PM_{10}	(1/9,1/8,1/7)	(1/6,1/5,1/4)	(1/5,1/4,1/3)	(1,1,1)	(3,4,5)
SO_2	(1/9,1/9,1/9)	(1/9,1/8,1/7)	(1/6,1/5,1/4)	(1/5,1/4,1/3)	(1,1,1)

Table 4: The geometric mean of fuzzy PCM.

Parameter	Fuzzy Geometric Mean values		
O ₃	3.76447	4.28225	4.75468
NO ₂	3.36	2.09128	2.459509
CO	0.83255	1	1.201124
PM ₁₀	0.40659	0.47818	0.568772
SO ₂	0.21032	0.00014	0.265641
$\Sigma \tilde{r}_i$	8.57393	7.85185	9.249727
$(\Sigma \tilde{r}_i)^{-1}$	0.11663	0.12736	0.108111

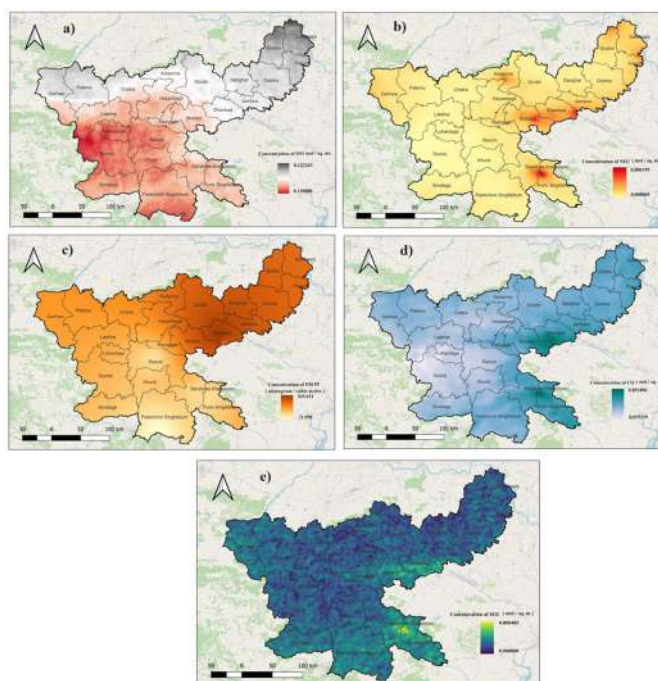
Table 5: Relative fuzzy weights of parameters.

Parameter	Fuzzy weights [$\tilde{W}_i$]		
O ₃	0.43906	0.54538	0.51403
NO ₂	0.39189	0.26634	0.2659
CO	0.0971	0.12736	0.12986
PM ₁₀	0.04742	0.0609	0.06149
SO ₂	0.02453	1.8E-05	0.02872

Table 6: Average weights of parameters.

Parameter	Weights [M _i]
O ₃	0.499
NO ₂	0.308
CO	0.118
PM ₁₀	0.057
SO ₂	0.018

district are depicted in Fig. 4. In the O₃ map, Sahibganj district has a very high concentration (0.123223 mol.m⁻²) of ground-level ozone (GLO), whereas the western parts of Latehar and Gumia districts have low concentrations (0.120968 and 0.120939 mol.m⁻²) of GLO in comparison to the rest of the state. Aside from these locations, other districts with a high concentration of GLO include Dhanbad, Giridih, and Deoghar. Select areas of Dhanbad, Bokaro, Kodarma, and Purbi Singhbhum have higher NO₂ concentrations (0.000159, 0.000169, 0.000139, and 0.000178 mol.m⁻²) than other districts since these areas have a high concentration of urban habitation and transportation mediums. The remainder of the districts, on the other hand, have an average concentration of NO₂. Dhanbad, Giridih, Bokaro, Jamtara, and Deoghar districts have quite high PM₁₀ concentrations (242.52, 221.89, 229.23, 225.30, and 228.10 µg.m⁻³) due to the position of mineral quarries, coalfields, or their near proximity to mining operations. Ranchi, Paschimi Singhbhum, and Kunti have relatively low PM₁₀ values in the atmosphere (114.90, 90.81, and 127.07 µg.m⁻³). Some areas of Bokaro, Dhanbad, Purbi Singhbhum, and Saraikela-Kharsawan districts possess elevated concentrations of CO (0.049906, 0.051123, 0.050991, and 0.047872 mol.m⁻²), while others, such as Gumia, Latehar, and Lohardaga districts, have low concentrations (0.036493, 0.036152, and 0.035781 mol.m⁻²). In the case of the SO₂ concentration map, Purbi Singhbhum, Saraikela-Kharsawan, Dhanbad, and Bokaro show comparatively significant concentrations

Fig. 4: Concentration of air pollutants across the districts of Jharkhand, (a) O₃, (b)NO₂, (c)CO, (d) PM₁₀ and (e) SO₂.

(0.000440, 0.000371, 0.000299, and 0.000299 $\text{mol}\cdot\text{L}\cdot\text{m}^{-2}$) of SO_2 in the atmosphere due to massive vehicular emissions in the area, and other districts show an average to low concentration of SO_2 in the atmosphere.

Fig. 5 shows the air pollution index (API) map by applying the FAHP technique and following the procedure referred to in Fig. 3. According to the analyzed index map of air pollution, places such as Dhanbad, Jharia, Giridih, Deoghar, and the eastern side of Bokaro district are situated on the highest and higher side of API value (14.40, 14.90, 12.51m and 13.07) and these places has very poor quality as well and the report published by CPCB also states that in December and January month, these places had very poor (315) and poor (201) air quality, respectively (CPCB 2020). On another side, Ranchi, Jamshedpur, Saraikela, and Barajamda have low API values (7.54, 6.27, 7.17, and 4.19) and fall under low health risk, and the report published by CPCB also confirms the finding by providing data for the December (102, 130, 136, and 136; moderate) and January (106, 135, 125, and 82; moderate to satisfactory), respectively (CPCB 2020). From the overall point of view, all 5 selected pollutants' concentrations are highest in the northeastern part of the state, and the southern part of the state shows the lowest concentration of the selected pollutants. Jharia coalfield, Bokaro Coal mine, and Koderma mica mine are located in the northeastern zone of Jharkhand, and their associated polluting factors contribute to such high concentration in Jharkhand. Noamundi Iron ore mine, Ghatsila Copper Mine, Chaibasa Bauxite mine, and Uranium mine of Jamshedpur and Jadugora are located in

the southeastern part of the state, though we observe from our mapping that air pollution contribution is comparatively less than the northern mines. Although SO_2 , CO, and NO_2 pollutants have high concentrations in the allocated southern part of the mining regions, the PM_{10} concentration is lower in the southern part; thus, it can be concluded that the respective mines take appropriate measures to reduce the concentration of PM_{10} .

Aerosol concentration had been studied, and the result depicts that high aerosols were observed in the north and eastern parts of Jharkhand, while low aerosols were observed along the south-western part of the state, which is similar to this study (Bandyopadhyay et al. 2021). In another research, it is observed that the "Air Quality Health Index" is much more critical in the southwestern part of the study (Panda et al. 2019).

Relationship Between TROPOMI AOD and PM_{10} Concentration

Fig. 6 presents the relationship between a selected parameter of API (i.e., PM_{10}) and TROPOMI AOD for different months in a year. According to the relationship, it is evident that the PM_{10} holds a significant association with TROPOMI AOD during average monthly data from January to June, with R^2 values of 0.0625, 0.1243, and 0.2858, respectively. Here, R^2 is the "coefficient of determination," and its value always falls between 0 and 1. R^2 is applied to measure the extent of the dependent variable that can be explained by the independent variable in a relationship.

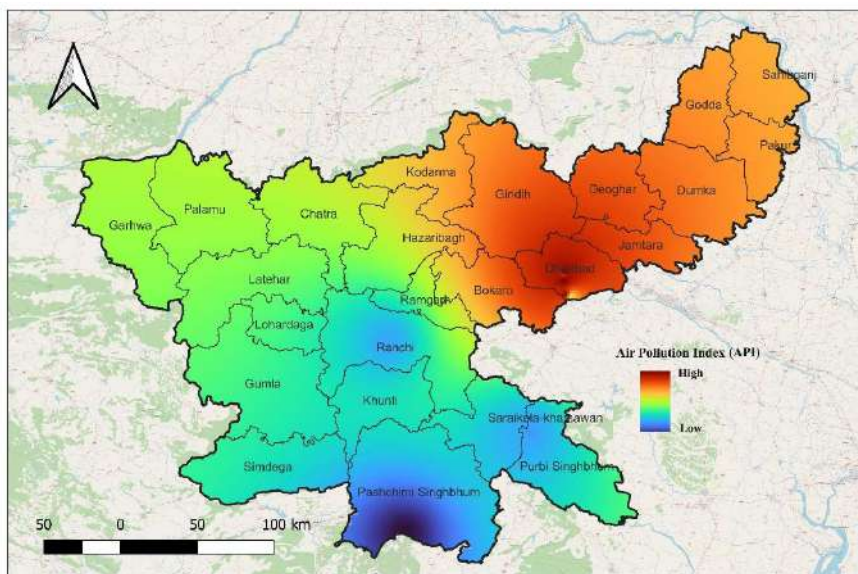


Fig. 5: Air pollution index (API) map of Jharkhand state.

Fig. 7 shows the relationship between PM_{10} and TROPOMI AOD from July to December, and among these

six months, October and November ($R^2 = 0.176$ and 0.0299) signify a good relationship between PM_{10} and TROPOMI

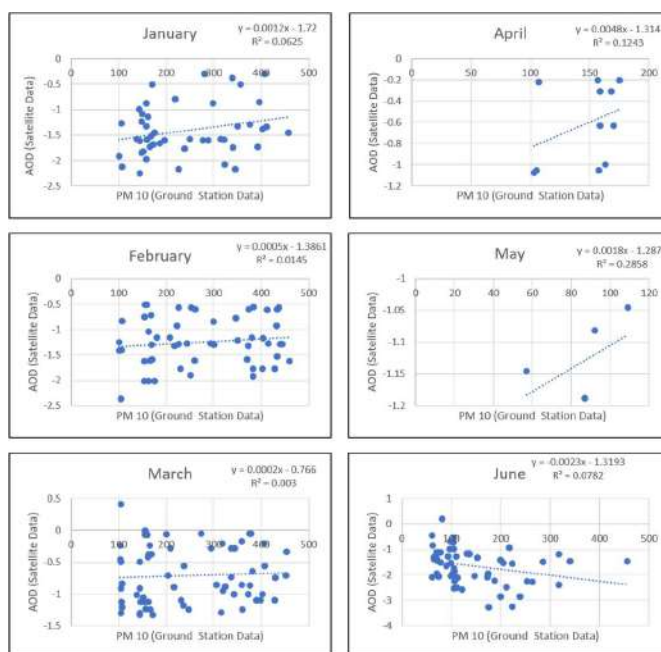


Fig. 6: Correlation between AOD and PM_{10} from January to June.

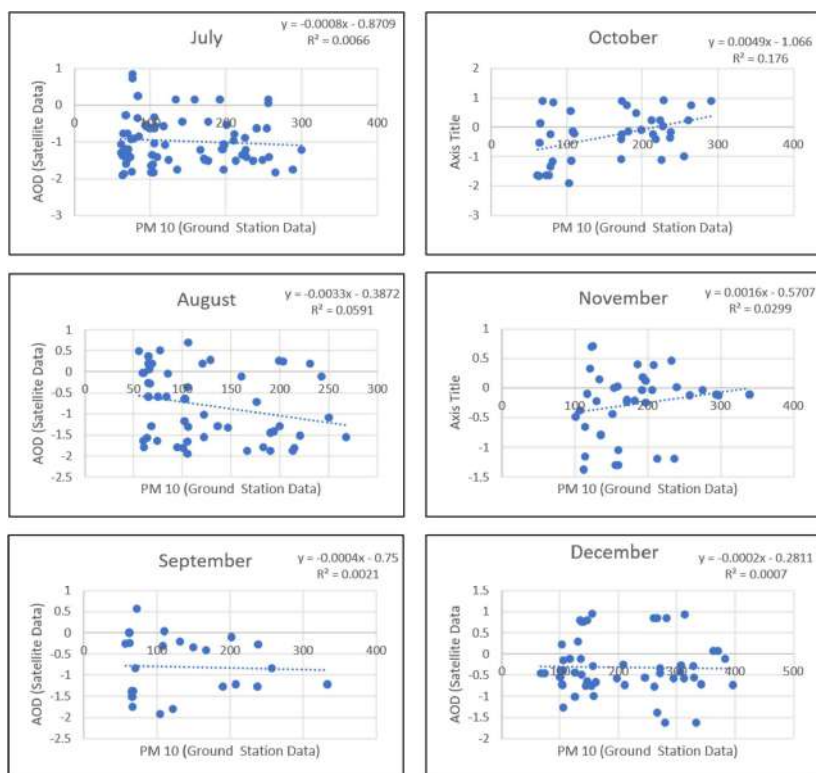


Fig. 7: Correlation between AOD and PM_{10} from July to December.

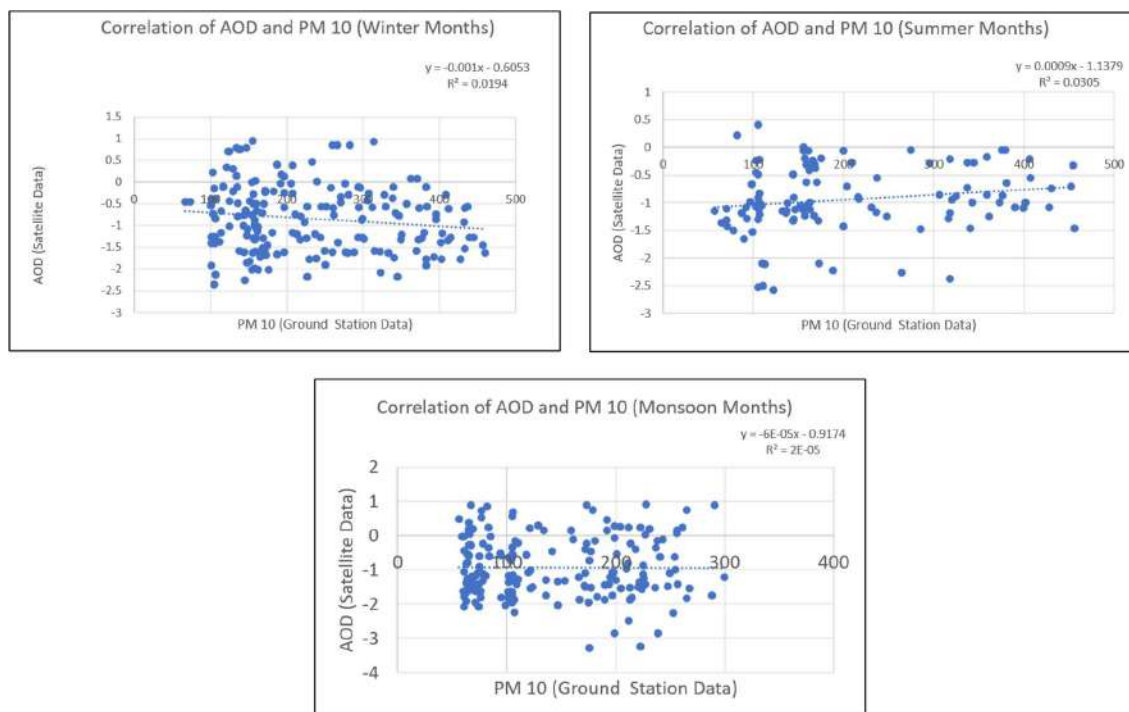


Fig. 8: Seasonal correlation between AOD and PM₁₀.

AOD. In January and February, the concentration of PM₁₀ particles in the atmosphere stays very high to the extent that it reaches a maximum value of approximately $450 \mu\text{g.m}^{-3}$. With increasing PM₁₀, the AOD in the atmosphere of Jharkhand state increases as well. In March, the association between PM₁₀ and TROPOMI AOD is very low, and more or less it stays neutral. April and May do provide a very good positive relationship ($R^2 = 0.1243$ and 0.2858); however, the availability of PM₁₀ data from the ground station is nearly none; hence, the small number of samples cannot explain the actual scenario of the relationship. In June, July, and August, the association between PM₁₀ and TROPOMI AOD is weakly negative (i.e., $R^2 = 0.0782$, 0.0066 , and 0.05961), which signifies the anomaly between the satellite AOD data and ground station data. September and December show no significant association ($R^2 = 0.0021$ and 0.0007) between PM₁₀ and TROPOMI AOD to the point where it can be considered as no association. Additionally, October and November show a small positive trend in the relationship between the two variables.

Fig. 8 shows the seasonal concentration of AOD and PM₁₀ and presents their relationship as well. For the winter season, the trend line shows a weak negative relationship ($R^2 = 0.0194$) between the two variables in the Jharkhand district. However, during the summer season, the concentration of both TROPOMI AOD and PM₁₀ in the atmosphere slightly

increases ($R^2 = 0.0305$), and in the monsoon season, it stays neutral, explaining no significant association and variance between the two variables.

There are some significant factors, temperature, relative humidity, the blowing direction of winds, as well as wind speed, influencing the correlation of AOD and PM₁₀, which might be responsible for weak correlations (Stirnberg et al. 2018, Kong et al. 2016). Low wind speeds show strong agreement between AOD and PM₁₀ and vice versa. The researchers have not incorporated the temperature and relative humidity in this study, which are limitations of this study; apart from that, the satellite data accuracy is another limitation of the study. PM_{2.5} data must also be incorporated and correlated with AOD, but due to the lack of availability of PM_{2.5} data, the researcher fails to incorporate it.

Being the region is mined, industries dominate the region is likely to be prone to air pollution; there is a need to check the pollution by incorporating appropriate planning strategies. Apart from the pollution measures adopted by management companies, local authorities, the pollution control board of the state government, and the central government of India should incorporate appropriate measures to mitigate or reduce the vulnerability of the health risk of the residents in sensitive areas. Some suggestive measures could be incorporated. SPM concentration could be reduced by imposing restrictions on the overloading trucks, regularly

cleaning roads, regularly spraying water using effective advanced sprinkling methods, regularly maintaining and repairing roads, and applying binding agents on unpaved roads.

Greenbelts must be constructed around the mining areas, the industries, and other sensitive polluting sources. "Greenbelt" is a model of a mass of pollutant-tolerant tree plantation incorporated to mitigate air pollution by absorbing, intercepting, and filtering pollutants, which was developed by Kapoor & Gupta (1984), incorporating distance from the pollution sources, height, and width of the green belts. *Butea monosperma*, *Ficus infectoria*, and *Anthocephalus cadamba* are some suggested plant species that can be planted in greenbelts to reduce SPM pollution (Chaulya et al. 2003).

CONCLUSIONS

In this study, the amount of air pollution in the Indian state of Jharkhand, a region known for its natural resource mining, was examined using a relatively new methodology for air pollution assessment. The goal of this study is to address the issue of elevated levels of particulate matter (PM_{10}) and $PM_{2.5}$ in the air, which are caused by dust deposition from nearby mining operations. Data on the five main air pollution parameters (CO , O_3 , NO_2 , SO_2 , and PM_{10}) were gathered, and API was computed using the combination of the five parameters in order to create a more thorough analysis of air pollution. The API presents a usually reliable evaluation of Jharkhand's air pollution and incorporates all the significant factors that contribute to it in the study area. Researchers can identify areas with high air pollution levels and associated threats to human health in the area with the aid of the air pollution evaluation. The API (i.e., using FAHP) showed a larger percentage of weight in O_3 and NO_2 pollutants in the mining pockets dispersed around the state, despite PM_{10} being the most problematic pollutant in the state of Jharkhand. Because mining activity is concentrated in the northeastern region of the state, the results of the API show that the region has a high concentration of API and a low concentration in the southern region. The association between TROPOMI AOD and PM_{10} concentration on a monthly and seasonal basis is also shown in the data. The concentration of PM_{10} particles in the atmosphere and the TROPOMI AOD present a mild to moderately positive association, and in contrast, a negative correlation was observed for June, August, December, and the winter season. This study used a novel approach to evaluate air pollution in Jharkhand by taking into account the assessment of AOD in relation to other air pollutants. The findings of this study can be utilized in various fields to promote sustainable mining practices, for example, strict enforcement of environmental regulations

(in cases of emission, dust control, and waste management), holding mining companies accountable for their share of environmental degradation and health impacts, incentives for mining companies that incorporate eco-friendly technologies, and investment in healthcare facilities to address issues related to air pollution except for these major implications some other broader implications also include sustainable extraction of mineral resources, usage of green energy, etc. Lastly, it can be concluded that this study only incorporated one of the most used MCDM methods (i.e., FAHP), which can be improved in the future by adding a machine learning technique as an extra edge to AQI calculation. Moreover, this study could have been more accurate if real-time in-situ sensor data could be utilized in the future to calculate and predict air quality in mining areas of India.

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Eco-friendly Management of Phytopathogens Through Nanopesticides: A Sustainable Approach

Shivam Maurya¹, Challapalli Bindu Damayanathi¹, Kavita Kansotia², Pasupuleti Sai Manvitha¹, Chedalla Anhuji¹, Donthireddy Joshna Sri¹, Lakshmi Bulla¹ and Seweta Srivastava^{1†} 

¹Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara-144 411, Punjab, India

²Department of Plant Pathology, Sri Karan Narendra Agriculture University (SKNAU), Jobner-303329, Rajasthan, India

†Corresponding author: Seweta Srivastava; seweta.21896@lpu.co.in

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ABSTRACT

Biopesticides have frequently been the focus of attention on a global scale as a safer alternative to chemical pest control that may provide less damage to both humans and the environment. The usage of biopesticides is rising rapidly worldwide, at 10 percent a year. With the idea of limited application for the most significant impact, nanotechnology has produced novel tools for pest management in agriculture, including nanopesticides and nanosensors. In contrast to conventional chemical pesticides, nanopesticides are formulations of a pesticide's active component in nanoform that have delayed degradation, targeted distribution, and controlled release of the active ingredient over longer periods. In accordance with lots of studies, incorporating certain biological agents in nanoparticulate systems increases their effectiveness against pests while lowering losses resulting from physical deterioration. The development and evaluation of nanobiopesticides have been the subject of laboratory-only research to date using techniques like the creation of nanocomposites, nanoengineered biopesticides, coating nanoparticles with bio-pesticides, etc. The formulation of appropriate, globally acceptable bio-safety and registration requirements is necessary to enable the effective use of these formulations for pest management at the field level.

INTRODUCTION

Nanotechnology is revolutionizing agricultural and food waste in underdeveloped countries via sustainable agriculture and circular economy applications (Preethi et al. 2024). In developing countries, more than 60% of the population relies upon agriculture for their livelihood, either directly or indirectly. In present days, agricultural scientists are facing challenges like climate change, stagnation in crop yields, multi-nutrient deficiencies, low nutrient use efficiency, declining soil organic matter, shrinking arable land and reduced water levels, and shortage of labor (Elizabeth et al. 2019). To counter these issues, many agriculturists and environmentalists have designed many practices for sustainable development, and bioformulations are one of them. In all the green practices developed, there were limitations in effectively delivering the crop growth requirements (Sahai et al. 2019). These delivery models are called carriers. These carriers can deliver pesticides, fertilizers, biocontrol agents, and so many others that are required for plants. Nanoparticles are small molecules with a size range of 1–100 nm with different physiochemical properties (El-Saadony et al. 2019). Nano-fertilizers have a high sorption capability, a large surface area, and regulated release kinetics to specific sites, making them a better delivery system to provide fertilizers to plants (Rameshaiah et al. 2015). The addition of fertilizers to soil may lead to loss of fertilizers, and therefore, more fertilizer should be added to compensate for the loss (Ombódi & Saigusa 2000), whereas foliar fertilizer application is effective, but nutrients might fail to penetrate cell wall pores as their

size varies from 5 to 20 micron (Benzon et al. 2015), while nanoparticles with a smaller diameter can easily pass through cell wall pores (Moore 2006). The features of nano-fertilizers are delivering the appropriate nutrients for enhancing plant growth through the soil and foliar applications, they are cost-effective and sustainable sources of plant nutrients, they help in preventing pollution, and they have a high fertilization efficiency (Guru et al. 2015).

Irrational use of pesticides poses a threat to the ecosystem and human health (Kuhlbusch et al. 2018). Nanoencapsulation of pesticides can help in the slow and controlled release of pesticides for a prolonged time, reducing the loss of unwanted pesticides (Agrawal & Rathore 2014). Nanoparticles were also used against viruses and in managing the maturation of fruits (Hawthorne et al. 2014). Nanotechnology is used in agriculture and food production as nanosensors for monitoring crop growth and pest control by early detection of disease-causing pests in plants (Bhagat et al. 2015). Remarkable opportunities were introduced in agriculture by using nanotechnology-based delivery systems, resulting in smart, controlled, slow release of fertilizers and agrochemicals required to enhance crop yield (Prasad et al. 2017). Agrochemical industries are mainly focusing on increasing crop yields regardless of the risk of nanotechnology, while the toxic effects of nanotechnology on the environment and human health have received little to no attention. Therefore, it is necessary to also focus on nanoparticle reactivity, biodegradation time, levels of bioaccumulation, retention time, and toxicity of waste (Chaud et al. 2021).

The integration of nanotechnology and agriculture has emerged as a ray of hope at a time when concerns about global sustainability are on the rise. This progressive approach might address some of the most urgent issues confronting the globe today, like the sustainable handling of food and agricultural waste, and encourage the adoption of circular economy principles, especially in poor nations. A substantial and frequently underutilized resource that is essential to the sustainability of the food supply chain is agricultural and food waste (Manna et al. 2023). Taking into account research findings worldwide, we found significant improvements with nanotechnology over traditional methods, which underscores the practical benefits of nanotechnology, including increased crop yields, efficient resource use, and reduced environmental footprint. This comprehensive review aims to investigate the association between agricultural systems and nanotechnology, emphasizing how it might improve resilience, sustainability, and production.

NANOTECHNOLOGY IN AGRICULTURE

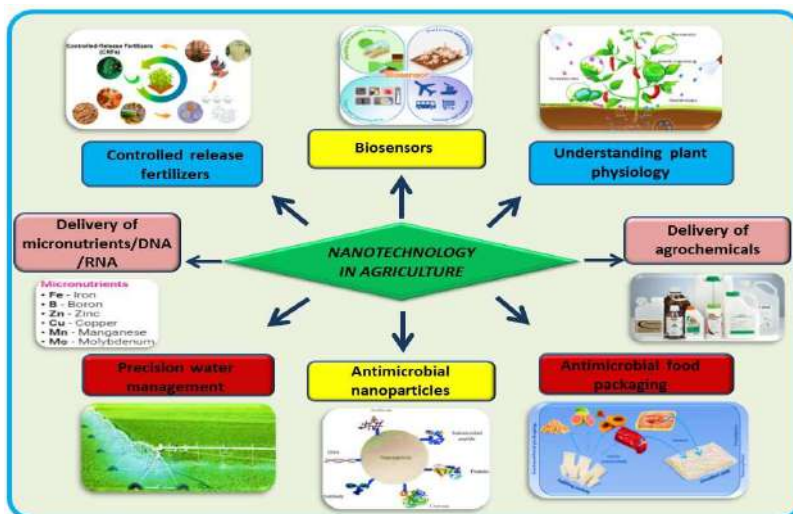
Nanoscience is being investigated in the areas of water

management, controlled applications of agrochemicals, plant hormone administration, seed germination, transfer of genes of interest, and nano-biosensors (Hayles et al. 2017). Additionally, by conjugation, adsorption, or encapsulation, nanoparticles are created to have the appropriate features (size, shape, surface area, etc.) for use as a protectant, therapeutant, or the site-specific delivery of active substances like fungicides (Khandelwal et al. 2016). Plants can be treated with nano-based materials through foliar spraying, soil application, seed treatment, and root dip treatment. In plants, metallic oxides, nonmetals, metalloids, polymeric, and carbon nanomaterials show growth-promoting and disease-suppressive properties (Elmer et al. 2018). The most often studied nanoparticles are made of iron, zinc oxide, silver, gold, and many more. The use of nanoparticles involves three primary mechanisms such as (a) using nanoparticles as biosensors, (b) using nanoparticles as therapeutics or protectants, and (c) using nanoparticles as intelligent delivery systems for active ingredients like target genes or fungicides. Against a range of soil-borne pathogens, such as *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Abdelrhim et al. 2021), *Ralstonia solanacearum* (Khairy et al. 2022), soil-borne viruses, such as Barley yellow mosaic virus (BaYMV) (Aref et al. 2012) etc., the nanoparticles may act as protective or therapeutic agents. The primary methods of combating microbes are agglutination and rupture of cell membranes, suppression of RNA, protein, toxin, and enzyme synthesis, including H⁺ATPase, as well as obstruction of nutrition flow (Dakal et al. 2016). Additionally, nanoparticles serve as carrier molecules, enabling the targeted release of active substances into the plant system and lowering the amount of chemicals released into the environment. Improved pesticide water solubility, site-specific distribution and uptake by target sites, longer shelf life, less impact on non-target organisms, and residual environmental effects are just a few advantages of the nano-based formulations (Hayles et al. 2017). Furthermore, nano-based formulations exhibit higher stability and activity in comparison to conventional pesticides, especially in adverse environmental circumstances like rainfall and UV exposure. The multifunctional role of nanotechnology is shown in Fig. 1.

Another important aspect of plant health management is the use of fertilizers. Nanofertilizers bear the potential to increase the release and uptake efficacy of nutrients, thereby boosting plant disease resistance. It has widely been explored in plant/disease systems viz., *Fusarium* wilt in tomato, chrysanthemum, root and crown rot in asparagus, red root rot in tea, *Verticillium* wilt in brinjal (Elmer et al. 2018).

NANOPARTICLES

Nanoparticles are characterized as particles in which at least



(Source: made by authors)

Fig. 1: The multifaceted role of nanotechnology in sustainable crop production and disease management.

Table 1: Different types of nanomaterials and their applications.

S.No.	Types of nano particles	Examples	Applications
1.	Metal-based nanoparticles	Copper, silver, silicon, Titanium oxide, zinc	Zinc-based nanoparticles inhibit fungal growth. Copper nanoparticles may act as bio-pesticides, and silicon-germanium is used as soil supplementation.
2.	Nanoscale polymers	Nylon-6/montmorillonite, montmorillonite-Alginate	Mainly used in catalytic processes and moisture removal
3.	Carbon-based nanomaterials	Carbon nanotubes [CNT], fullerenes	Improving plant health and environmental cleanup
4.	Nano emulsions	Beta-cypermethrin methyl laurate, alkyl polyglycolides	Nano emulsions are finding application in diverse areas such as drug delivery, food, cosmetics, pharmaceuticals, and materials synthesis
5.	Clay encapsulations	Organic layered double hydroxides, anionic inorganic	Clay tubes are excellent miniature containers for encasing chemical agents and are attractive for applications in drug delivery, antimicrobial materials, self-healing polymeric composites, and regenerative medicine
6.	Nanogels	Polyethyleneimine-PEG-polyplex nanogel	(N-isopropylacrylamide) (PNIPAM)-based nanogels with high deformability structures were successfully constructed for smart pesticide delivery and effective pest control
7.	Dendromers (Nanoparticles)	poly(L-lysine) (PLL), and triazene	potential applications of dendrimers include gene transfection, a catalyst for nanostructures, rheology modification

half have dimensions ranging from 1 to 100 nm. Compared to bulk materials, nanoparticles have a substantially greater surface-to-mass ratio (Modena et al. 2019). Various types of nanoparticles are tabulated in Table 1. Nanoparticle characteristics, such as their optical, magnetic, and electrical qualities, differ greatly from those of traditional materials (Khan et al. 2017). Nanoparticles can be found in diverse chemical compositions, spanning from micelles to metal (oxide) and synthetic polymers to large biomolecules. Each of these materials exhibits distinct chemistry, analyzable through various methods such as optical spectroscopy,

X-ray fluorescence and absorbance, Raman spectroscopy, and solid-state NMR (Ma 2016). While they are commonly assumed to be spherical, nanoparticles exhibit a wide range of geometric and irregular shapes (Li et al. 2014).

Nanoparticles can be characterized using high-resolution microscopy technologies, such as electron and scanning probe microscopy, capable of detecting features with sub-nanometer resolution (Park et al. 2015). The potential to synthesize nanoparticles with porous frameworks has significantly broadened the application scope of nanomaterials (Baeza et al. 2017). The surface-to-volume

ratio of the nanoparticles is dramatically increased by porosity, exceeding that of solid particles with identical diameters by many orders of magnitude (Modena et al. 2019). Various types of nanoparticles exist, including zerovalent metal nanoparticles, metal oxide nanoparticles, carbon nanotubes, nanocomposites, and quantum dots, among others. Each of these holds distinct significance in the realm of nanotechnology (Singh et al. 2019). Various techniques are employed for synthesizing nanoparticles, including biological, chemical, and physical methods. However, chemical and physical approaches are not cost-effective and often involve the use of hazardous and toxic compounds. It is widely acknowledged that these methods pose risks to human and environmental health due to harmful radiation, synthetic reductants in concentrated forms, and stabilizing agents. In contrast, biological methods relying on plants and microbes are more efficient, cost-effective, and environmentally friendly. They entail a one-step bio-reduction process, offering a safer alternative with fewer adverse impacts (Ul Haq & Ijaz et al. 2019).

HISTORY AND TIMELINE

In response to the growing difficulties associated with sustainable production and ensuring food security, substantial technological progress and creative innovations have emerged in the agricultural sector in recent years (Dwivedi et al. 2016, Kou et al. 2018, Xiao et al. 2013). Nanotechnology is one such technological advancement that stands out as one of the most promising advancements in the 21st century. The concept of nanotechnology was first time introduced by an American physicist and Nobel Prize recipient, Richard Feynman, in 1959. He delivered a lecture titled “There’s Plenty of Room at the Bottom” at the annual meeting of the American Physical Society, which took place at the California Institute of Technology (Caltech) (Bayda et al. 2019). The term ‘nanotechnology’ was first time used and defined by the Japanese scientist Norio Taniguchi, who served as a professor at the Tokyo University of Science in 1974 (Khan & Rizvi 2014). According to him, “nanotechnology primarily involves the manipulation, assembly, and alteration of materials at the scale of individual atoms or molecules” (Taniguchi et al. 1974). After Feynman discovered this new field of research, it piqued the interest of many scientists. The invention of the scanning tunneling microscope in 1981 and the discovery of fullerene/carbon “buckyballs” (C60) in 1985 led to the emergence of nanotechnology (Kroto et al. 1985). In 1986, K. Eric Drexler published the first book on nanotechnology, titled “Engines of Creation: The Coming Era of Nanotechnology.” This publication contributed to the increasing popularity of the concept of

“molecular engineering” (Drexler 1986). Later on, in 1991, Drexler, Peterson, and Pergamit released another book titled “Unbounding the Future: The Nanotechnology Revolution.” In this book, they introduced the terms “nanobots” and “assemblers” to describe nano processes in the field of medicine. It was after this publication that the well-known term “nanomedicine” was first coined and used (Drexler et al. 1991).

SYNTHESIS OF NANOBIOFORMULATIONS

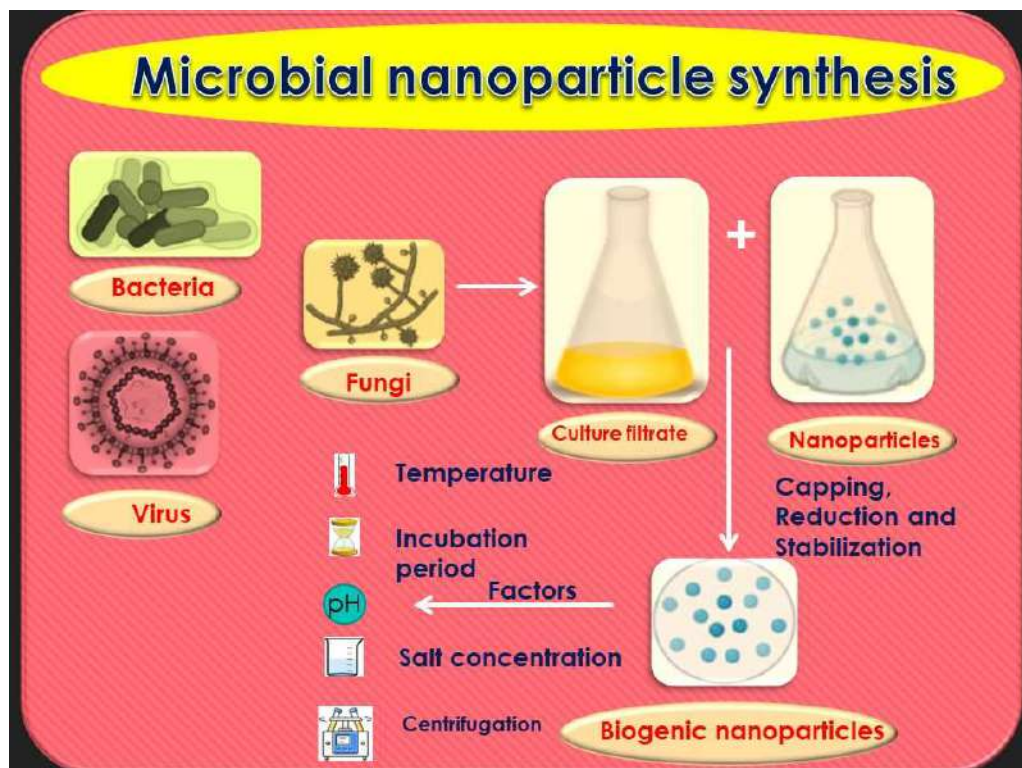
Biological Synthesis of Nanoparticles by Microorganisms

Since the beginning of life on earth, biological beings and inorganic materials have been in constant contact with one another. With a well-organized mineral deposit, life may exist on this planet because of this regular contact. The relationship between inorganic molecules and living species has attracted the curiosity of scientists more and more recently. Numerous bacteria can create inorganic nanoparticles through extracellular or intracellular pathways, according to investigations. The synthesis of different nanoparticles using biological methods is covered in this section. The mechanism involved in the synthesis of microbial nanoparticles is shown in Fig. 2. The categories of nanoparticles covered are metallic (such as gold, silver, and other metal nanoparticles), oxide (which includes both magnetic and nonmagnetic oxide nanoparticles), sulfide, and other miscellaneous.

Gold Nanoparticles

The use of gold nanoparticles or AuNPs for elegant glass staining dates back to the Roman era, and their history in chemistry is extensive. AuNPs have been utilized for ages to treat a variety of diseases. Michael Faraday, who may have been the first to notice that the properties of colloidal gold solutions differ from those of bulk gold, is credited with starting the present age of AuNP synthesis more than 150 years ago (Hayat 1989).

The growing need to develop environmentally benign technologies in materials synthesis has brought the biosynthesis of nanoparticles—the nexus of nanotechnology and biotechnology—to significant attention (Subbarayudu & Kubendiran 2024). The extracellular production of gold nanoparticles by the actinomycete *Thermomonospora* sp. and the fungus *Fusarium oxysporum* has been reported by Ahmad et al. (2003). Additionally, they found that the fungus *Verticillium* sp. was able to synthesize gold nanoparticles inside cells (Mukherjee et al. 2001). Bacterial cells can easily precipitate gold particles with nanoscale dimensions when



(Source: made by authors)

Fig. 2: Depicting various steps involved in the synthesis of microbial nanoparticles.

they are incubated with Au^{3+} ions (Southam & Beveridge 1996).

Silver Nanoparticles

Similar to its bulk counterpart, silver nanoparticles have potent antibacterial activity against both Gram-positive and Gram-negative bacteria, including strains of the latter that are extremely multi-resistant, like methicillin-resistant *Staphylococcus aureus* (Panáček et al. 2006). The creation of biomimetic methods for the formation of sophisticated nanomaterials is a result of the secrets found in nature (Gareev et al. 2022). Scientists have recently been working to employ microbes as potentially environmentally benign nano factories for the manufacture of silver nanoparticles. It is known that a variety of bacteria can decrease the Ag^+ ions to produce silver nanoparticles, the majority of which are spherical in shape (Fayaz et al. 2010). The bacterium *Pseudomonas stutzeri* AG259, isolated from a silver mine, was shown by Klaus and colleagues to play a significant role in the reduction of Ag^+ ions and the development of distinct sized and topographically defined AgNPs within the periplasmic space of the bacteria when it was placed in a concentrated aqueous solution of silver nitrate (Klaus et al. 1999). When the fungus *Verticillium*, *Fusarium oxysporum*, or *Aspergillus flavus* were used, AgNPs

were created as a film in solution or deposited on the cell's surface (Jain et al. 2011).

Oxide Nanoparticles

Over the past ten years, there has been a sharp increase in the industrial use of metallic oxide nanoparticles in a wide range of applications. These applications involve the utilization of metallic oxide nanoparticles such as silicon, titanium, iron, and others, increasing the sensitivity of humans and other animals to these nanoparticles through occupational and environmental exposure (Lai et al. 2007). However, because regulatory oversight has not focused on the environmental impact of metallic oxide nanoparticles, the health implications of exposure to these particles in humans and other species have not been thoroughly studied (Lai et al. 2007). One significant class of microbial nanoparticles produced by microorganisms is the oxide nanoparticle. The process of biogenesis of oxide nanoparticles is in this section. The majority of the biological systems and magnetotactic bacteria are utilized to produce magnetic oxide nanoparticles and nonmagnetic oxide nanoparticles (Li et al. 2011).

Copper Oxide (CuO) Nanoparticles

Copper has been shown in multiple investigations

conducted over the past two years to have exceptional antibacterial activity at the nanoscale (Cuevas et al. 2015). Copper as a metal or copper oxide exhibits broad-spectrum biocidal activity. Since copper is a necessary metal for all living things, it may find use in biomedical applications, unlike silver nanoparticles, which have been the subject of substantial research for their antibacterial properties (Rubilar et al. 2013). It's vital to remember that copper is currently around ten times less expensive on the market than silver, so a process that makes use of copper would end up being quite economical. However, it has been noted that compared to silver nanoparticles, copper nanoparticles are less hazardous (Bondarenko et al. 2013). Under ambient conditions, microorganisms like *Fusarium oxysporum* can extract copper from integrated circuits found on electronic boards (Cuevas et al. 2015). No significant polydispersity was found in the pH range of 5–9 in the investigation of the biogenic synthesis of copper oxides from CuSO_4 by *Penicillium aurantiogriseum*, *P. citrinum*, *P. waksmanii*, and *F. oxysporum* (Honary 2012, Hosseini et al. 2012). Only a few numbers of research evaluating various fungal strains for copper nanoparticle production have been published. It has been observed that certain strains of *F. oxysporum* and *Penicillium* sp. are capable of biosynthesizing Cu_2S and copper oxide nanoparticles (Honary et al. 2012, Hosseini et al. 2012). In the presence of three different copper salts. Ability to synthesize copper and copper oxide nanoparticles using a mycelium-free extract made by the white-rot fungus *Stereum hirsutum* (Cuevas et al. 2015). They also assessed the ability to characterize and evaluate the role of proteins in the formation of nanoparticles.

BIOFORMULATIONS

Bioformulation is a green practice developed by environmentalists and agriculturists to address issues related to biomagnification and chemical toxicity in the environment (Sahai et al. 2019). Any biologically active chemicals generated from microbial biomass or products containing microorganisms and their metabolites that might be utilized to promote plant development, acquire nutrients, and manage disease in an environmentally acceptable way are referred to as bioformulations. Essentially, a bioformulation is a blend of an active ingredient within a formulated product composed of inert (inactive) substances (Aamir et al. 2020). Bioformulation is a ready-to-use formulation that contains living cells or their metabolites (of one or more strains), supported by nontoxic and inert compounds called carriers to sustain the viability and efficiency of cells or metabolites and to lengthen their shelf life (Usta 2013). For promoting plant development, controlling phytopathogens, preserving soil fertility, and preventing disease, bio-formulated

solutions provide environmentally friendly substitutes to commonly used chemical fertilizers and pesticides (Arora & Mishra 2016, Khatri & Bhateria 2023). A bioformulation is a preparation of microorganisms or their active gradient that can be used in place of chemical fertilizers/pesticides. However, for the formulation to be successful, a true bio-formulated product needs to have an active component and be made of living microbes, spores, or their derivatives. Talc, Peat, carboxymethylcellulose, vermiculite, and polymers, particularly xanthan gum and diatomaceous earth, are some of the most popular inert active materials. The antagonistic microbial cells have been discovered to be more effectively incorporated into the plant system and rhizosphere by using inert carrier-based bioformulations, which could be applied both foliarly and topically (Aamir et al. 2020). Starch, methylcellulose, silica gel, and gum are a few additives that help bioformulated products withstand extreme weather conditions while also enhancing their physical, chemical, and nutritional qualities (Schisler et al. 2004). Some of the microorganism that is used in bioformulation are the strains of *Trichoderma* spp., *Rhizobium*, *Bacillus*, *Glomus*, *Lactobacillus*, *Azospirillum*, *Burkholderia*, *Rhodococcus*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Azotobacter*, *Pseudomonas* spp., *Actinobacteria*, *Bradyrhizobium*, *Acetobacter*, *Paenibacillus*, *Serratia*, *Herbaspirillum*, Blue-green algae, *Paenibacillus elgii*, *Pseudomonas putida*, mycorrhizal fungi, etc. (Bidyarani et al. 2022).

VARIOUS NANOBIOFORMULATIONS

Nanobioformulation as a Nanobiofertilizer

Nano-biofertilizer refers to a product comprising nano-scale materials paired with a particular microbial inoculant. Its nanoscale properties influence its application method, allowing for precise and timely nutrient delivery to field crops. Simultaneously, it enhances the functional advantages of the bio-fertilizer component within the formulation. Bio-fertilizers encompass crucial root-associated microorganisms, such as fungal mycorrhizae, *Azospirillum*, blue-green algae (BGA), and *Bacillus* species (Shanware & Taiwade 2022). The utilization of nano-biofertilizers has reduced the indiscriminate use of agrochemicals and contributed to substantiating integrated nutrient management practices for the consistent productivity of crops (Mir et al. 2015). The surface area of nutrients is increased through the process of making nano-biofertilizers, which involves coating the surface of bio-fertilizers with nanoparticles. This improves both their stability and the efficiency with which soil nutrients are taken up (Mala et al. 2017). This approach entails the live formulation of microorganisms that stimulate bacterial activity for improved plant growth. This formulation is then coated with a nanoscale

polymer, a method commonly known as nanoencapsulation (Shebl et al. 2019). One of the notable advantages of nanoencapsulation technology lies in its flexibility, particularly in protecting the components of bio-fertilizers containing PGPR (plant growth-promoting rhizobacteria). This innovation has resulted in an extended shelf life and has also enabled precise control over the release of PGPR mechanisms (Gouda et al. 2018). Silicon, zinc, copper, iron, and silver are among the most frequently employed nanoparticles in the formulation of nanobiofertilizers (Akhtar et al. 2022). Three crucial steps are involved in the creation of nanobiofertilizers: (1) the growth of the biofertilizer culture, (2) its encapsulation with nanoparticles, and (3) the assessment of its efficacy, quality, purity, and shelf life (Panichikhal et al. 2021). Various formulations have been developed to improve crop production, such as the combination of nano-Zn and biofertilizer, which has been shown to enhance the physiochemical properties of sugarbeet plants. Additionally, the application of a combination of nanopharmax and humic acid in black cumin may boost the nutritional content of cumin (Shanware & Taiwade 2022). The application of Nano-biofertilizer containing specific bio-inoculants, such as the *Azotobacter* strain, *Pseudomonas putida*, and *Pantoea agglomerans* strain P5, has been studied. This application has been found to raise nutrient concentrations and increase pigment content. Moreover, it enhances various plant parameters, including photosynthetic productivity, leaf area, sucrose content, and other factors on different parts of the plants.

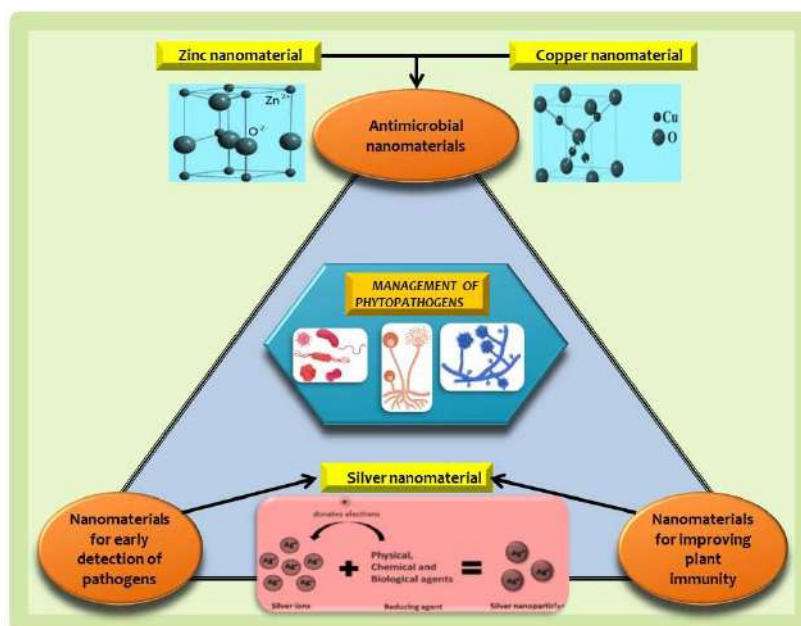
Nanobioformulation as a Nanobiopesticide

Despite their pesticidal effectiveness, biopesticides have some drawbacks. Innovative formulations have been created to increase their efficacy and fix current problems (Ayilara et al. 2023). This includes nanoformulations with a smaller amount of active ingredients that are appropriate for diverse crop protection applications. Management of plant pathogens by use of various nanoparticles is depicted in Fig. 3.

The resulting biopesticidal nanoformulations can be used directly as pesticides when nanomaterials are stabilized with biopesticides and nanobiopesticides are incorporated. By gaining efficiency at lower dosages of active components, these nanobiopesticides aid in successful pest management (Vimala Devi et al. 2019).

Examples of Various Nanobioformulations as Nanobiopesticides

Silver nanoparticles were developed and capped with the leaf extract of *Aristolochia indica* (Siva & Santhosh Kumar 2015). Management of various plant diseases by using different nanoparticles tabulated in Table 2. The resulting nanoparticles exhibited toxicity against the larva of *Helicoverpa armigera*. In field conditions within a cotton crop, it was observed that the combination of silver nanoparticles with the pyrethroid compound bifenthrin, extracted from *Chrysanthemum*, demonstrated greater toxicity to *Lygus hesperus* compared to *Acheta domesticus*. Essential oils were



(Source: made by authors)

Fig. 3: Management of plant pathogens by use of various nanoparticles.

extracted from *Zanthoxylum rhoifolium* leaves (Christofoli et al. 2015). Subsequently, nanospheres were developed with these oils using nanoprecipitation, resulting in a notably high encapsulation efficiency of 96 percent. The effectiveness against adult *Tribolium castaneum* (Herbst) was examined by utilizing polyethylene glycol nanoparticles as carriers for garlic essential oil. The eco-friendly production of silver nanoparticles and lead nanoparticles, utilizing extracts from *Euphorbia prostrata* and *Avicennia marina* (a mangrove plant), demonstrated pesticidal activity against *Sitophilus oryzae*. The outcomes revealed a 100% mortality rate after a 4-day treatment period (Vimala et al. 2019). Coating derived from cashew gum was applied to an insecticide extract obtained from Moringa seeds. This coating led to a notable enhancement in entrapment efficiency, rising from 39% to 60%. Remarkably, the released insecticide remained effective for up to 55 days following application (Paula et al. 2012). Chitosan nanoparticles (ChNPs) loaded with silver metal ions exhibit heightened antibacterial efficacy, demonstrating a low minimum inhibiting concentration (MIC) of 3 µg/ml against *Escherichia coli* and 6 µg/ml against *Staphylococcus aureus* (Du et al. 2008). Titanium dioxide nanoparticles (TDNPs), synthesized through *T. viride*, were evaluated for their bioefficacy against larvae and pupae of *Helicoverpa armigera*. The TDNPs exhibited remarkable effectiveness, causing 100 percent mortality in first, second, and third instar larvae of *H. armigera* at a concentration of 100 ppm (Kamaraj et al. 2018). Extracellular synthesis of silver nanoparticles (AgNPs) utilizing the bacterium *Serratia* sp. BHU-S4 revealed an inhibitory impact on the fungus *Bipolaris sorokiniana*. The AgNPs produced through biogenic means displayed complete inhibition of conidial germination, both on the growth medium and on wheat leaves artificially inoculated with fungal conidia (Mishra et al. 2014). Nanoemulsions formulated with neem and citronella essential oils demonstrated potent antifungal activity against *Rhizoctonia solani* and *Sclerotium rolfii* (Ali et al. 2017). *Conyza dioscoridis*, *Melia azedarach*, and *Moringa oleifera* extracts were used to make silver nanoparticles (Ag-NPs), which showed increased nematocidal action against eggs and young of the root-knot nematode *M. incognita* (Abbassy et al. 2017). The prospective use of mycogenic zinc oxide nanoparticles (ZnONPs) as a preventative measure against potato late blight looks promising (Singh et al. 2022). Silver and gold nanoparticles were biosynthesized using *Trichoderma atroviride*, demonstrating the potential for biological control of *Phomopsis* canker disease in tea plants. The nanoparticles exhibited antagonistic activity against the pathogen (Ponmurugan 2017).

Bacillus-Based Nanobiopesticide for Disease and Pest Reduction

Due to its great entomopathogenic potential, the genus *Bacillus* is frequently used as a biocontrol agent for managing both diseases and insects (Mampallil et al. 2017). The *Bacillus* group of bacteria is found in a diverse range of environments and is renowned for its ability to produce numerous antimicrobial compounds with diverse structures. Approximately 5–8% of its genome is dedicated to the synthesis of secondary metabolites (Fira et al. 2018). The creation of a nano-formulation using Bt relies on top-down methods of micronization, including ball and jet milling, as well as high-pressure homogenization. These processes are employed to convert coarse powder into fine particles within the size range of 2-5 µm (Vimala Devi et al. 2019). A nanobioformulation, functioning as a nanobiopesticide, is a composite of nanoparticles and microbial biocontrol agents (such as *Bacillus* sp.) (Padmakumar et al. 2023). This combination capitalizes on the higher surface area/volume ratio of nanoparticles, leading to enhanced effectiveness, reduced toxicity, increased solubility, durability, and versatility. Various types of nanoparticles, including Ag, Al, Au, MnO, ZnO, etc., can be integrated with different *Bacillus* species like *B. thuringiensis*, *B. subtilis*, *B. licheniformis*, etc. This integration aims to create an effective, efficient, and environmentally friendly nanobiopesticide capable of suppressing the growth of diseases and pests (Kumar et al. 2021). Strains of *B. subtilis* have been identified for their ability to inhibit the growth of various plant pathogens, including *Phomopsis*, *Colletotrichum truncatum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides*, *Phytophthora infestans* and others (Widnyana & Javandira 2016).

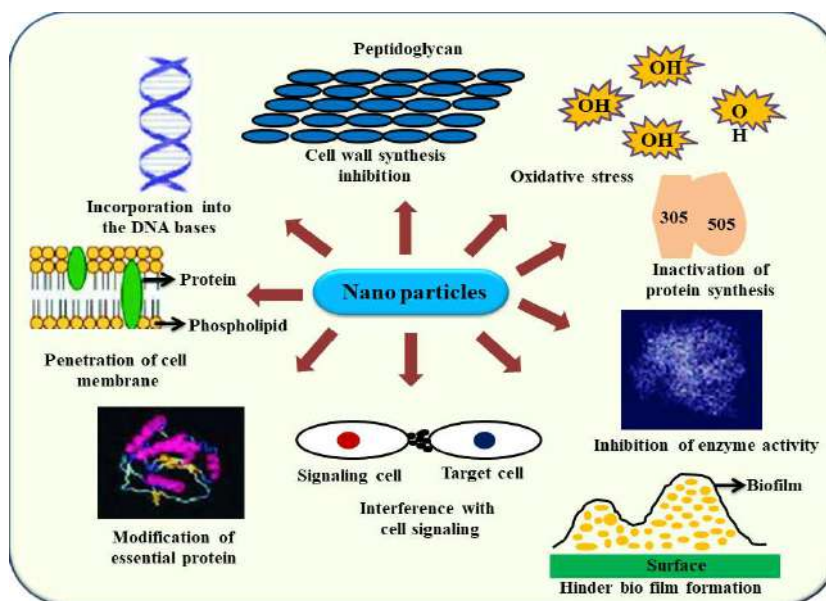
Mode of Action of Nanobioformulation

When beneficial bacteria are introduced into the soil as biofertilizers, they colonize and multiply quickly. This microbial activity plays a crucial role in converting typically insoluble and organic nutrients into soluble forms, making it easier for plants to absorb them. Biofertilizers that include nitrogen-fixing bacteria like *Rhizobium*, *Frankia*, *Xanthomonas*, and others can produce the nitrogenase enzyme. The various modes of actions of bionanoformulation are shown in Fig. 4.

This enzyme, in turn, facilitates the conversion of nitrogen into ammonia (Patel et al. 2023). Phosphate-solubilizing microorganisms like *Bacillus*, *Aspergillus*, and *Pseudomonas* produce enzymes or organic acids that play a catalytic role in converting insoluble phosphate complexes, such as aluminum and tricalcium phosphates, into soluble

Table 2: Management of soil-borne plant pathogenic organisms by using various nanoparticles.

Nanomaterial	Type of pathogen	Target pathogen	Crop	Effect	Reference
AgNPs	Fungi	<i>Macrophomina phaseolina</i> , <i>Fusarium fujikuroi</i> , and <i>Rhizoctonia solani</i>	Cotton (<i>Gossypium herbaceum</i>)	Reduction in mycelial growth and disease of cotton seedlings	(Zaki et al 2022)
MgONPs	Fungi	<i>Phytophthora infestans</i>	Potato (<i>Solanum tuberosum</i>)	Inhibition of <i>Phytophthora infestans</i> by cell membrane distortion, oxidative stress, disruption of metabolic pathways, and membrane transport activity with no harmful effect on potato	(Wang et al. 2022a)
Copper oxide nanoparticles (CuONPs)	Fungi	<i>Phytophthora nicotianae</i>	Tobacco (<i>Nicotiana tabacum</i>)	33.69% increase in control efficacy and tobacco black shank disease suppression without inducing phytotoxicity at 100 mg L ⁻¹ of CuO NPs treatment	(Juan et al. 2022)
Capped AgNPs	Fungi	<i>Sclerotinia sclerotiorum</i>	Vegetables	Inhibition of mycelial growth and sclerotia germination	(Guilger et al. 2021)
Chitosan NPs	Fungi	<i>Fusarium oxysporum</i> , <i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i>	Cereals, Vegetables	Reduction in mycelial growth	(Boruah & Dutta 2021)
Carboxymethyl cellulose coated core/shell SiO ₂ @Cu nanoparticles	Fungi	<i>Phytophthora capsici</i> Host: Black pepper	Black pepper (<i>Piper nigrum</i>)	Antifungal activity against <i>P. capsici</i> with MIC 75 ppm	(Hai et al. 2021)
Zinc oxide (ZnO), Iron oxide (FeO), and Copper oxide (CuO) nanoparticles	Bacteria	<i>Ralstonia solanacearum</i> Host: Tomato	Tomato (<i>Solanum lycopersicum</i>)	Reduced incidence of tomato bacterial wilt disease	(Jiang et al. 2021)
AgNPs	Fungi	<i>Sclerotinia sclerotiorum</i>	Mustard (<i>Brassica juncea</i>)	Inhibition of hyphal growth, sclerotial formation, and myceliogenic germination of sclerotia	(Tomah et al. 2020)
Magnesium oxide nanoparticles (MgONPs)	Fungi	<i>Phytophthora nicotianae</i> and <i>Thielaviopsis basicola</i>	Tobacco (<i>Nicotiana tabacum</i>)	Inhibition of fungal growth, spore germination, and impediment of sporangium development	(Chen et al. 2020)
AgNPs	Nematodes	<i>Meloidogyne javanica</i>	Tomato (<i>Solanum lycopersicum</i>)	Nematicidal activity on egg hatchability and juvenile mortality. Reduction in the number of galls, egg masses, number of females per root/plant, and mortality of juveniles.	(Ghareeb et al. 2020)
Silver Nanoparticles (AgNPs)	Fungi	<i>Sclerotium rolfsii</i> , <i>Rhizoctonia solani</i> , <i>Sclerotinia sclerotiorum</i> and <i>Fusarium oxysporum</i>	Cereals, Pulses, and vegetables	Mycelial growth inhibition at 100 ppm AgNP	(Kaman & Dutta 2019)
Copper/Iron NPs	Nematodes	<i>Meloidogyne incognita</i> and <i>M. javanica</i>	Tomato (<i>Solanum lycopersicum</i>)	Nematicidal activity such as paralysis, biological cycle arrest, and reduction in the number of galls with the lowest EC ₅₀ value as compared to commercial nematicides	(Gkanatsiou et al. 2019)
AgNPs	Fungi	<i>Fusarium solani</i> and <i>Macrophomina phaseolina</i>	Strawberry (<i>Fragaria ananassa</i>)	The nanoparticle showed broad spectrum antagonism against <i>M phaseolina</i> (67.05%) and <i>F. solani</i> (83.05%)	(Ruiz-Romero et al. 2018)
Gold nanoparticles (AuNPs)	Virus	Barley yellow dwarf virus (BaYDV)	Barley (<i>Hordeum vulgare</i>)	A high yield of ruined virus-like particles (VLPs) was also observed	(Alkubaisi & Arif 2017)
AgNPs	Fungi	<i>Phomopsis</i> sp. Host: Soybean seeds	Soybean (<i>Glycine max</i>)	Absolute inhibition of the pathogen was observed at 270 and 540 ppm concentrations.	(Mendes et al. 2014)



(Source: made by authors)

Fig. 4: Illustrating various modes of action of bionanoformulation.

forms that plants can readily absorb (Tian et al. 2021). These microorganisms, known as Plant Growth-Promoting Rhizobacteria (PGPR), also release substances like iron, vitamins, and hormones that are essential for plant growth. Additionally, the relationship between PGPR and plants is strengthened through cellular communication methods, such as quorum sensing. This serves as a signaling mechanism through which microorganisms assess their environment and coordinate their activities within the rhizosphere (Sarhani & Yahaya 2022).

ADVANTAGES AND DISADVANTAGES OF NANOBIOFORMULATIONS

Advantages

Nanobioformulations are the combination of biofertilizers with nanonutrient particles. These nanobioformulations may incorporate one or more beneficial microorganisms that enhance soil productivity, including nitrogen-fixing bacteria, phosphate-solubilizing/mobilizing organisms, and plant growth-promoting stimulators (Tarafdar 2022). Compared to traditional pesticides, nanopesticides offer superior performance and are better suited for widespread use in the global context of climate change and resource scarcity (Wang et al. 2022b). Nanoformulations stand out as a vital component in contemporary agricultural technology (Sahu et al. 2021). Nanoformulations encompass the process of coating, encapsulating, adsorbing, or entrapping fertilizers, pesticides, and nutrient supplements within the voids, pockets,

or pores of nanomaterials (Bhardwaj et al. 2022). There are various advantages of using nanobioformulation instead of conventional chemical fertilizer. The inoculation of plants with NBFs improves plant development and resistance to stress, utilizing nano biofertilizer formulations can contribute to the advancement of sustainable agriculture development (Garg et al. 2023). The application of nanoformulations in agriculture presents an opportunity to enhance global food production sustainability. The significant advantages of nanofertilizers over traditional chemical fertilizers revolve around their nutrient delivery systems (Yadav et al. 2023). For instance, nanofertilizers can release nutrients gradually over a period of 45 to 50 days, in contrast to the 5 to 10 days typical of conventional fertilizers. This extended-release improves nutrient use efficiency up to 20 times, reducing nutrient requirements and cutting down on transportation and application costs. Nanoparticles are considered effective carriers for delivering nutrients to specific locations through methods such as encapsulation or creating nanoscale emulsions. Interestingly, the surface coating of nanomaterials on fertilizer particles offers better adhesion due to their higher surface tension compared to conventional surfaces. This improved adhesion facilitates controlled nutrient release (Brady & Weil 1999, DeRosa et al. 2010, Bhattacharyya et al. 2016). Nanobioformulations possess a great advantage over conventional fertilizers as they exhibit specificity, reduced toxicity, and gradual release of nutrients (Cui et al. 2010). Another advantage of using nano biofertilizers is they prevent soil from becoming excessively loaded with

salts, a common issue associated with the over-application of conventional fertilizers (Tarafdar 2022). Moreover, the application of nanotechnology in agriculture counteracts problems such as crop yield, food security, climate change, and sustainability (Mishra et al. 2014).

Disadvantages

Although the future of nanopesticides in agriculture development appears promising, human exposure to dangerous agrochemicals able to cross biological barriers (e.g., blood-brain barrier, blood-placental barrier, and blood-retinal barrier) is a significant concern as it can cause irreversible damage to vital organs. The risks posed by the exposure to hazardous nanopesticides, which are able to

induce toxic and genotoxic events, are currently receiving great attention by studying the effect not only on the chemical composition of the bulk material but also on the physicochemical properties of nanopesticides such as size, electrical charge, and surface properties (Grillo et al. 2012, Zielinska et al. 2020).

According to studies, nanoparticles may have a negative impact on soil, plant, and animal health. Nanoparticles have the potential to alter the composition and structure of soil, potentially leading to reduced water retention and nutrient accessibility. Moreover, there is a possibility that plants may absorb these nanoparticles, which could impact food chains and affect plant growth (Ahmed et al. 2021). Nanomaterials may enter aquatic habitats, potentially affect aquatic

Table 3: The pros and cons of different nanopesticides with reference to specific case studies.

Type of Nano-pesticide	Pros	Cons	References
Nano-Encapsulation	- Controlled release reduces application frequency and enhances efficacy. - Enhanced targeting minimizes non-target exposure. - Protects active ingredients from degradation.	- High cost of nano-carrier synthesis. - Potential residue persistence of encapsulation materials. - Limited biodegradability.	Arratia-Quijada et al. (2024), Dinh et al. (2024)
Case Study:	Nano-encapsulated carbendazim effectively controlled <i>Fusarium oxysporum</i> with 50% lower doses. Phytotoxicity evaluation of nanoformulated fungicide confirmed that the nanoformulated carbendazim is safer for germination and root growth of the seeds of <i>Cucumis sativa</i> , <i>Zea mays</i> , and <i>Lycopersicon esculantum</i> .		Sandhya et al. (2017)
Nano-Emulsions	- Improved solubility of hydrophobic pesticides, enhancing efficacy. - Uniform distribution leads to better plant coverage. - Reduces environmental impact by lowering active ingredient runoff.	- Stability issues like phase separation during storage. - Energy-intensive production processes (e.g., ultrasonication).	Mustafa and Husseins (2020)
Case Study:	Citronella-oil (1%), biosilica (2%) nanoemulsion, and neem oil formulas (1%) effectively suppressed nematode populations (99.33%, 97.65%, and 99.66%, respectively), increasing the sinensetin content/production in comparison with control, and could be evaluated further in the plantation in the field.		Djiwanti et al. (2022)
Nano-Metal Oxides (e.g., CuO, ZnO)	- Broad-spectrum activity with antifungal, antibacterial, and insecticidal properties. - Low chance of resistance development due to multiple action modes.	- Potential phytotoxicity and bioaccumulation risks. - Expensive and energy-intensive to produce at scale.	Saqib et al. (2019)
Case Study:	CuO nanoparticles inhibited <i>Pseudomonas syringae</i> in tomato plants effectively. Another study reported that <i>Pseudomonas syringae</i> was inhibited after exposure to 200 mgL ⁻¹ of Cu-based NPs without any negative effect on other microbes, indicating its selective effect.		Banik and Pérez-de-Luque (2017), Tortella et al. (2024)
Nano-Clays	- Economical and readily available materials. - Reduces pesticide leaching and improves soil retention. - Environmentally benign.	- Limited compatibility with certain active ingredients. - Farmers' unfamiliarity with application techniques.	Margal et al. (2023), Rana et al. (2024)
Case Study:	Clay-composite nano-formulations reduced herbicide leaching by 40% in field trials.		
Nanosilver	- Highly effective against a wide range of pathogens like bacteria, fungi, and some viruses. - Compatible with biocontrol agents. - Long-lasting efficacy.	- Risk of toxicity to beneficial microorganisms and aquatic ecosystems. - Expensive production and regulatory challenges.	Zahoor et al. (2021), More et al. (2023)
Case Study:	Silver nanoparticles controlled by <i>Alternaria solani</i> cause early blight on potatoes more effectively than conventional fungicides. Lower concentrations of silver nanoparticles increased phenolics, PO, PPO, and PAL production. These defensive mechanisms demonstrate the fungicidal potential of AgNPs and recommend their utilization in different crop protection programs.		Ansari et al. (2023)

ecosystems, and may become part of the aquatic food chain. Nanoparticles can easily enter into the biological systems, which is not possible for the larger particles. The ability of nanoparticles to cross cell membranes depends on their size. In situations where farm workers are spraying nanoparticles, there is a potential risk of inhalation, which could allow these nanoparticles to enter the bloodstream and potentially reach various target sites within the body, including the brain, liver, or heart (Suppan 2017). These nanoparticles might affect the regulatory mechanisms of enzymes and other proteins (Saptarshi et al. 2013). There is a possibility of nanoparticles entering food chains, with potential implications for human health. Therefore, it is crucial to prioritize the development of a skilled workforce that comprehends the intricacies of agricultural production systems, enabling the successful integration of nanotechnology applications in agriculture (Siddhartha 2014). However, the exact consequences of their presence on human health and aquatic ecosystems remain uncertain at this time (Li et al. 2014). It may adversely affect biodiversity and ecosystem health as even small changes may upset sensitive balances. There are worries about how long-term usage of nano-materials will affect biodiversity and ecosystem dynamics (Ahmed et al. 2021).

Nanopesticides, which are nano-formulated pesticides, offer innovative solutions to pest and disease management. Their effectiveness, however, depends on formulation, target pests, and environmental interactions. Table 3 gives the pros and cons of different nano-pesticides with reference to specific case studies:

FUTURE DIRECTIONS

Research into nanotechnology applications for use in agriculture, especially in the field of crop protection, has become increasingly popular over the past decade. This analysis of the latest research trends provides a useful basis for identifying research gaps and future priorities. The development of novel plant-protection products has received greater attention than other applications, such as those related to nanosensors or fertilizers. Remarkable opportunities to renew agriculture practices have been introduced by using nanotechnology-based delivery systems, attributed to the smart controlled release profile of fertilizers and agrichemicals required to enhance crop productivity (Prasad et al. 2017). Such systems play a critical role in agriculture, improving fertilizers and agrochemicals performances (Fraceto et al. 2016). Presently, numerous research initiatives of nanopesticides prioritize performance optimization, including enhancing activity (Li et al. 2022), targeted deposition (Su et al. 2023), improved safety (Gao et al. 2018), and controlled release (Xiao et al. 2022).

However, some nanopesticides might be designed without initial consideration for specific application scenarios or are limited to traditional spraying methods. This research idea contradicts the need for pesticide application in future diversified agriculture. Furthermore, there is a scarcity of reviews covering existing nanopesticides developed for diverse agricultural scenarios. It should be noted that the plant cultivation environment is diverse, 125, and future application scenarios for pesticides might be more extensive, such as cities, oceans, and even deserts. This is crucial information that developers and manufacturers of nanopesticides must not overlook. As agricultural mechanization advances, pesticide application methods are also evolving. Therefore, this review focuses on ‘Scenario-oriented Nanopesticides’, highlighting the importance of prioritizing practical and specific application scenarios in the future development of nanopesticides to enhance sustainable development potential and resource utilization.

In addition to the application methods, experimental research targeting specific scenarios should consider environmental conditions when pests and diseases occur (such as temperature, humidity, and pH) as well as microenvironmental changes within the plant (such as enzymes, ROS, and hormones). More importantly, these products need to be validated in the field to bridge the gap between the scientific and industrial circles and provide the public with a more objective and realistic understanding. In addition, it is crucial to consider the environmental impact and biosafety of nanopesticides. Manufactured nanopesticides require a comprehensive evaluation system. 126 Scenario-oriented nanopesticides can more accurately target actual production needs and accelerate the industrialization process. In the context of sustainable agriculture, this concept is expected to lead the future development of nanopesticides.

CONCLUSIONS

Nanopesticides, as a promising technology, bring scientific and technological impetus to sustainable development and the green revolution of agriculture. The excellent physicochemical properties, beneficial biological effects, and functional potential of nanopesticides have significantly contributed to improving utilization rates of pesticides, enhancing pest and disease management, and alleviating stresses. In conclusion, the development and production of nanopesticides will necessarily lead to an increased amount of nanopesticides applied in agriculture. A reliable and comprehensive risk assessment before widespread application is the first line of defense to ensure environmental safety and human health. There have been serious environmental issues, like DDT or neonicotinoids, in human history, which were

allowed to occur due to a lack of a comprehensive assessment of environmental risk in advance. For nanopesticides, we should learn the lesson and avoid a similar outcome in the future. The unexpected risk of nanopesticides comes from their possible toxicity to nontarget organisms, transportation, and bioaccumulation and from interactions with other pollutants in the environment. These processes should be the focus of the regulatory framework for nanopesticide risk assessment. In order to lessen the amount of chemicals that are released into the environment, nanomaterials can also be employed as intelligent pesticide, fungicide, and fertilizer delivery systems. Beyond comparing these technologies with existing ones, further research is needed to evaluate the real-world viability, sustainability, efficiency, applicability, and releasability of nanotechnology-based products.

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Implementation of the Circular Economy Model in Coconut White Copra Production

Budy Rahmat[†], Iqbal Akmalludin and Tedi Hartoyo

Department of Agrotechnology, Siliwangi University, Tasikmalaya City 46115, Indonesia

[†]Corresponding author: Budy Rahmat; budyrahmat@unsil.ac.id

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ABSTRACT

This study implemented a circular economy model in processing coconuts into white copra as the main product to achieve a zero-waste production system. Simultaneously, other fruit components, such as shells, fibers, and coconut water, are also processed into products that play a role in supporting the main product directly or indirectly. The processed products obtained were liquid smoke, charcoal briquettes, and tar. The processing of white copra was carried out in the following stages: (i) coconut meat was processed into white copra, while the shell was pyrolyzed into liquid smoke, charcoal, and tar; (ii) liquid smoke was used as a preservative for copra, (iii) charcoal was formed into charcoal briquettes which were used for pyrolysis heating, and (iv) the tar produced was used as a wood preservative. The entire series of research was conducted as a laboratory experiment. The treatment of coconut meat dipping for copra in the concentration of liquid smoke solution was arranged in a randomized block design (RCBD). The data were analyzed by ANOVA and continued with Duncan's multiple comparisons. Meanwhile, the determination of the components of the results of peeling coconuts and the results of pyrolysis of coconut shells and fibers, as well as the characterization of liquid smoke and charcoal briquettes formed, were carried out by observational experiments. The results of this study indicate that the weight of 100 coconuts of the tall variety sample was 175.1 kg, or an average weight of 1,751 g per coconut. After peeling all the coconuts, the components of meat, shell, fiber, and fruit water were obtained, each weighing 48.9 kg, 23.2 kg, 70.6 kg, and 32.4 kg. Furthermore, 23.2 kg of shells were pyrolyzed and produced liquid smoke, charcoal, and tar of 9,126.70 g, 7,155.52 g, and 574.64 g, respectively, which was the average of three pyrolysis repetitions. Coconut shell charcoal briquettes were formed by mixing charcoal flour with tapioca and water in a ratio of 80:5:10, which formed a homogeneous mixture. The mixture was molded by a hydraulic press with a pressure of 2,000 g.cm⁻². Furthermore, the test results of water content, volatile matter, ash, fixed carbon, specific gravity, compressive strength, and calorific value were 7.79%, 13.75%, 2.76%, 68.66%, 0.92 g.cm⁻³, and 64.22 kg.cm⁻², 6,521 cal.g⁻¹, respectively. All the results of the charcoal briquette test parameters met Indonesian and Japanese Standards. In the processing of coconut meat, 25.67 kg of white copra was obtained, which was treated with 12.5% liquid smoke with a quality equivalent to the results of sulfur fumigation, namely, free from fungal infection and the highest oil yield and copra brightness. The results of this study provide new findings that, from one coconut of the tall variety, 489 g of coconut meat and 232 g of shell were produced. From the shell, 91.13 g of liquid smoke and 82.15 g of charcoal briquettes were produced, and 26.67 g of white copra was produced as the main product. This study provided new findings on the circular economy model and the principle of zero waste in white copra production, with the fact that each tall coconut variety produced 489 g of coconut meat and 232 g of shell. From the shell, 91.13 g of liquid smoke and 82.15 g of charcoal briquettes were produced, and 26.67 g of white copra was produced as the main product. This evidence provided new enthusiasm in the business of producing white copra that is financially profitable and sustainable. This study opens up many further studies and studies on the circular economy and zero waste, especially in the processing of coconut products, for example, in the coconut oil, desiccated coconut, brown sugar, and virgin coconut oil industries etc. In addition, it does not rule out the possibility of research accompanied by financial studies.

INTRODUCTION

Global food demand is being driven by sustained population growth and rising consumption, and agricultural activity is growing to meet this demand. The waste

produced by modern agricultural systems is staggering; in Europe alone, agri-food waste amounts to almost 700 million tonnes annually. The circular economy seeks to minimize waste in the agri-food chain while maximizing the utilization of waste produced by employing commercially feasible methods and techniques to raise its value (Toopa et al. 2017, Stelte et al. 2023). Achi et al. (2024) stated that the rapid increase in global waste production and the urgent demand for sustainable energy alternatives have highlighted the potential of advanced Waste-to-Energy (WtE) technologies. These cutting-edge systems transform waste into a valuable resource, offering a practical approach to sustainable energy recovery while significantly supporting the circular economy framework.

Indonesia has the potential for abundant natural resources in the agricultural sector, especially coconut plantations, which cover an area of 3,728,600 ha, which is equivalent to 18.3 million tons of coconuts per year, making Indonesia the largest coconut producer (Kambey et al. 2023). In Indonesia, 15 billion coconut fruits are harvested per year (Bradley & Conroy 2019). Based on initial research, each coconut fruit has an average weight of 2.83 kg, and the waste obtained is 2.15 kg (75.97%). Based on these two factors, the biomass capacity of coconut fruit waste will be obtained at 32,250,000 tons per year.

Coconut waste management is essential for sustainable environmental practices, especially in countries that produce and consume large amounts of coconuts. The reasons behind coconut waste management involve various environmental, economic, and social factors (Rizal et al. 2022). Viera et al. (2024) stated that coconut husk and fibers are often discarded as waste. Without proper management, they contribute to landfills, which not only take up valuable space but also cause long-term pollution. Another consequence, as stated by Nordahl et al. (2023), is that organic waste, if not properly decomposed, releases methane, a potent greenhouse gas. Coconut waste management through composting or bioenergy production helps reduce methane emissions. Chen et al. (2024) stated that coconut by-products can be recycled or reused for other purposes, thereby reducing the need for new raw materials, supporting sustainable consumption.

Coconut waste management is in accordance with the concept of a circular economy, where waste materials are not only discarded but also reintroduced into the production cycle in useful ways. This approach reduces environmental impact, minimizes waste, and maximizes resource efficiency (Viera et al. 2024).

The product components of the pyrolysis or carbonization process of coconut shells are charcoal and liquid smoke, which have benefits as vegetable preservatives (Mulyawanti

et al. 2019). Liquid smoke contains several components that contribute to its preservative qualities. The key components of liquid smoke that make it effective as a preservative include phenols, acids, carbonyls, and alcohols (Lingbeck et al. 2014, Saputra et al. 2021).

Liquid smoke can be used for various purposes, one of which is to preserve or extend the shelf life of a product (Assidiq et al. 2018). Traditionally produced copra is blackish brown because it absorbs a lot of tar, the smoke flavor is very strong, and it can cause air pollution. To overcome this problem, the use of re-distilled liquid smoke was pioneered to reduce the tar content (Umami et al. 2023). Organic acid compounds contained in liquid smoke can inhibit the formation of spores and suppress the growth of microbes in food products, namely bacteria and fungi. Phenolic compounds in liquid smoke show effective antimicrobial activity against various organisms such as bacteria, yeast, and fungi (Suryani et al. 2020). Liquid smoke treatment is expected to replace the sulfur fumigation treatment of copra, which is a production input that requires financing and is not environmentally friendly (Amperawati et al. 2012).

Puspanantasari (2024) stated that charcoal briquettes have several advantages compared to other fuels, such as firewood or coal. Here are some of the advantages: environmentally friendly, stable combustion, economical, high energy, low ash, waste utilization, easy to store and transport, and almost smokeless.

The novelty of this study was the knowledge of the potential data of coconut shell and coconut fiber waste as by-products of copra production. Both by-products were produced through pyrolysis to form liquid smoke and charcoal briquettes, which would later become input for white copra production in the circular economy model, namely as botanical preservatives and copra heating oven fuel, respectively. Therefore, this study also determined the effectiveness of liquid smoke as a preservative to replace sulfur fumigation and coconut shell charcoal briquettes as fuel. The specific objective of the study was to determine the equivalence of the quantity of coconuts from white copra as the main product and liquid smoke and charcoal briquettes as by-products to realize production efficiency, a circular economy, and zero waste.

MATERIALS AND METHODS

Materials

The materials used in this experiment were coconuts of the tall variety obtained from coconut plantations in Mangkubumi District, Tasikmalaya City, Dunia Kimia Lestari natural sulfur powder for copra fumigation, NaOH pellets Merck CAS Number 215-185-5 to make 0.1 N NaOH

solution, FeCl_3 powder Merck CAS Number 10025-77-1 to made 1% FeCl_3 solution, and distilled water for solvents and rinsing tools.

The tools used in this experiment were biomass pyrolysis reactor equipment with a volume of 200 dm^3 which was a product of previous research, distillation equipment, Pyrex Erlenmeyer flasks (100, 250, 500 mL), Pyrex chemical bottles (100, 250, 1,000 mL), funnels, Duran distillation kits and analog heating mantle AHM 3000-S (3,000 mL), digital precision balance ACIS type AD-300 ($300 \times 0.01 \text{ g}$), digital humidity tester type R&D MT-10, hygrometer temperature humidity test HTC-2, Thermometer Pudax, magnetic stirrer Spinbar Polygon type f37120-0060, measuring cups (10 and 500 mL), label paper, filter paper, stationery and other supporting tools.

Methods

Determination of coconut fruit components: Determination of the weight of each fruit component was carried out by observing the results of peeling 100 ripe coconuts of the tall variety as samples so that data was obtained per coconut for the proportion of coconut fiber, shell, water, and flesh.

Determination of pyrolysis product: Pyrolysis is a thermal decomposition process ranging from 300 to 350°C of lignocellulosic biomass in conditions without oxygen, then liquid smoke, charcoal, and tar are produced. Pyrolysis of the entire shell from the peeling stage, then the quantity of liquid smoke, charcoal, and tar is recorded until the end of pyrolysis.

Characterization of liquid smoke: Liquid smoke directly resulting from pyrolysis is crude or grade 3 liquid smoke, so it needs to be purified through two distillations, which produces grade 1 liquid smoke.

Here is how to determine the four quality parameters of liquid smoke:

- (1) *pH value:* Testing the pH value using a pH meter by dipping the tool into a coconut shell liquid smoke solution. The scale value was read after the pointer was constant.
- (2) *Specific gravity:* Specific gravity was tested using a pycnometer that can measure the volume of the solution objectively. The results of the measurement were then calculated using the following formula:

$$\text{Specific gravity} = \frac{\text{Mass of liquid smoke (g)}}{Z \times \text{Volume of pycnometer (mL)}}$$

- (3) *Acid content:* The acid content was tested using the liquid smoke titrimetric method by a 0.1N NaOH standard solution in a burette. The calculation of this acid content used the formula (Diatmika 2019):

$$\text{Acid content} = \frac{V \times N \times \text{Mr}}{S \times 1,000} \times 100\%$$

where V is the volume of NaOH (mL), N is the concentration of NaOH (meq.L^{-1}), Mr is the relative molecular weight of CH_3COOH , and S is the sample weight (g)

- (4) *Phenol content:* The content of phenol compounds tested in liquid smoke was proven by the reaction of 1% FeCl_3 solution. The occurrence of a change in the color of liquid smoke to a brownish-purple color means that liquid smoke contains phenol compounds.

Characterization of charcoal briquettes: The resulting charcoal, as much as 850 g, was mixed evenly with 50 g of tapioca flour as a binder and 100 mL of clean water with a weight ratio of 85:5:10 to form a homogeneous dough. Then, the mixture was pressed hydraulically with a pressure of 80 kg.cm^{-2} so that briquettes were formed.

The pyrolysis process produces coconut shell charcoal, which is subsequently ground and filtered. 5% starch binder is combined with the powdered charcoal until a homogenous mixture is achieved. The mixture is run through a manual hydraulic press machine to make briquettes. After that, the sun is used to dry the briquettes for two days (Arafah & Harsono 2021).

Following the procedure of ASTM D5142-02 (2017) and Ajimotokan et al. (2019) determination of the characteristics of coconut shell charcoal briquettes were determined as follows:

- (1) *Moisture content:* The water content (M_c) of 0.5 g of sample (W_0) was determined by heating each sample at 105°C for 1 h in an oven. The samples were removed from the oven and allowed to cool in a desiccator. The samples were reweighed to obtain the final (dry) weight (W_d). The water content was then calculated using the equation :

$$M_c = \frac{W_0 - W_d}{W_0} \times 100\%$$

- (2) *Volatile matter content.* The cup with known mass and its lid were filled with the test sample and placed in the furnace. It was heated in the furnace at a temperature of 900°C for 7 min, then cooled in a desiccator and weighed. The volatile matter (V_m) was then evaluated as the percentage loss in mass of the sample using the equation:

$$V_m = \frac{B - C}{B} \times 100\%$$

where B is the weight of the oven-dried sample, and C is the weight of the furnace-dried sample.

- (3) *Ash content*: One gram of each pulverized briquette sample was measured and then placed in a container of known mass and dried in an oven to constant mass. These samples were then heated in a furnace at 800°C for five hs and weighed after cooling. The ash content was then estimated from the percentage loss of mass of the sample using the following equation:

$$A_c = \frac{D}{B} \times 100\%$$

where A_c is the percentage ash content, D is the weight of ash (furnace-dried), and B is the weight of the oven-dried sample.

- (4) *Fixed-carbon content*: Fixed carbon is the fraction of carbon in briquettes, in addition to the fraction of water, volatile matter, and ash. The fixed carbon content is calculated using the equation:

$$F_c = 100 - (V_m + M_c + A_c)$$

Where F_c is the fixed carbon (%), V_m is the volatile content (%), M_c is the moisture content (%), and A_c is the ash content (%).

- (5) *Briquette density*: Briquette density. Generally speaking, density is expressed as a weight-to-volume ratio, which is determined by weighing and measuring the volume in dry air. The following formula could be used to determine the density of briquettes:

$$D = \frac{M}{V}$$

Where D is the briquettes density (g.cm^{-3}), M is the briquette weight (g), and V is the briquettes' volume (cm^3)

- (6) *Compressive strength*: The briquette is compressed until it breaks to perform the compressive strength test. It is possible to compute compressive strength using the following formula:

$$S = \frac{P}{A}$$

Where S is the compressive strength (kg.cm^{-2}), P is the compressive load (kg), and A is the surface area (cm^2).

- (7) *Calorific value*: A silica cup containing one gram of the material was placed inside the bomb calorimeter tube. A manual peroxide bomb calorimeter was the instrument used to calculate this calorific value. The following formula could be used to calculate the calorific value:

$$C_v = T_2 - T_1 - 0.05 \text{ m } 0.24$$

Where T_1 is the water's initial temperature ($^{\circ}\text{C}$), T_2 is the water's temperature after burning ($^{\circ}\text{C}$), and C_v is the calorific

value of charcoal briquettes. Temperature owing to wire heat increase (0.05), m is the calorimeter specific temperature ($m = 73529.6 \text{ (kJ.kg}^{-1}\text{)}$), and 0.24 constant ($1 \text{ J} = 0.24 \text{ cal}$).

Effectiveness of liquid smoke as a preservative for white copra: Grade 1 liquid smoke was dissolved with distilled water at the concentrations tested, namely concentration levels of 5%, 7.5%, 10%, 12.5%, and 15%, which were compared with the existing preservation method with sulfur fumigation. The seven treatments were applied in a laboratory experiment arranged in a randomized block design (RBD) with four replications.

The response data in the form of percent intensity of fungal infection, copra color level score, and oil yield from copra were subjected to ANOVA. Because there was a significant response to the treatment, it was continued with Duncan's multiple tests.

- (1) *Intensity of fungal infection*: The growth of fungal mycelium on the surface of copra is observed visually during drying. Calculation of the intensity of fungal attacks (Rahmat & Albaki 2019):

$$I = \frac{\sum(n \times V)}{Z \times N} \times 100\%$$

Where I is the damage intensity (%), n is the number of each category of attacks, V is the scale value of each category, Z is the scale value of the highest category, and N is the number of samples observed.

- (2) *Copra color*: Determining the color of copra visually was a direct observation of copra based on the scale, namely a rating range of 0 to 4 (Apriyanto & Rujiah 2019), with scale details 1 = white, 2 = somewhat white, 3 = slightly yellow, and 4 = yellow.
- (3) *Oil yield*: Calculation of the yield of coconut oil produced from each unit weight of copra (Hernawati & Jirana 2018), with the following formula:

$$\text{Oil yield} = \frac{\text{Mass of coconut oil produced (g)}}{\text{Mass of copra}} \times 100\%$$

RESULTS AND DISCUSSION

Zero Waste and Circular Economy Efforts in Coconut Processing

This study attempts to implement the principle of zero waste by designing a circular economic model for processing coconuts into white copra. Meanwhile, other components of the fruit, such as shells, fibers, and coconut water, are also processed into products that are valuable financially and in terms of production and environmental sustainability. The white copra industry in Indonesia has a high potential to

increase farmers' income if the utilization of by-products and waste is pioneered to be processed into valuable materials.

The processed products are liquid smoke, charcoal briquettes, and tar, as illustrated in Fig. 1. However, products from coconut water were not studied in this study.

The following are several ways of utilization that can increase the added value and income of coconut farmers.

- Coconut shells can be carbonized to produce charcoal, which has a high calorific value and can be used as fuel, briquettes, or even activated charcoal for the food and pharmaceutical industries (Labombang & Nirmalawati 2017, Pomalingo et al. 2022).
- Coconut shell processing also produces liquid smoke that can be used in the food industry. This liquid smoke not only adds value to the product but also reduces environmental pollution due to burning (Pojo 2017, Pomalingo et al. 2022).
- Coconut fiber, which is usually considered waste, can also be utilized in the following ways: Coconut fiber can be processed into coco fiber, which is used in various applications, including textile products and raw materials for handicrafts (Stelte et al. 2023).

- Water from coconut processing can be used to make Nata de Coco, a popular food product with high economic value (Basuki & Fahadha 2020).

Quantity of Coconut Fruit Components

Table 1 shows the average data of the peeling test results on 20 coconuts of the tall variety. The main components of coconuts were the meat and its by-products, which were often wasted from the copra industry, namely shells and fibers. The proportion of fibers and shells in tall coconut varieties was 13.25% and 40.32%, respectively. Based on these proportions, if 100 fruits from tall coconut varieties are peeled, the shells and fibers will be obtained as much as 232 g and 706 g, respectively. If these two coconut components are not processed into useful products, they will become biomass waste, with a proportion of 62% (Obeng et al. 2020). This quantity of biomass has the potential to be turned into valuable and useful products, such as tar, charcoal briquettes, and liquid smoke, through the pyrolysis process. Moreover, if these two products are included again as one of the inputs for white copra production, they will play a role in improving the quality and increasing the efficiency of white copra production.

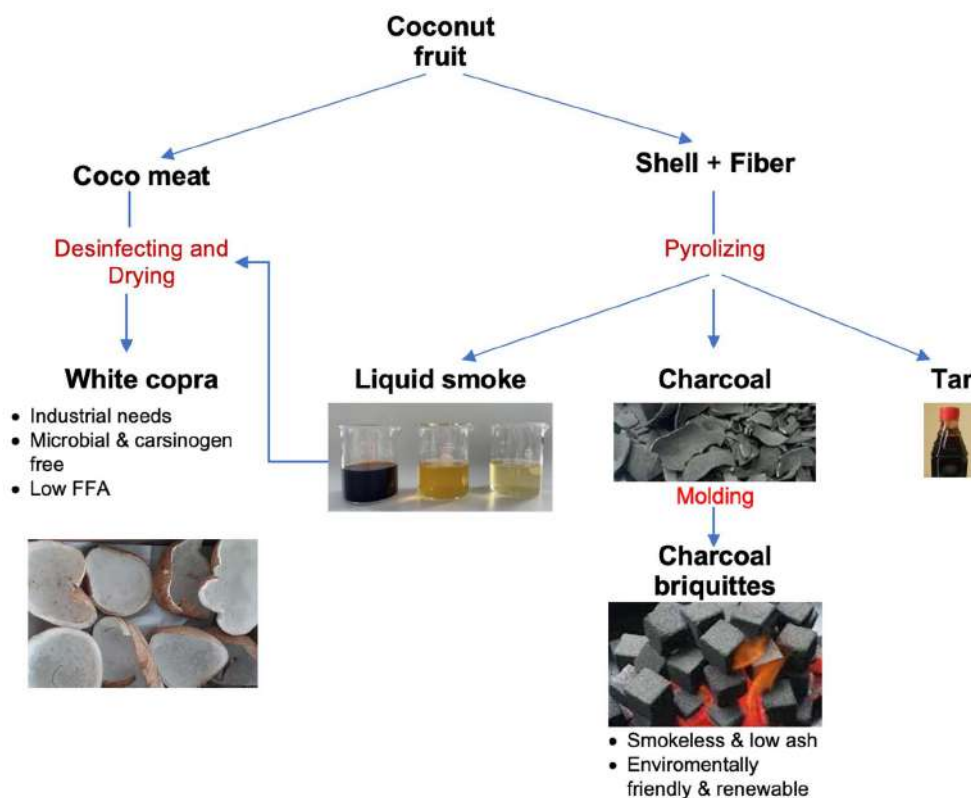


Fig. 1: Implementation of the zero waste and circular economy model in processing coconut into white copra, liquid smoke, charcoal briquettes, and tar.

Table 1: Quantity of 100 coconut fruit components.

Coconut fruit components	Weight total of 100 coconut fruits [kg]	Average Weight per fruit [g]	Proportion [%]
Fruit meat	48.9	489	27.93
Shell	23.2	232	13.25
Fiber	70.6	706	40.32
Cocowater	32.4	324	18.50
Total	175.1	1,751	100.00

Table 2: Quantity of pyrolysis products from each 23,2 kg of coconut shells.

Pyrolysis replication	Quantity of product		
	Liquid smoke [g]	Charcoal [g]	Tar [g]
1	8,965.00	7,540.14	563.08
2	9,276.26	6,751.06	563.09
3	9,138.70	7,175.35	597.76
Average	9,126.65	7,155.52	574.64
Proportion (%)*	39.34	30.84	2.48

* The total percentage of pyrolysis products does not reach 100%, because there are non-condensable gas components at the end of the pyrolysis reactor, with a proportion of 27.43%.

Quantity of Coconut Shell Pyrolysis Products

This quantity of biomass has the potential to be turned into valuable and useful products, such as tar, charcoal briquettes, and liquid smoke, through the pyrolysis process. Table 2 shows that the pyrolysis occurred at 250 to 300°C of 20 kg of coconut shells produced liquid smoke, charcoal, tar, and non-condensable gases of 7,867.81 g (39.35%), 6,168 g (30.99%), 295.405 g (2.16%), (27.51%), respectively. This percentage is comparable to the findings of other studies, which showed that the yields of char, liquid, and gas were, respectively, 22 to 31%, 38 to 44%, and 30 to 33%. The findings show that heating rate and residence time have less of an impact on the pyrolysis yield than temperature and particle size (Sudaram & Natarajan 2009). The pyrolysis process consists of multiple stages, with significant degradation of hemicellulose and cellulose occurring between 230°C and 400°C. Above 400°C, lignin starts to decompose,

affecting the overall product yields (Wang & Sarkar 2018).

In addition, this pyrolysis product is also influenced by smaller particle sizes, leading to higher surface area-to-volume ratios, enhancing heat transfer, and potentially increasing bio-oil yields (Anggraini et al. 2024). However, excessively small particles may lead to operational challenges such as clogging. Moisture must be removed before effective pyrolysis can occur. High moisture content can lower the overall yield of bio-oil and other products due to energy being used for evaporation rather than pyrolysis (Wang & Sarkar 2018).

Properties of Redistilled Liquid Smoke

The distillation process aimed to remove toxic substances contained in liquid smoke. In liquid smoke, there are differences in content indicated in the color of each grade of liquid smoke, as seen in Fig. 2.

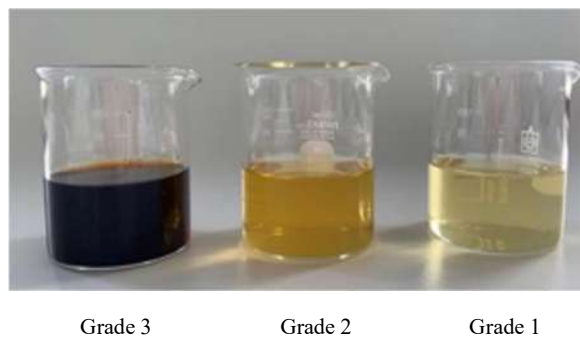


Fig. 2: Comparison of visual appearance on increasing purity of liquid smoke in succession grade 3 (crude), grade 2 (result of one distillation), and grade 1 (result of two distillations).

Table 3: Properties of grade 1 liquid smoke.

Quality parameters	Value	Japenese standard
Yield [mL.kg ⁻¹]	248,25	-
Color	pale yellow	yellowish brown
pH	2	1.3 – 3.7
Transparency	transparent	transparent
Density [g.mL ⁻¹]	1.02	> 1.001
Total acid content [%]	7.56	1 - 18
Phenol content [%]	2.78	-
Benzo(a)pyrene [%]	naught	-

Crude liquid smoke, also known as grade 3, is the liquid smoke that results from the direct pyrolysis process. This grade 3 liquid smoke still contains toxic tar and benzo(a) pyrene, which is carcinogenic, so two redistillation processes must be carried out, which respectively produce grade 2 and grade 1 (food grade) liquid smoke.

The results of the physical and chemical properties test show that the liquid smoke from coconut shells is of good quality and meets the standards. This can be seen in Table 3. Namely, the liquid smoke has a specific gravity, color, acid content, pH, and no dispersed substances according to international standards (Wu et al. 2015).

Grade 1 liquid smoke, which can be used as a direct food preservative to make food safe for consumption, was used in the investigation. The entire batch of grade 1 coconut shell liquid smoke was produced with a quality parameter value that complies with international criteria for liquid smoke quality. The characterization of the liquid smoke aimed to determine the composition of liquid smoke as an antimicrobial for copra.

The quality parameters of liquid smoke are crucial for its application in food preservation and flavoring. These parameters include pH, titratable acidity, phenolic content, and specific chemical characteristics. Liquid smoke typically exhibits a pH range of 1.5 to 5.5, depending on the wood source and processing methods used. For example, a study noted that full-strength liquid smoke had a pH of 2.3, while

refined products had higher pH values ranging from 4.2 to 5.7 (Montazeri et al. 2013). Phenolic compounds are critical for the flavor and preservative qualities of liquid smoke. For instance, the phenolic content in liquid smoke produced from Lamtoro wood was measured at 481.2 ppm, while corn cob liquid smoke had 335 ppm (Swastawati 2007). These compounds contribute to the antimicrobial properties and overall flavor intensity of the product.

The color of liquid smoke can vary significantly, with good quality liquid smoke typically exhibiting a pale yellow to reddish-brown hue. The chemical composition is influenced by the type of wood used, and hardwoods generally yield different profiles compared to agricultural residues like corn cob (Budaraga et al. 2016). Quality assessments also include checking for undesirable compounds such as Benz(a)pyrene, a known carcinogen found in some smoked products. Studies have shown that certain types of liquid smoke do not contain this compound, enhancing their safety for consumption (Murcovic 2004).

Properties of Charcoal Briquettes

Table 4 presents the Characteristics of the sample briquettes and standard values. The water content of the sample charcoal briquettes in this test was lower than the average water content in Indonesia, which is 8% (BSN 2019). The water content of the briquettes increases with increasing concentration of binder and decreasing compaction pressure in the molding process. The hygroscopic nature of the carbonized material and the water's availability in the binder are assumed to be the causes of the high water content (Aransiola et al. 2019).

The test sample charcoal briquettes had a 13.75% volatile content. Compared to Japanese and Indonesian standards, this value is superior. This demonstrates that the created coconut shell charcoal briquettes satisfied the specifications. Materials with higher stability levels produce less smoke when briquettes burn. Volatile matter is a component that evaporates when briquettes are burned, including hydrocarbon compounds such as methane and carbon monoxide. This content is directly related

Table 4: Characteristics of coconut shell charcoal briquettes.

Test Parameters	Units	Sample Briquette Values	Standards	
			Indonesian	Japanese
Water content	%	7.79	8	6 - 8
Volatile content	%	13.75	15	15 - 30
Ash content	%	2.76	8	3 - 6
Fixed carbon content	%	68.66	77	60 - 80
Density	g.cm ⁻³	0.92	-	1.0 - 1.2
Compress strength	kg.cm ⁻²	64.22	-	60 - 65
Calorific value	cal.g ⁻¹	6,521	5,000	6,000-7,000

to the amount of smoke produced during combustion (Rusman et al. 2023).

The test sample's charcoal briquettes had an ash percentage of 2.83%, which is still within the range allowed by the Japanese standard but below the Indonesian norm. It is imperative to maintain a low ash content in the briquettes to prevent the heating level from dropping. The biomass material's density and mineral content have an impact on the charcoal briquettes' ash content. Low-density biomass will contain a lot of minerals. The varying inorganic components of the raw materials are assumed to be the cause of the variation in ash content (Alpian et al. 2020).

The fixed carbon content of the coconut shell charcoal briquette sample tested at 68.66%, which is within the Japanese Standard's required range but less than the Indonesian Standard. Fixed carbon is the carbon fraction remaining after the combustion process and is the main indicator of the quality of charcoal briquettes. The higher the fixed carbon content, the better the quality of the briquettes (Abimanyu et al. 2024). Briquettes with high fixed carbon content have better calorific value, making them more efficient when used as fuel. In research, the ideal fixed carbon content to meet quality standards is above 77% (Dewi et al. 2020).

Coconut shell charcoal briquettes have a density of 0.92 g.cm^{-3} , although, in reality, it is below the Japanese standard. According to earlier studies, the density of charcoal briquettes manufactured from coconut trash is 0.86 g.cm^{-3} , whereas the density of charcoal briquettes made from Madan wood is 0.68 g.cm^{-3} . This analysis suggests that the material's specific gravity accounts for the majority of the density (Kongprasert et al. 2019).

The test sample's coconut shell charcoal briquettes had a compressive strength of 64.22 kg.cm^{-2} , falling between the 60 and 65 kg.cm^{-2} quality range specified by the Japanese Standard. Compressive strength is the ability of the briquettes to withstand axial loads without failure. It is a crucial mechanical property that indicates how much

compressive force a briquette can endure before collapsing. This property is significant for ensuring the durability and stability of briquettes during handling, storage, and transportation (Putri & Andasuryani 2017). This degree of compressive strength is critical to the briquette's integrity during packing and transit. Small particle size, low water content, and high molding pressure all have an impact on compressive strength. Additionally, at 150 MPa applied pressure, the briquette unit density increases from 2.8 to 3.0 kg.dm^{-3} when the binder content is increased from 10% to 30% (Khelifi et al. 2020).

According to the test results, the charcoal briquette sample had a calorific value of 6.521 cal.g^{-1} . This value is more than the Indonesian Standard and falls within the range of the Japanese Standard value (ASTM 2017). The calorific value, often expressed in megajoules per kilogram (MJ.kg^{-1}) or kilocalories per kilogram (kcal.kg^{-1}), indicates the energy content of the briquettes (Utami et al. 2024). Briquettes with higher calorific values provide more energy per unit mass, which translates to better fuel efficiency. This means less material is required to achieve the same heating effect, making them more economical and environmentally friendly (Suluh et al. 2022). The calorific value significantly affects market pricing and competitiveness. Fuels with higher energy content are generally more desirable and can command higher prices due to their efficiency and lower consumption rates (Pari et al. 2023).

Effectiveness of Liquid Smoke as a Preservative for White Copra

Based on the analysis of variance and Duncan's test (Gomez & Gomez 1983), it was proven that the treatment of liquid smoke concentration had a significant effect on the intensity of fungal infection, color, and yield of copra oil. Furthermore, the difference test between the treatments tested was as shown in Table 5.

The acidic compounds in liquid smoke inhibit the growth of microorganisms by penetrating cell walls and causing cell

Table 5: Effect of liquid smoke treatments on the fungal infection, copra color, and oil yield.

Treatment	Fungal Infection [%]	Copra color *	Oil yield [%]
A = sulfur fumigation dosage 30 g.m^{-3}	10.16 a	1.22 a	47.33 b
B = liquid smoke concentration 5%	24.22 b	1.94 c	39.84 a
C = liquid smoke concentration 7.5%	21.09 b	1.97 c	41.34 a
D = liquid smoke concentration 10%	11.72 a	1.91 bc	49.34 b
E = liquid smoke concentration 12.5%	7.81 a	1.13 a	51.51 b
F = liquid smoke concentration 15%	7.81 a	1.22 a	48.22 b

Note: in each column, Duncan's multiple range test indicates that there is no significant difference between numbers that are followed by the same letters at the 5% level. *: Color score ranges from white to brown: 1 to 4

lysis. This property is particularly beneficial in preventing spoilage caused by bacteria and fungi, thus enhancing food safety (Desniorita & Maryam 2015). While studies on the antifungal effects of liquid smoke are less extensive, some evidence suggests it can inhibit fungal growth as well. For instance, liquid smoke has been shown to prevent mold growth on fibrous food casings and inhibit specific molds like *Aspergillus oryzae* and *Penicillium spp.* on cheese (Gracia & Ismail 2020). The minimum inhibitory concentrations for molds such as *Aspergillus niger* have been reported to range from 1.5% to 5% (Deliephan et al. 2023).

Liquid smoke has been shown to prolong the shelf life of various food products, including sauces and meats. For instance, adding liquid smoke powder to sauces can increase their shelf life significantly. Sauces treated with liquid smoke showed an increase in shelf life from 3 days (without treatment) to 13 days (with 6% liquid smoke) due to reduced microbial growth and flavor retention (Keryanti et al. 2020).

The organic acids found in liquid smoke made from coconut shells include acetic and carboxylic acids, phenols, furans, aldehydes, and ketones (Rizal et al. 2020). Thus, coconut shell liquid smoke can be one of the materials to prevent and inhibit the growth of microorganisms in coconut meat.

The color of copra was impacted by sulfur fumigation and the treatment of coconut shell liquid smoke. In the treatment of sulfur fumigation, 12.5% liquid smoke and 15% produced

the same copra color, which was close to pure white. While the treatment of 5, 7.5, and 10% liquid smoke produced a yellowish-white copra color (Fig. 3). The yellowish color was caused by the intensity of fungal growth attacks on the surface of the copra meat (Subramanian et al. 2018).

On the second day of copra drying, fungi are seen starting to grow on the surface of the copra. Fungi that grow in the early stages appear white, which is their mycelium. From the next day until the seventh day, the fungi develop and begin to experience changes in the color of the infection zone spots, namely yellow, green, brown, reddish brown, gray, to black until the 7th day.

This result is in line with previous research that copra with sulfur fumigation produced white copra. Liquid smoke treatment that was comparable to sulfur fumigation was 12.5% and 15% treatment, which correlated with the results of the effect of liquid smoke in inhibiting fungal infections. Copra that was given a soaking treatment in a sulfite solution for 6 to 24 h was grayish-white. The grayish color was caused by the oil content in the copra meat. But the copra that was given treatment without washing was slightly white to slightly yellow. This was thought to be because there was still sugar on the surface of the coconut, so when heated, it browned (Kaseke 2016).

Liquid smoke treatment with concentrations of 10, 12.5, and 15% gave the same value as the comparison treatment of sulfur fumigation. This proved that the three liquid smoke

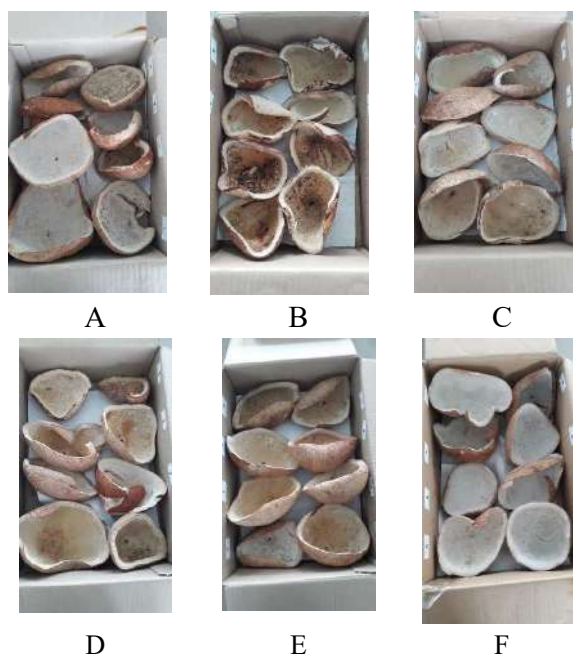


Fig. 3: Copra color in treatment: (A) sulfur fumigation, (B) liquid smoke treatment of 5%, (C) liquid smoke treatment of 7.5%, (D) liquid smoke treatment of 10%, (E) liquid smoke treatment of 12.5%, and (F) liquid smoke treatment of 15%.

treatments were able to provide protection against copra damage caused by microbes that infected copra, which was correlated with oil yield from copra. However, liquid smoke treatment with concentrations of 5 and 7.5% was not effective enough to protect copra from damage by microbes. The above facts prove that the application of liquid smoke can prevent a decrease in oil yield due to microbial infections that damage the coconut meat tissue. The liquid smoke can maintain the yield of copra oil which can decrease due to the growth of fungi such as *Rhizopus sp.*, *Aspergillus flavus*, *Aspergillus niger*, and *Penicillium glaucum*, and the oil produced from the copra has the lowest levels of protein, cholesterol and free fatty acids (FFA) (Amperawati et al. 2012).

Based on the description above, several advantages are obtained from using coconut shell liquid smoke as a preservative for white copra, including: (a) Liquid smoke is more economical than traditional smoking methods, saving time and energy and reducing the need for extensive equipment and labor. It streamlines the smoking process, making it suitable for large-scale production (Pojo 2017). (b) It effectively reduces the risk of food contamination by inhibiting the growth of various foodborne pathogens. This property helps in maintaining food safety during storage (Deliephan et al. 2023). (c) Unlike traditional smoking, which can result in variable flavors and colors, liquid smoke provides a consistent product quality. This is crucial for commercial applications where uniformity is important (Nithin et al. 2018). (d) The production of liquid smoke is generally less harmful to the environment compared to traditional methods, as it produces fewer emissions and reduces waste associated with wood burning (Wibowo et al. 2023).

CONCLUSIONS

The results of this study indicate that the weight of 100 coconuts of the tall variety sample was 175.1 kg, or an average weight of 1,751 g per coconut. After peeling all the coconuts, the components of meat, shell, fiber, and fruit water were obtained, each weighing 48.9 kg, 23.2 kg, 70.6 kg, and 32.4 kg. Furthermore, 23.2 kg of shells were pyrolyzed and produced liquid smoke, charcoal, and tar of 9,126.70 g, 7,155.52 g, and 574.64 g, respectively, which was the average of three pyrolysis repetitions.

Coconut shell charcoal briquettes were formed, and quality tests of water content, volatile matter, ash, fixed carbon, specific gravity, compressive strength, and calorific value were 7.79%, 13.75%, 2.76%, 68.66%, 0.92 g.cm^{-3} , and 64.22 kg.cm^{-2} , 6,521 cal.g^{-1} , respectively. All the results of the charcoal briquette test parameters met Indonesian and Japanese Standards.

In the processing of coconut meat, 25.67 kg of white copra was obtained, which was treated with 12.5% liquid smoke with a quality equivalent to the results of sulfur fumigation, namely, free from fungal infection and the highest oil yield and copra brightness.

This study provided new findings on the circular economy model and the principle of zero waste in white copra production, with the fact that each tall coconut variety produced 489 g of coconut meat and 232 g of shell. From the shell, 91.13 g of liquid smoke and 82.15 g of charcoal briquettes were produced, and 26.67 g of white copra was produced as the main product.

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Optimization of HHO Gas Production in Alkaline Dry-Type HHO Kit Using Various Electrode and Electrolyte Configurations

M. B. D. K. Siriwardena¹, A. R. Nihmiya^{1†}, Udara S. P. R. Arachchige² and Gamunu S. Arachchige³

¹Department of Civil and Environmental Technology, Faculty of Technology, University of Sri Jayewardenepura, Sri Lanka

²Department of Mechatronic and Industrial Engineering, Faculty of Engineering, National School of Business Management (NSBM), Green University, Sri Lanka

³Department of Process, Energy and Environmental Technology, Faculty of Technology, Natural Sciences and Maritime Sciences, University of South-Eastern Norway, Norway

†Corresponding author: A.R. Nihmiya; nihmiya@sjp.ac.lk

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ABSTRACT

This research investigates the optimization of a dry-type alkaline HHO kit for efficient oxyhydrogen (HHO) gas production, targeting applications in small (two-wheel vehicle) internal combustion engines (ICE). Key experimental parameters were evaluated to enhance gas production and system efficiency, including voltage, electrode configuration, electrolyte type, and concentration. Sodium hydroxide (NaOH) was identified as a more effective electrolyte than potassium hydroxide (KOH) due to its lower electronegativity, which contributes to accelerating HHO gas production. The highest overall efficiency, 24.6%, was achieved with a 0.1M NaOH solution using stainless steel (SS) as the anode and Titanium (Ti) as the cathode, and SS paired with graphite scored 23.1%. Voltage levels positively influenced gas production, although higher potentials resulted in electrode surface oxidation and decreased efficiency. The optimum voltage range of 4.5V to 5.2V for SS with graphite and 4.2V to 5.2V for SS with Ti configurations was provided. The study concludes that the SS-Ti and SS-Graphite configurations are optimal options for HHO gas production, minimizing heat generation and energy consumption while enhancing gas output. These findings suggest significant potential for improving fuel efficiency and reducing greenhouse gas emissions in two-wheel vehicle four-stroke gasoline engines (100cc to 150cc).

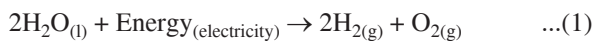
INTRODUCTION

The trend of renewable energy resource utilization has increased with higher demand, driven by increasing awareness of the harmful effects of fossil fuel consumption. It has been identified as a significant contributor to greenhouse gas (GHG) emissions, leading to climate change and global warming in transportation. The growing concern over fossil fuel depletion of non-renewable energy sources has further highlighted the urgent need to transition to green energy sources, such as solar, wind, and hydropower (Jones 2014). These have appeared as viable and cleaner energy options over general fossil fuel-consumed internal combustion engines (ICE). It has been blown up by the global focus on emission reductions and meeting international climate milestones (Zohuri 2018, Chinguwa et al. 2020).

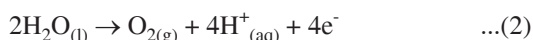
Furthermore, hydrogen (H₂) plays a vital role in the evolution of alternative fuels because of its purity and higher energy content compared with other fuels. Hence, it can be supported to achieve the circular economy through sustainable development goals (SDGs), affordable and clean energy (SDG 7), industry innovation (SDG 9), and climate action (SDG 13) (Rinawati et al. 2022). H₂ can lower GHG emissions, aligning with global efforts to address climate change

(Khandal et al. 2022). H_2 offers a unique advantage in providing a closed-loop energy system, utilizing renewable resources to meet energy demands in a cleaner, more efficient manner (Yurukcu et al. 2021).

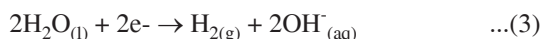
Different technologies can be applied to produce H_2 , such as Steam Methane Reforming (SMR), Partial Oxidation, Coal Gasification, and Electrolysis (Bard & Faulkner 2001, Zohuri 2018). The electrolysis process by water splitting is better aligned with the ICE application, most commonly due to its purity and applicability (Petrovic & Hossain 2020). Direct current (DC) electricity is required as the power source to generate HHO gas (Newman & Thomas-Alyea 2012). Hence, this method is named green H_2 gas (Bard & Faulkner 2001). HHO generators comprise an electrolyte consisting of two electrodes, the anode and cathode, configured as a sandwich, and electrolytes enter inside that chamber, fixed with a gasket. As mentioned, the type of specific electrolyte solution used in HHO generators may differ from the purpose of the application (Huang & Wang 2020, Wang 2022). During electrolysis, an electric current is passed through the electrolyte solution, leading to the decomposition of water molecules into H_2 and Oxygen (O_2), and the whole process of HHO generation is represented in the following 1, 2, and 3 Equations from (Bard & Faulkner 2001, Fuller & Harb 2018).



Half-cell chemical reaction of the Anode (Oxidation)



Half-cell chemical reaction of the Cathode (Reduction)



In the electrolysis process, there are two main types of alkaline generators separated by considering cell configuration, such as Wet-type and dry-type HHO generators in Fig. 1. Alkaline-wet HHO generators mainly use electrolytes of sodium hydroxide (NaOH) or potassium hydroxide (KOH) in a liquid state. The electrode stack sink

is filled with electrolyte solutions in the container. Dry-type HHO generators only touch electrolytes inside the gasket with the electrode as a sandwich, which is used to fix wells except for leakages (Wilberforce & Olabi 2020). They operate with a dry cell configuration with liquid electrolyte. For instance, dry-type HHO generators are preferred due to their efficiency. Unlike wet cells, they do not submerge electrodes in liquid but instead use conductive material to facilitate the reaction (Sivakumar et al. 2021).

While HHO gas can be integrated with internal combustion engines (ICEs), it has been explored as a fuel additive to enhance ICE performance and improve the purity of transportation emissions. Studies by Arjun et al. (2019) and Chinguwa et al. (2020) reported that integrating HHO gas into gasoline engines improves combustion efficiency and minimizes harmful emissions of GHGs due to the improved engine performance of brake power by reducing fuel consumption. Specifically, Chinguwa et al. (2020) demonstrated a 10% increase in thermal efficiency and a 34% reduction in fuel consumption when HHO gas was blended with gasoline in a 0.1M NaOH electrolyte solution by following alkaline electrolysis. Integrating HHO gas into ICEs also addresses common challenges like detonation and flashback. Blending HHO gas with gasoline at a suitable ratio (1:8) to prevent engine knock, detonation, pre-ignition, and flashback could reduce CO and HC emissions by 26% and 27%, and SAC levels decreased to 9%, respectively (Arjun et al. 2019). This improvement is attributed to the higher energy density of H_2 , which promotes a more complete combustion process.

Nevertheless, several challenges remain in optimizing the HHO system with ICEs. Typically, electrode degradation due to oxidation is a critical issue, as it affects the long-term durability and efficiency of the system. Studies by Bockris et al. (2002) and Fuller & Harb (2018) explored the impact of electrode material choices on system performance, emphasizing the need for corrosion-resistant materials

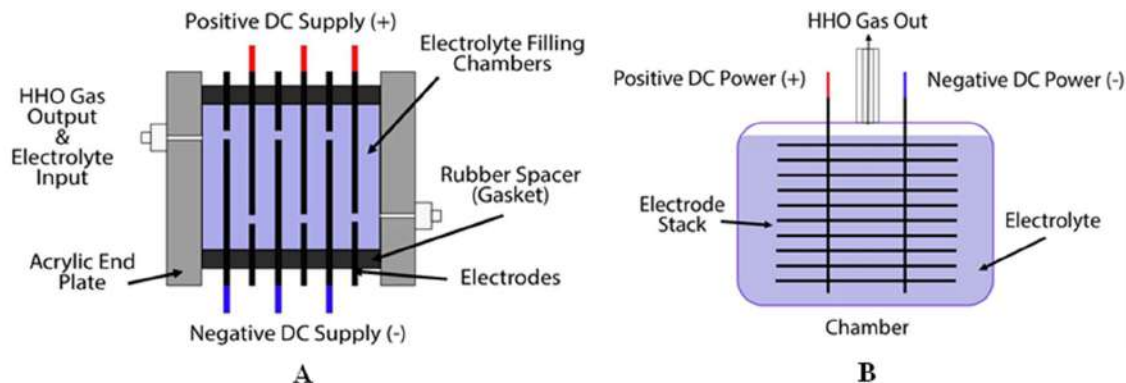


Fig. 1: Dry-Type Alkaline Electrolyser (A), Wet-Type Alkaline Electrolyser (B).

such as titanium (Ti) and stainless steel (SS). A significant challenge is heat and vapor production during electrolysis. Hence, it requires improvements in critical areas to enhance the efficiency of HHO production by electrode materials and electrolyte solutions (Arjun et al. 2019, Rusdianasari et al. 2019).

Additionally, Khandal et al. (2022) highlighted that maintaining an optimal electrolyte concentration is crucial to minimizing energy losses and heat generation. Hence, recent innovations are being conducted to improve the combination of electrode materials to leverage their complementary properties. For instance, Rimkus et al. (2023) found that combining SS with graphite resulted in a higher gas production rate than single-material configurations.

This study aims to improve the design of the alkaline dry-type HHO kit and its efficiency by examining the effects of various electrode materials, electrolyte types, and their concentrations on HHO gas production. This research seeks to enhance the performance of the HHO kit system integrated with selected ICEs by minimizing heat and vapor generation during electrolysis. It facilitates the prevention of engine failures by identifying significant issues and contributes to a more sustainable and environmentally friendly transportation sector.

MATERIALS AND METHODS

Reagents and Materials

SS sheets, Graphite sheets, and Ti sheets were purchased from the AliExpress retail service by Alibaba.com in China. EPDM rubber sheet and copper sheet were used. The chemicals NaOH and KOH, as well as distilled water, were used with more than 99.98% purity. Plexiglass (Acrylic) sheet and tube (transparent), polyvinyl-chloride (PVC) endcap, pneumatic fittings, and hose were used. YAXUN 1502DD⁺ bench power supply (12V/2A DC), UNI-T UT136⁺ digital multimeter (potentiometer), Peristaltic DC pump, and AFM0725H00 gas flow meter were utilized. The solvent cement of S-Lon 10min rapid was used.

Table 1: Specifications of Developed Alkaline Dry-Type HHO Kit Parameters.

Parameter	Unit	Specification
Operating Voltage (DC)	V	1.4-7.0
Working Current range	mA	1-700
Max Cell Power	W	4.9
The volume of the HHO generator	cm ³	1.6
Reactive area of electrodes	cm ²	4
Solution Molarity	Moles.cm ⁻³	0.08-0.12
The volume of the gas separator	cm ³	157

Design and Construction of the HHO Generator

The developed HHO kit consisted of two central units: the generator unit and the separator/bubbler unit. Table 1 presents the specifications of the developed Alkaline Dry-Type HHO kit.

The generator unit was built using a single cell, which included one anode and one cathode. The electrode plates were arranged in layers and separated by gaskets made from EPDM rubber. The EPDM rubber is known for its resistance to high temperatures and helps prevent leaks. Electrode plates and gaskets were rigidly connected to copper terminals, which were used to supply power. The frame plate of the generator was made from transparent plexiglass, and it had eight holes drilled to connect the electrolyte pipes and gas outlets. The generator unit was assembled using SS nuts and bolts, ensuring a tight and secure fit. The gas outlet's hole size was 4M, and the others were 2 M. The bubbler unit was constructed from PVC end caps and a transparent acrylic tube. The Bubbler unit was fixed with an acrylic tube and PVC endcaps. It was bonded by solvent cement to make seals to prevent electrolyte leakages. Fig. 2 illustrates the design followed to construct the kit, and Table 2 illustrates the measures of all materials used for the HHO kit.

Pneumatic fittings were fixed to the 4M tread hole, and solvent cement was applied to make the systems of the generator and bubbler units fully closed. Fig. 3 illustrates the constructed HHO kit with materials in sub-units.

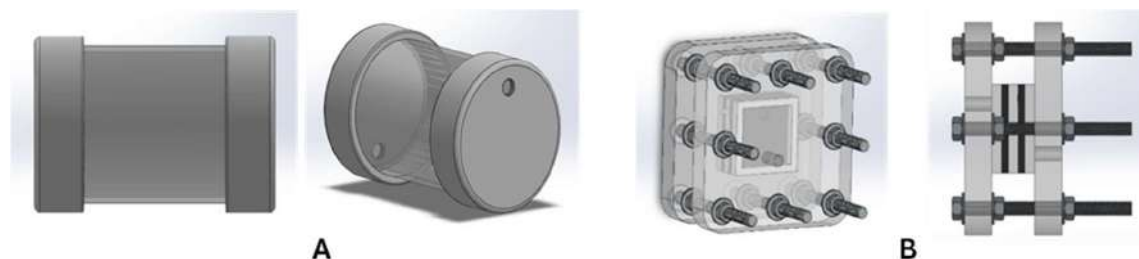


Fig. 2: Design of Bubbler Unit (A), Design of Generator Unit (B).

Table 2: Materials and Equipment for HHO Kit and Associated Components.

Component/Application	Materials and Equipment	Dimensions/Quantity
HHO Generator Unit	Plexiglass (Acrylic) 8mm thick	80cm ²
	SS grade 306 sheet	25mm × 25mm
	Graphite sheet 2mm thick	25mm × 25mm
	Ti grade II sheet	25mm × 25mm
	EPDM rubber sheet 3mm thick	25mm × 25mm
	copper sheet 1mm thick	4mm × 2mm
Bubbler Unit	Polyvinyl chloride (PVC) endcap	Φ 50mm
	Plexiglass (Acrylic) tube	Φ 50mm
Chemicals and Solvents	NaOH and KOH	6.2g + 4.8g
	Distilled water	120mL
Fitting and Fixings	Pneumatic fittings	6M
	Pneumatic hose	Φ 4mm
	SS nuts and bolts	4M
Bonding Glue	S-Lon solvent cement	5mL
Testing Equipment	Bench power supply	12V/2A
	Potentiometer	-
	Peristaltic pump	12V
	Gas flow meter	-
	Measuring cylinder	250mL
	Petri dish	-
	Beaker	100mL
	Plastic basing	5000dm ³



A



B



C

Fig. 3: Work Completed Electrodes of SS, Graphite and Ti, EPDM Gaskets, Copper Terminals, Acrylic Fixing Plates with SS Nuts, Bolts and Washers (A), Finalized HHO Generator Unit (B), Finalized Bubbler Unit (C).

Laboratory Testing

The laboratory testing was conducted in two phases. The first phase was conducted to study the behavior of the constructed HHO generator unit. There, the HHO generator was connected to the bench power supply, and its performance was observed under different voltage levels in 1-minute intervals. A peristaltic pump was used to supply the electrolyte at a constant rate of 11 mL.min⁻¹. Current and voltage readings were taken using a potentiometer with a bench power supply display to measure the voltage addition of the potentiometer. The voltage varied from 0V to 7V. An

electrolyte of 0.1M KOH was used, with a 3 mm gap between the electrodes. Generated HHO gas was released by the outlet line of the bubbler unit; that line was connected to the exhaust line to remove it safely due to the highly flammable gas. The goal was to monitor how the generator behaved under different electrical conditions. Fig. 4 presents phase one of the experimental work.

In the second phase, tests were conducted using both KOH and NaOH hydroxides as the electrolytes with 0.08M, 0.1M, and 0.12M molar concentrations because (Chinguwa et al. 2020) say that the range of KOH and NaOH had resulted

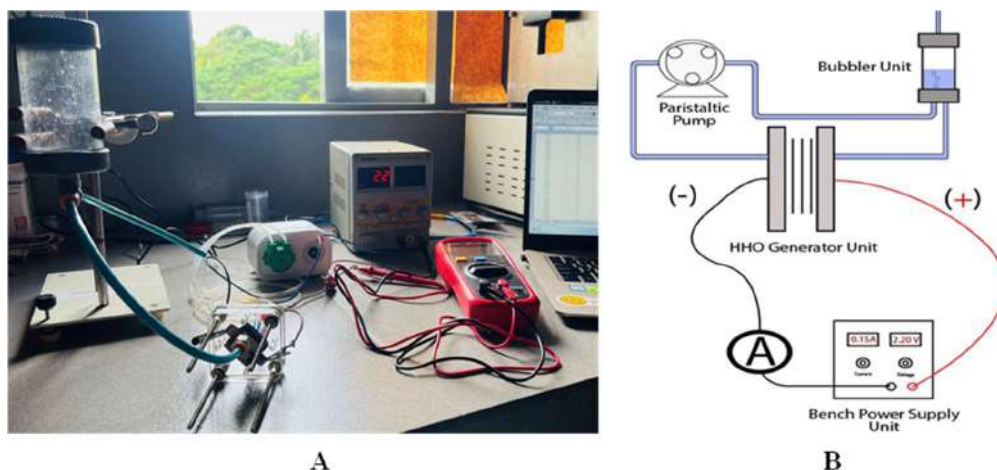


Fig. 4: Apparatus of the whole System (A), Schematic Diagram of the Work (B).

ideally. The measuring cylinder apparatus was used to capture and measure the produced gas, and the gas flow meter was used to measure the flow further to reduce errors. The electrolyte's flow rate remained $11 \text{ mL} \cdot \text{min}^{-1}$ throughout the tests. Various factors, including electrolyte type, electrode material, and voltage (1.4V to 6.4V), were adjusted to determine their effects on the gas production rate and energy consumption during the same time frames to observe the optimum configuration. Fig. 5 illustrates phase two of the research work.

Efficiency Calculation

The efficiency of the HHO generator was determined using Faraday's laws of electrolysis. According to Faraday's law, the amount of gas produced depends on the current passing through the electrolyte. The theoretical volume of gas was calculated using the following Equation 4:

$$V_{\text{theoretical}} (\text{L}) = \frac{I \times t}{n \times F} \times 22.4 \quad \dots(4)$$

Where I is the current in Amperes (A), t is the time in seconds (s), n is the number of electrons involved, F is Faraday's constant ($96485 \text{ C} \cdot \text{mol}^{-1}$), and 22.4 L is the molar volume at Standard conditions.

The actual amount of gas produced was compared to the theoretical value using Equation 5 to calculate how efficiently the generator converted electricity into gas.

$$\text{Faradaic Efficiency } \% (\eta_F) = \frac{V_{\text{actual}} (\text{L})}{V_{\text{theoretical}} (\text{L})} \times 100 \quad \dots(5)$$

Where η_F represents Faradaic efficiency, $V_{\text{(actual)}}$ is the exact volume of gas produced, and $V_{\text{(theoretical)}}$ is the expected volume of gas, calculated using Equation 4.

Next, Equation 6 calculated the thermodynamic efficiency to determine how much applied voltage was

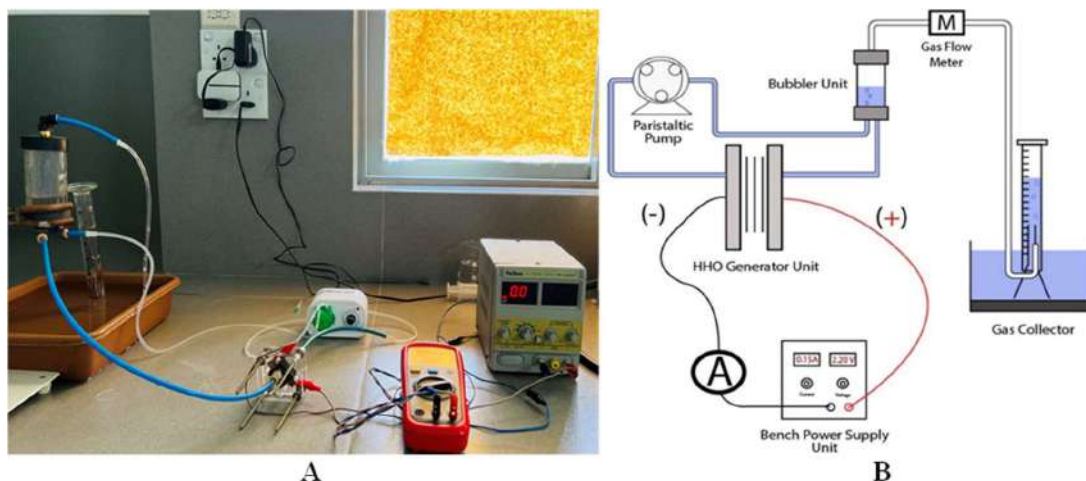


Fig. 5: Apparatus of the Testing (A) Schematic Diagram (B).

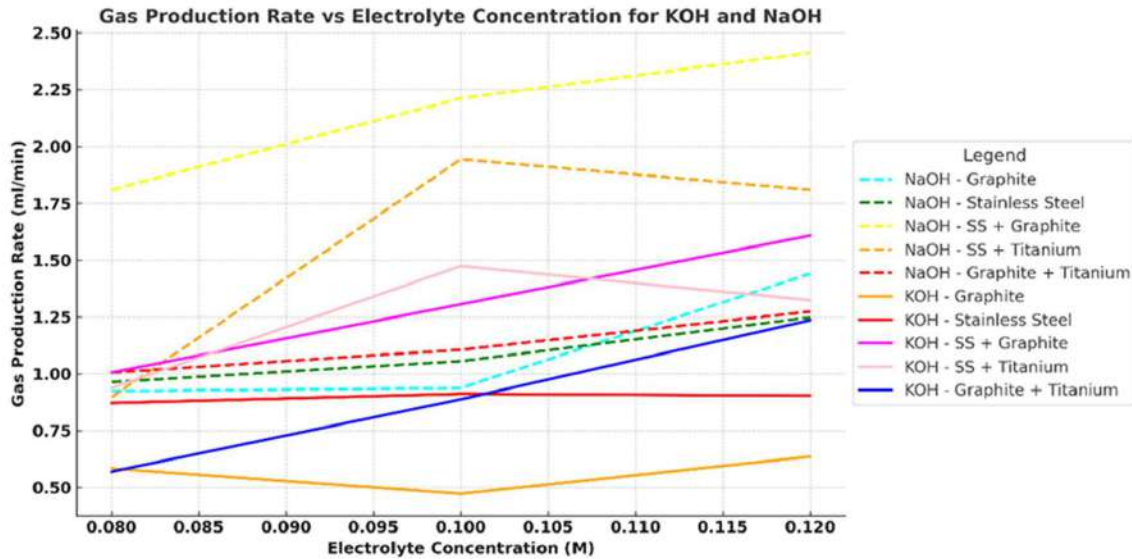


Fig. 6: Gas Production Rate vs Electrolyte Concentration ($T = 25\text{ }^{\circ}\text{C}$, $p = 1\text{ atm}$, $q = 11\text{ mL}\cdot\text{min}^{-1}$).

necessary to split water into hydrogen and oxygen.

$$\text{Thermodynamic Efficiency } \% (\eta_{th}) \frac{1.23V}{V_{supply} (V)} \times 100\% \dots(6)$$

Where 1.23V is the theoretical voltage needed for the water-splitting reaction.

Finally, the overall efficiency of the generator was determined by combining both the Faradaic and thermodynamic efficiencies that represent Equation 7.

$$\eta_t = \eta_{th} \times \eta_F \dots(7)$$

Overall efficiencies of each electrode configuration helped to conclude how well the HHO generator converts electrical energy into gas, considering both the actual gas production and the energy used in the result and discussion sections.

RESULTS AND DISCUSSION

Gas Production Rate vs Electrolyte Concentration

The gas production rate increased with the higher concentration of both Potassium Hydroxide (KOH) and Sodium Hydroxide (NaOH) across Graphite, SS, and Ti electrode configurations. The graph (Fig. 6) shows production that increased at concentrations above 0.10M due to the higher ion mobility and placing optimum point. The reaction rate is boosted with gas generation. Further, NaOH steadily produced more gas than KOH for all electrode setups. The combination of SS and Graphite also performed flawlessly at a $2.4\text{ mL}\cdot\text{min}^{-1}$ gas production rate at 0.12 M concentration, especially with NaOH. Further, the SS and graphite electrode configuration

reached about $1.3\text{--}1.4\text{ mL}\cdot\text{min}^{-1}$ at the same concentration of KOH, which was the second-best performance.

Other setups of the SS and Ti combination performed moderately. Combined with Ti, Graphite combined and graphite alone appeared to have the lowest gas production rates for both electrolytes. When graphite acts as an anode, it could accelerate corrosion in nearby metals. Conversely, Ti has lower metal activity than graphite in the Galvanic series. Hence, graphite acts as the cathode, and Ti acts as the anode in this configuration. However, Ti has excellent corrosion resistance due to its ability to form a stable oxide layer. Overall, the SS and Graphite setup with NaOH at 0.1M proved to be the most effective for gas production by graph (Fig. 6).

Gas Production Rate vs Voltage

The results in Fig. 7 show that the effect of the gas production rate increases proportionally with the voltage of all electrode configurations. The SS and Graphite combination demonstrated the highest gas production rate of approximately $0.234\text{ mL}\cdot\text{min}^{-1}$ at 2.6 V. This setup consistently outperforms others, indicating high efficiency in generating gas at higher voltages. Graphite alone and graphite combined with Ti show much lower gas production rates, with minimal gas output at lower voltages and slower increases as voltage rises. SS alone shows moderate performance but is significantly outperformed by the combination of SS Steel and Graphite. This trend suggests that pairing different electrode materials, particularly SS with Graphite, greatly enhances gas production efficiency as voltage increases.

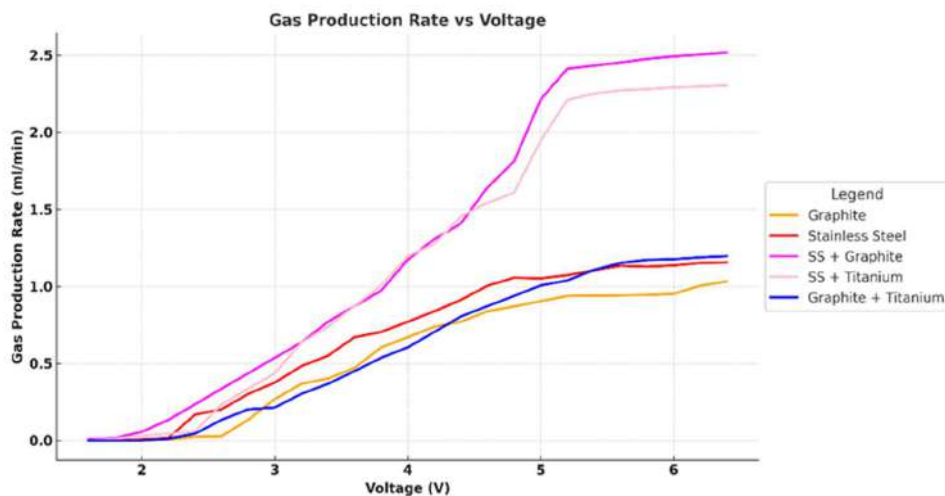


Fig. 7: Gas Production Rate vs Voltage ($C = 0.1M$, $T = 25\text{ }^{\circ}C$, $p = 1\text{ atm}$, $q = 11\text{ mL}\cdot\text{min}^{-1}$).

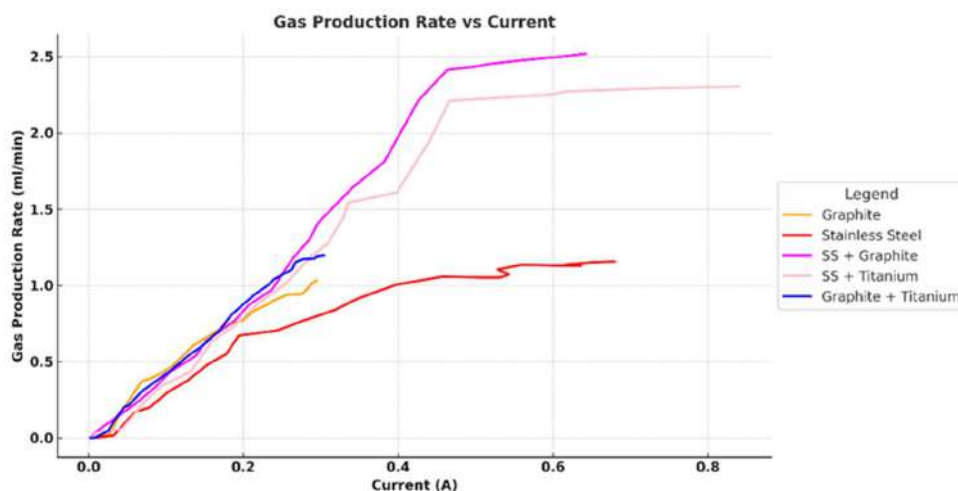


Fig. 8: Gas Production Rate vs Current ($C = 0.1M$, $T = 25\text{ }^{\circ}C$, $p = 1\text{ atm}$, $q = 11\text{ mL}\cdot\text{min}^{-1}$).

Gas Production Rate vs Current

The gas production rate increases with the current across different electrode configurations of Graphite, SS, and Ti. Higher current levels require more energy to drive the electrochemical reactions responsible for more gas generation. Including all setups, the combination of SS and Graphite steadily produced the most HHO gas of approximately $0.234\text{ mL}\cdot\text{min}^{-1}$ at a current of 0.06 A , which is shown in Fig. 8. SS alone performed moderately, with its gas production rate steadily rising to around $0.168\text{ mL}\cdot\text{min}^{-1}$ at similar current levels. Other setups, such as Graphite alone and Graphite combined with Ti, showed significantly lower gas production rates. These configurations generated minimal gas at lower current levels and exhibited slower increases in production as the current rose. The combination of SS and Ti

also performed moderately, with a maximum gas production rate of around $0.058\text{ mL}\cdot\text{min}^{-1}$.

Current vs Voltage

The current increased with rising voltage for all electrode configurations due to Ohm's law. The combination of SS and Ti shows the most significant current enhancement, especially at higher voltages above 2.6 V , with 0.09 A . This combination emphasizes higher current flow, potentially due to improved conductivity between the two materials. Graphite alone shows the least current, remaining relatively low even as voltage increases. Other SS and Graphite combinations show steady increases in current, but not higher than the SS and Ti combination. This pattern highlights that the choice of electrode material affects the current flow, with

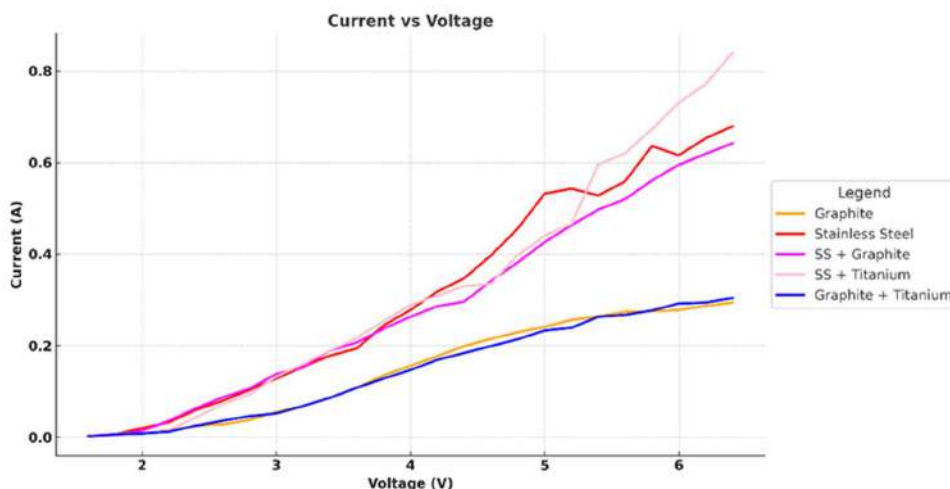


Fig. 9: Current vs Voltage ($C = 0.1M$, $T = 25\text{ }^{\circ}C$, $p = 1\text{ atm}$, $q = 11\text{ mL}\cdot\text{min}^{-1}$).

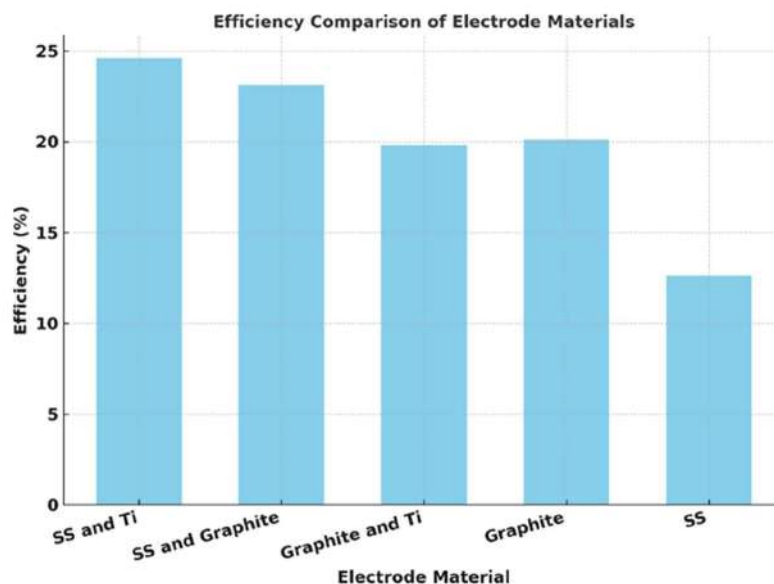


Fig. 10: Efficiency Comparison of Electrode Materials ($C = 0.1M$, $T = 25\text{ }^{\circ}C$, $p = 1\text{ atm}$, $q = 11\text{ mL}\cdot\text{min}^{-1}$).

the material Ti offering better conductivity when paired with others. According to Fig. 9, SS and Graphite yield the best gas production performance compared with SS and Ti because SS and Graphite configuration performed more similarly leaner distribution with more sooner to one of the R2 values considering operational priorities. Hence, SS and Ti performed as second-best configurations.

Efficiency Comparison of Electrode Materials

The bar chart (Fig. 10) compares different electrode materials based on overall efficiency calculated from Faradaic efficiency (η_F) and thermodynamic efficiency (η_{th}). The

data shows that SS and Ti achieved the highest efficiency, 24.6%, and the second highest was SS and Graphite, 23.1%. The combination of Graphite and Ti had a moderate efficiency of 19.8%. Graphite alone reached 20.1%, and SS alone had the lowest efficiency, at 12.6%.

Furthermore, efficiency reduction was significantly attributed to high-potential scenarios like heat generation and electrode surface oxidation. Surface reduction degrades materials and reduces performance. Electrodes can oxidize on their surface according to the electrochemical dynamics. In this environment, NaOH acts as a strong electrolyte, dissociating into Na^+ and OH^- ions and facilitating



Fig. 11: Electrolyte Color Change by Metal Oxidation (A), Appearance of Graphite and Ti electrode surfaces after oxidation (B).

electrolysis. Hence, due to their intrinsic material properties and electrochemical behavior, graphite electrodes oxidize more readily than SS and Ti when used as an anode in an alkaline HHO generator. Fig. 11 illustrates the oxidation results of graphite as an anode application and Ti electrode as a cathode at 20 to 30 minutes of operation. Graphite comprises carbon atoms arranged in a layered structure with weak van der Waals forces between the layers. This structure provides high electrical conductivity but also exposes edge sites and defects, which are highly reactive. In an alkaline NaOH solution, these reactive sites are susceptible to attack by OH^- ions, forming CO_2 or carbonate ions, which degrade the graphite structure.

In contrast, SS and Ti form stable, protective oxide layers of Cr_2O_3 for SS and TiO_2 for Ti on their surfaces when exposed to oxidative environments. These passive layers significantly reduce the rate of further oxidation and protect the underlying material from degradation. Graphite, lacking such a protective mechanism, remains exposed to continuous attack by the electrolyte, leading to higher oxidation rates. Moreover, graphite's relatively lower overpotential for anodic reactions facilitates oxidation at a lower applied voltage, making it less durable than SS and Ti under similar conditions. It makes graphite less favorable for long-term use as an anode in highly oxidative environments.

CONCLUSIONS

The study successfully optimized the performance of a dry-type alkaline HHO electrolyzer, emphasizing the effects of electrode materials, electrolyte concentration, and applied voltage on gas production efficiency. Among various configurations, SS paired with Ti and a 0.12M NaOH solution achieved the highest gas production efficiency at 24.6%. SS paired with graphite scored 23.1%, getting the second-highest result. Further, the optimum voltage range is 4.5V to 5.2V for SS with graphite and 4.2V to 5.2V for SS with Ti configurations. The results demonstrate that increasing the voltage enhances gas output and leads to electrode degradation due to surface oxidation, which limits long-term efficiency.

Furthermore, with 0.12M NaOH, NaOH was a more suitable electrolyte than KOH, promoting higher gas production rates. This research provides a clear path for enhancing HHO gas production while minimizing energy consumption and preventing electrode deterioration. The findings support optimized HHO systems integrating into two-wheeler vehicle four-stroke ICEs to improve fuel efficiency and reduce GHG emissions. The next phase of this research will involve integrating the system with 100cc gasoline internal combustion engines (ICE) in two-wheeled vehicles. This approach could support broader adoption and contribute to further reductions in emissions. Hence, it can extend its positive application to three-wheel and four-wheel vehicles in future improvements.

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Addressing Climate Change Through Renewable Energy Generation for Sustainable Development in India: Challenges, Current and Future Status

Mohammed Sharif^{1†}  and Owais Ul Hassan² 

¹Department of Civil Engineering, Jamia Millia Islamia, New Delhi-110025, India

²Department of Civil Engineering, Indian Institute of Technology, Jammu-181221, India

†Corresponding author: Mohammed Sharif; Mohammed Sharif, msharif@jmi.ac.in

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ABSTRACT

The rapid population and economic growth in India have led to a significant rise in energy demand. Meeting this demand through renewable energy is critical, as fossil fuel-based energy generation contributes heavily to greenhouse gas emissions. Renewable energy development offers a sustainable and comprehensive approach to addressing the dual challenges of climate change mitigation and energy security. This paper evaluates the current status of renewable energy development in India and its role in mitigating the adverse effects of climate change. A detailed analysis of trends in solar, wind, and hydropower development reveals that India's installed solar capacity is 82 GW, with an additional 88 GW under development. Wind energy accounts for approximately 46 GW of installed capacity, with 32 GW under development. Hydropower contributes 47 GW of installed capacity, with 18 GW under development. Together, these sources amount to approximately 175 GW of renewable energy capacity. Despite significant progress, India faces substantial challenges in achieving its ambitious renewable energy target of 500 GW by 2030. This paper highlights various programs initiated by the Government of India to promote renewable energy and ensure sustainable development. Additionally, it identifies key challenges in meeting the targets and recommends innovative solutions to overcome these hurdles, paving the way for a sustainable energy future.

INTRODUCTION

The United Nations Department of Economic and Social Affairs (UN DESA) has projected the global population to range between 9.4 and 10.1 billion by 2100, depending on the fertility rate scenario (UN DESA 2019). As this growth continues, the demand for essential resources such as food, water, and shelter will increase, creating significant environmental challenges. To ensure our continued existence, we must adapt and embrace a lifestyle that fosters harmony with the natural world (Pandey 2002). A major driver of economic growth is energy. The International Energy Agency (IEA 2022) predicts a 1.2% annual increase in global primary energy demand from 12,300 million tons of oil equivalent (MTOE) in 2008 to 16,700 MTOE by 2035, marking a 40% increase overall (IEA 2010). Environmental concerns are intricately intertwined with various factors such as population dynamics, technological advancements, economic paradigms, and socio-political considerations. Changes in driving forces, including population growth, lifestyle choices, technological innovations, and socio-economic strategies, have significantly impacted the global climate system. The Intergovernmental Panel on Climate Change (IPCC) has warned that human activities, particularly the burning of fossil fuels, have led to a significant rise in global temperatures (IPCC 2021).

The driving forces described by Doos (1995) have notable impacts, particularly on greenhouse gas (GHG) emissions, affecting both the natural environment and the ecosystem crucial for human sustenance. The proliferation of industries has led

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to heightened utilization of energy resources, consequently escalating the emission of GHGs into the atmosphere, thereby playing a pivotal role in climate change. Various GHGs such as CO₂, CH₄, and N₂O are generated through natural and human-induced activities. While natural mechanisms exist for removing substantial quantities of GHGs from the atmosphere, anthropogenic activities have surpassed the Earth's natural capacity to mitigate these gases effectively. A major contributor to CO₂ emissions is the power generation sector, mainly due to the burgeoning demand for fossil fuel-powered plants in developing nations (Soares 1999). Anthropogenic CO₂ emissions are principally attributable to suboptimal combustion of fossil fuels and biomass. Additionally, non-methane hydrocarbons are generated through anthropogenic activities of biomass and fossil fuel burning and solvent usage. These pollutants undergo atmospheric reactions with sunlight, contributing to the formation of tropospheric Ozone (O₃) - a significant GHG (Soares 1999). To reduce the adverse effects of climate change, transitioning to green energy sources is crucial. By decreasing dependence on fossil fuels, this shift can manage climate change challenges and energy security concerns. Renewable energy presents a promising solution as it generates power without emitting GHG and relies on abundant, sustainable resources. This approach aligns with global efforts to promote sustainability and combat climate change, encouraging policymakers to prioritize renewable energy in their strategies. For a country like India, with abundant solar, wind, and biomass resources, transitioning to renewable energy is not only feasible but also economically advantageous.

India has demonstrated a strong commitment to combating climate change by aligning its energy and environmental policies with the agenda of the Paris Agreement. As a signatory, the country has pledged to achieve 40% of its installed power capacity from non-fossil fuel sources by 2030 and to reduce the emissions intensity of its GDP by 33-35% from 2005 levels. These targets underscore India's proactive role in addressing the global climate crisis while maintaining its developmental trajectory. Renewable energy development is central to fulfilling these commitments. By transitioning to renewable energy, India aims to reduce its reliance on fossil fuels, which are not only major contributors to GHG emissions but also a significant source of air pollution. Air pollution, primarily from the combustion of fossil fuels, poses severe health risks, including respiratory and cardiovascular diseases. The adoption of cleaner energy sources is expected to significantly improve air quality, particularly in urban areas where pollution levels are alarmingly high, thereby enhancing public health and reducing healthcare burdens.

The development of the renewable energy sector has a significant impact on socio-economic status, as well.

Therefore, the government of India (GOI) has identified renewable energy as a major driver for job creation. From manufacturing and installation to maintenance and research, the sector offers diverse employment opportunities, contributing to economic development. Given the multifaceted advantages of renewable energy, the major objective of the present research is to critically evaluate the status of renewable energy development in India with a focus on its contribution to combating climate change. The intent is to provide a comprehensive understanding of the role of renewable energy in India's climate action strategy, offering insights that can inform future policies and initiatives. This paper also reviews several programs designed to mitigate the adverse impacts of climate change in India, with a focus on achieving Sustainable Development Goals (SDGs). The challenges associated with achieving the renewable energy target of 500 GW by 2030, and innovative solutions to address these challenges, are also discussed in the paper.

MATERIALS AND METHODS

The methodology of this research involves a comprehensive approach to addressing climate change in India through renewable energy development. The first step is to describe the causes and impacts of climate change, focusing on key drivers such as GHG emissions from industrial activities, deforestation, and fossil fuel consumption. The environmental and socio-economic impacts, including rising temperatures, extreme weather events, and threats to agriculture and water resources, are described to underscore the urgency of mitigation efforts. Next, the study discusses India's plan for climate change mitigation, highlighting national strategies like the National Action Plan on Climate Change (NAPCC) and commitments under the Paris Agreement. Key initiatives such as the National Solar Mission, National Water Mission, and the Green India Mission are reviewed to assess their contributions toward achieving climate goals. The role of Sustainable Development Goals (SDGs), particularly SDG 7 (Affordable and Clean Energy) and SDG 13 (Climate Action), in combating climate change is discussed next. The alignment of these SDGs with India's renewable energy and sustainability objectives is examined to emphasize their importance.

The next step in the methodology is to include an evaluation of the current status of renewable energy development in India, analyzing progress in solar, wind, and hydropower capacities and identifying regional and sectoral gaps. The study then identifies challenges in meeting renewable energy targets, such as infrastructure limitations, financial constraints, and policy inconsistencies. Finally, innovative solutions are recommended to overcome these

challenges and ensure the achievement of India's renewable energy target of 500 GW by 2030.

The data on installed capacity and capacity under development for wind, solar, and hydropower has been sourced from the Central Electricity Authority (CEA 2023) and the Ministry of Power, GOI, recognized as the most reliable sources for information on these forms of renewable energy. Other sources of data on installed capacity include the NITI Aayog, the apex policy-making body of India. The data of the National Action Plan on Climate Change has been obtained from the Ministry of Environment and Forest, GOI.

Causes and Impacts of Climate Change in India

Climate change refers to long-term alterations in climate patterns driven by natural processes, external factors, or human activities that modify atmospheric composition. The IPCC (IPCC 2022) emphasizes variability over extended periods, while the UNFCCC specifically attributes change to human actions. The Environmental Protection Agency (EPA) highlights significant shifts in temperature, precipitation, and wind patterns over decades. The fifth assessment report of IPCC (IPCC 2022) suggests that extreme hydrological events are more likely to intensify due to climate change. These changes are expected to impact natural ecosystems and socio-economic systems, as noted in India's National Communications Report to the UNFCCC (INC 2004). The second working report by the United Nations anticipates significant coastal erosion due to the escalating sea levels, projected to rise by approximately 40 cm. This surge is attributed to the accelerated melting of glaciers in the Himalayas and the Hindu-Kush ranges. The ramifications include the potential displacement of around half a million individuals in India, primarily due to heightened flooding in coastal regions.

Additionally, there are concerns about the escalation of salinity levels in both groundwater in the Sundarbans and surface water in coastal areas (Bindoff 2007). Research indicates a decline in mountain glaciers and snow cover across both hemispheres. This widespread reduction is strongly linked to climate change, a contributing factor to the observed rise in sea levels. In the Indian mainland, it is estimated that a rise in sea levels would inundate approximately 5,763 square kilometers of coastal land, displacing an estimated 7 million residents.

The dominance of fossil fuel-based power generation, including coal, oil, and gas, has led to significant adverse climatic changes in India. With significant population growth in recent decades, energy demand has grown substantially, leading to a sharp increase in carbon dioxide emissions

(Asumadu-Sarkodie & Owusu 2016). Traditional fossil fuel-based energy production emits between 0.5–0.97 kg of carbon dioxide (CO₂) per kWh (Abdallah & El-Shennawy 2020). As of March 2019, atmospheric CO₂ levels have risen to 414.8 ppm, far exceeding the recommended optimal level of 350 ppm. Global CO₂ emissions currently surpass 36.8 Gt, with India contributing approximately 6.7% (based on 2016 data) (O'Neill 2020, Suresh & Rao 2017). In 2007, India emitted 1221.76 Mt of CO₂, 20.56 Mt of CH₄, and 0.24 Mt of N₂O, totaling approximately 1727.71 Mt CO₂ equivalent. The largest share (37.8%) was contributed from electricity generation, followed by industry (21.7%), agriculture (17.6%), transportation (7.5%), and residential (7.2%) sectors (MoEF 2010).

While a thorough compilation detailing the effects of climate change in Table 1 on India remains elusive, three key impacts stand out: (1) the rising sea levels resulting in the inundation of coastal regions, (2) the detriment to agriculture and infrastructure caused by more frequent extreme weather events, and (3) the various other environmental concerns. Each of these phenomena, among others, presents significant threats to India's well-being.

India's Plan for Climate Change Mitigation

To mitigate the impacts of climate change driven by high GHG emissions, renewable energy sources hold significant potential for replacing fossil fuels in electricity generation. This transition plays a vital role in reducing GHG emissions and combating climate change (Edenhofer 2011). However, reducing these emissions is quite challenging, given that around 16 million people in India still lack access to electricity (IEA 2022), highlighting the ongoing need for equitable development and energy access. Sharma et al. (2020) emphasize the perspectives of developing nations on justice, advocating for the fair distribution of mitigation responsibilities alongside considerations of efficiency and cost-effectiveness. Additionally, Halsnaes & Shukla (2008) explore international cooperative mechanisms and financing strategies to support climate mitigation policies in developing countries.

Table 2 presents different plans for mitigation of climate change impacts in India. These plans aim to address the challenges posed by climate change through legislative and policy initiatives. For instance, India's first National Action Plan on Climate Change (NAPCC), launched on June 30, 2008, outlines the current and future strategies for climate mitigation and adaptation. The plan includes eight national missions: the National Solar Mission, the National Mission for Enhanced Energy Efficiency, the National Mission on Sustainable Habitat, the National Water Mission, the

Table 1: Climate change impact parameters.

S. No.	Causes	Details	Impacts
01.	Meteorological effects	Sea-level rise and extreme weather events altered rainfall patterns in India, leading to more frequent droughts and floods.	Such changes cause altered weather patterns, irregular rainfall, extended droughts, and higher evaporation rates, contributing to water scarcity, as evidenced in many parts of India (Bhaktikul & Sharif 2020).
02.	Greenhouse emissions	The combustion of fossil fuels for energy production, transportation, and industrial activities releases GHGs like CO ₂ , CH ₄ , and N ₂ O into the atmosphere, traps heat, and causes global warming (IPCC 2021).	Rising global temperatures and sea levels (IPCC 2021). Heightened frequency and severe weather events like heatwaves, droughts, and storms (IPCC 2014b). Ocean acidification due to increased absorption of CO ₂ (Doney et al. 2009)
03.	Himalayan Glacial Retreat	Glaciers in the Himalayas are retreating at an alarming rate due to rising temperatures, threatening water resources and increasing the risk of glacial lake outburst floods (Bajracharya et al. 2015).	Reduced water availability for agricultural, domestic, and industrial uses (Immerzeel et al. 2010) Increased risk of glacial lake outburst floods, threatening downstream communities (Bajracharya et al. 2015)
04.	Deforestation and Land-Use Changes	The destruction of forests and other natural habitats, along with changes in land use, reduces the ability of the earth to absorb and store carbon, contributing to increased atmospheric CO ₂ levels (Houghton 2012).	Loss of biodiversity and habitat fragmentation (Newbold et al. 2015). Disruption of ecosystems and their services (Foley 2005). Increased soil erosion and desertification (Lal 2004).
05.	Agricultural Activities	Livestock farming, rice cultivation, and fertilizer utilization release CH ₄ and N ₂ O, which are intoxicating GHGs (Tubiello et al. 2015).	Contribution to GHG emissions, particularly methane and nitrous oxide (Tubiello et al. 2015). Soil degradation and water pollution from excessive fertilizer use (Tilman et al. 2002).
06.	Extreme Heat Events	India has experienced an increase in the frequency and intensity of heatwaves, with severe impacts on human health, agriculture, and ecosystems (Rohini et al. 2016).	Heat-related illnesses and increased mortality, especially among vulnerable populations (Rohini et al. 2016) Crop yield losses and livestock productivity declines (Chand et al. 2021)
07.	Industrial Processes	Industrial operations of cement production, chemical manufacturing, etc., release GHGs (IPCC 2014).	Release of GHGs, such as carbon dioxide and hydrofluorocarbons (IPCC 2014) Air and water pollution from industrial waste and emissions (Worrell et al. 2001)
08.	Increasing Population and Urbanization	The growing global population and urbanization lead to increased energy demand, transportation needs, and waste generation, contributing to GHGs (Seto et al. 2014).	Increased energy demand and resource consumption (Seto et al. 2014) Urban heat island effect and air pollution (Grimmond 2007)
09.	Coastal Flooding and Erosion	Sea-level change has altered rainfall patterns in India, leading to more frequent droughts and floods, affecting agriculture, water resources, and livelihoods (Nandargi & Dhar 2012).	Displacement of coastal communities and loss of infrastructure (Kumar & Tholkappian 2019) Damage to coastal ecosystems, such as mangroves and coral reefs (Spalding et al. 2014)
10.	Changing Rainfall Patterns	Climate change is contributing to the loss of biodiversity in India, with species facing habitat loss, changes in migration patterns, and increased extinction risks (Chaudhary & Bawa 2020).	Water scarcity and drought conditions affecting agricultural production (Nandargi & Dhar 2012) Increased risk of floods and landslides, causing damage to property and loss of life (Hirabayashi 2013)
11.	Biodiversity Loss:	The influence of climate change on Indian agriculture is profoundly significant (Shiva 2010). With 62% of cultivated land still reliant on rainfall, as documented by the Ministry of Environment and Forests in 2002, the agricultural sector in India remains fundamentally tethered to weather patterns.	Species extinctions, such as the Critically Endangered Pygmy Hog (<i>Porcula salvania</i>), due to habitat loss (Chaudhary & Bawa 2020) Disruption of ecosystems and their services, including pollination and pest control (Chapin 2000)
12.	Risk of Lower Agricultural Production and Food Security		According to reports, approximately 150,000 Indian farmers took their own lives within a span of nine years from 1997 to 2005. Although farm suicides have been recorded across various states, a staggering two-thirds of these tragedies are concentrated in Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh, and Kerala, despite these states accounting for only a third of the nation's population, as highlighted by The Hindu in 2007.
13.	Impact on Agricultural Productivity:	Escalating temperatures, shifting precipitation dynamics, and heightened frequency of severe weather phenomena are exerting detrimental effects on agricultural yields in India (Chand et al. 2021).	Reduced crop yields, particularly for wheat, rice, and pulses (Chand et al. 2021) Increased pest and disease incidence, affecting crop health (Chakraborty & Newton 2011)

National Mission for Sustaining the Himalayan Ecosystem, the National Mission for Sustainable Agriculture, the National Mission for a Green India, and the National Mission on Strategic Knowledge for Climate Change. Among these, the Jawaharlal Nehru National Solar Mission plays a pivotal role. This mission is supported by the Renewable Energy Procurement Obligation (RPO), which was amended under the National Tariff Policy in 2011 to increase the solar-specific RPO from 0.25% in 2012 to 3% by 2022.

The Energy Conservation Act of 2001 introduced mandatory measures like the energy conservation building code for commercial buildings, energy audits for energy-intensive industries, demand-side management initiatives, and industrial energy benchmarking (The Energy Conservation Act, 2001). The Electricity Act of 2003, the National Electricity Policy of 2005, and the Tariff Policy of 2006 encouraged the production and procurement of power from non-conventional and renewable sources. The National Rural Electrification Policies of 2006 aimed to provide off-grid renewable power to remote areas (MNRE 2011). Similarly, the Integrated Energy Policy (2006) emphasized energy efficiency and the promotion of renewable energy (IEP 2006).

India has committed to voluntary climate action through its Nationally Appropriate Mitigation Actions (NAMAs). The NAMAs target a 20-25% reduction in the GHG intensity of GDP from 2005 levels by 2020 (UNFCCC 2010). This commitment was further reinforced in India's Intended Nationally Determined Contribution (INDC), submitted on October 1, 2015. India pledged to derive 40% of its power generation capacity from non-fossil fuel sources and reduce emissions intensity by 33-35% from 2005 levels by 2030 (UNFCCC 2015). To support these goals, platforms like the Akshay Urja Portal and the India Renewable Idea Exchange (IRIX) Portal foster the exchange of environmentally sustainable ideas and promote global collaboration on renewable energy advancements. These comprehensive efforts underscore India's proactive approach to achieving a sustainable energy future while addressing climate change.

Role of SDGs in Combatting Climate Change

To achieve sustainability, it is imperative to identify crucial component systems and establish indicators that offer essential and dependable insights into the viability of each system and the overall structure. Sustainability

Table 2: India's plan for climate change mitigation.

S. No.	Plan and year	Specification
01.	National Action Plan on Climate Change (NAPCC, 2008)	Outlined eight national duties to address climate change (MOEF 2010). Focused on enhanced energy efficiency, sustainable habitats, water conservation, and increasing forest cover.
02.	National Mission for Enhanced Energy Efficiency (NMEEE) - 2008	Part of the NAPCC Aimed to achieve energy efficiency through various policies and programs.
03.	Jawaharlal Nehru National Solar Mission (JNNSM) - 2010	Part of NAPCC Aimed to promote solar energy and increase its share in the energy mix. The target of deploying 20 GW of grid-connected solar power by 2022.
04.	National Mission for Sustainable Agriculture (NMSA) - 2010	Part of NAPCC Focused on promoting sustainable agriculture practices to adapt to climate change
05.	National Mission on Strategic Knowledge for Climate Change (NMSKCC) - 2010	Part of NAPCC Aimed to build a knowledge network and support research on climate change
06.	Intended Nationally Determined Contribution (INDC) - 2015	Indian commitments under the Paris Agreement. sought to reduce the emissions from 2005 by 33-35% through 2030 achieve about 40% cumulative electric power installed capacity from non-fossiliferous resources by 2030
07.	National Clean Air Programme (NCAP) - 2019	Aimed to reduce particulate matter emissions by 20-30% by 2024 across 122 non-attainment cities
08.	India Cooling Action Plan (ICAP) - 2019	Aimed to reduce cooling demand, enhance energy efficiency, and reduce GHG emissions from the cooling sector.
09.	National Electricity Mobility Mission Plan (NEMMP) - 2020	Aimed to promote electric mobility and increase the adoption of electric vehicles. Targets a 30% market penetration in sales of electric vehicles by 2030.
10.	Updated Nationally Determined Contribution (NDC) - 2022	NDC target updation of India under the Paris Agreement. achieve about 50% cumulative electric power installed capacity through non-fossiliferous sources by 2030 Targeted to reduce the emissions from 2005 levels by 45% through 2030.

involves maintaining the core identity of a system amidst continual change, fostering dynamic interactions with its external environment (Gallopín 2003). It is not merely a characteristic but a continual process of positive change, enhancing the system over time in a sustainable manner (Gallopín 2003). When sustainability is coupled with development, it necessitates addressing the key issues vital to human progress (Markandya 2002). Economic development is sustainable if, in relation to its population, a society's productive foundation does not dwindle (Dasgupta 2007). Energy savings, efficiency improvements, and substitutes for fossil fuels are three major technological changes involved in sustainable development. An example of this approach is the concept of solar cities, which aim to develop economically and environmentally sustainable energy systems. These cities address challenges such as limited financial resources, workforce constraints, and uncertainty about future economic and technological developments. The operationalization of sustainability policies often involves addressing the sustainability of what and whom, leading to a high degree of conflict in sustainability issues (Munda 2008).

Several researchers have described the role of SDGs in combating climate change. Fujimori (2020) introduced a new concept known as marginal SDG-emissions-reduction values (MSVs), which measure the incremental effects on SDG indicators from each unit of reduced CO₂ emissions. The authors observed significant correlations between CO₂ emission reductions and several SDG objectives. For example, cutting CO₂ emissions by 1% could lead to a 0.57% decrease in premature deaths due to air pollution (SDG 3) and a 0.026% decrease in mean species richness (SDG 15). According to Soergel et al. (2021), despite current initiatives, the efforts in different regions of the world are insufficient to meet the SDG targets. Wu et al. (2021) analyzed the impact of a cleaner environment in boosting the economic output of E7 and G7 nations over time while minimizing environmental pollution and transitioning to green and innovative energy sources. Liu et al. (2023) explored the relationship between CO₂ emissions, economic growth, and employment composition in 297 Chinese cities using machine learning. The authors argue that manufacturing and construction industries can achieve this decoupling while also leading to job creation. India is presently prioritizing two key Sustainable Development Goals: SDG 7 (Affordable and Clean Energy) and SDG 13 (Climate Action).

SDG 7 (Affordable and Clean Energy): SDG 7 aims to provide access to affordable, reliable, sustainable, and modern energy for all. In India, this involves promoting the use of renewable energy sources like solar, wind, and hydropower, decreasing dependency on fossil fuels,

and enhancing energy efficiency. India is contributing to reducing GHG emissions through substantial investments in clean energy technologies. India has made substantial progress in increasing access to electricity. As of January 2023, over 99% of Indian villages were electrified. The “Saubhagya” scheme, launched in 2017, aimed to provide electricity connections to all households in the country. Due to high electricity demand, India has set ambitious goals for its renewable energy capacity, aiming for 175 GW by 2022 and 500 GW by 2030.

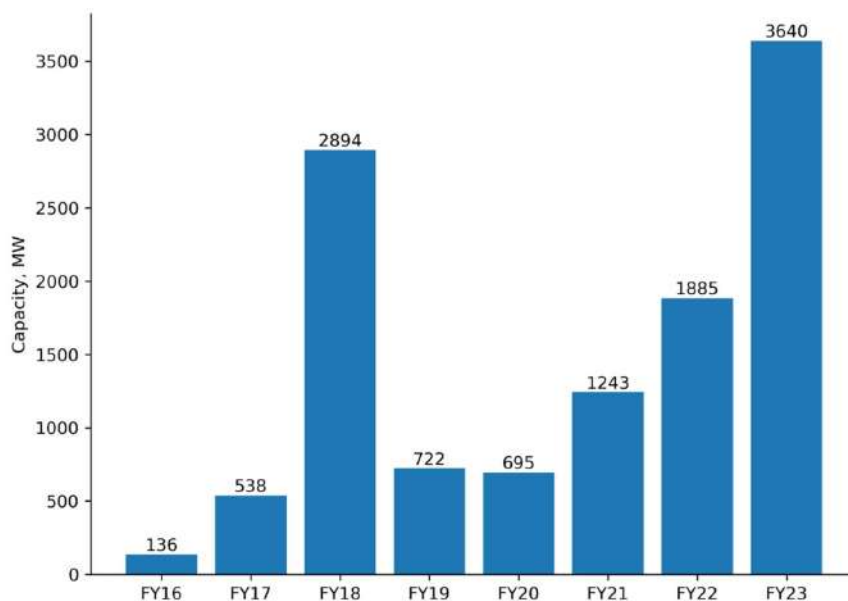
SDG 13 (Climate Action): SDG 13 directly addresses climate change and its impacts. India has committed to various international fora to reduce its emissions and enhance its resilience to climate change through different initiatives, including the National Action Plan on Climate Change (NAPCC) and the development of a National Mission on Enhanced Energy Efficiency. India has been making significant progress in the installation of renewable energy plants. India has set a goal to reach a renewable energy capacity of 500 GW by the year 2030. This includes solar, wind, and hydroelectric power, with substantial investments and initiatives to promote these technologies. India has launched initiatives like the Green India Mission and afforestation programs to increase forest and tree cover, thereby contributing to climate change mitigation through carbon sequestration. India has taken measures to address air pollution, which is closely related to climate change. India has been focusing on reducing air pollution from the transportation sector through promoting electric vehicles (EVs) and improving public transportation systems. Initiatives like the National Clean Air Program (NCAP) aim to improve air quality and reduce emissions of air pollutants.

Status of Renewable Energy Development

India's open-access renewable energy growth is shown in Fig. 1. It can be seen from Fig. 1 that there has been a clear upward trend in open-access renewable energy capacity over the years. Beginning in FY2017, the Indian renewable energy market experienced a strong annual increase in capacity addition. The year FY-2018 marked the peak of this trend with the installation of 2894 MW, the highest annual capacity addition to date. The addition of 3640 MW during FY23 indicates a continued strong growth in open-access renewable energy capacity during the period from FY16 to FY23.

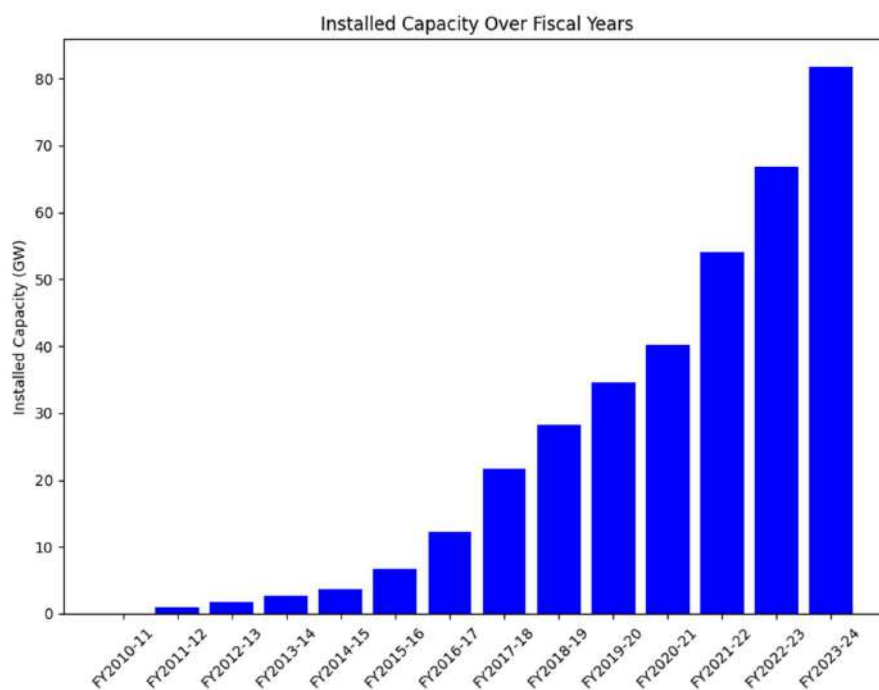
Solar Energy

The installed solar capacity in India has seen a massive increase from 40 MW in 2011-12 FY to 81810 MW in 2023-24 FY (Fig. 2), making India the 4th largest country



(Source: Gulia & Garg 2022).

Fig. 1: India's open access renewable energy growth.



(Source: NITI Aayog, 2024, India's Climate and Energy Dashboard).

Fig. 2: Solar energy capacity in India from FY 2010-11 to FY 2023-24.

globally in terms of solar power capacity. The Solar Park Scheme initiated by the GOI aims to establish 50 solar parks with a cumulative capacity of around 38 GW by 2025-26. Till the end of 2024, 11 parks with a capacity of 8521 MW

have been completed. The PM-KUSUM scheme is set to add 30.8 GW by March 2026, focusing on agricultural sector transformation by setting up decentralized solar power plants. The International Solar Alliance of India

Table 3: Installed solar power capacity in various states.

State	Installed Capacity, MW
Rajasthan	18,089.21
Gujarat	10,417.56
Karnataka	9,347.18
Tamil Nadu	7,164.59
Telangana	4,712.98
Andhra Pradesh	4,555.20
Madhya Pradesh	3,167.92
Maharashtra	5,064.46
Uttar Pradesh	2,632.61
Punjab	1,266.55
Haryana	1,224.30
Kerala	858.68
Uttarakhand	575.53
Chhattisgarh	1,015.24
Odisha	465.23
West Bengal	194.06
Bihar	223.54
Jharkhand	121.77
Assam	155.81
Himachal Pradesh	111.55
Delhi	236.41
Jammu & Kashmir	54.98
Goa	35.76
Tripura	18.47
Meghalaya	4.19
Manipur	13.03
Nagaland	3.04
Sikkim	4.69
Mizoram	30.43
Arunachal Pradesh	11.79
Ladakh	7.80
Andaman & Nicobar Islands	29.91
Chandigarh	64.05
Dadra & Nagar Haveli and Daman & Diu	46.47
Lakshadweep	4.77
Puducherry	43.27
Others	45.01
Total	72,020.00

Source: CEA (2023)

focuses on enhancing the widespread adoption of solar technologies. India's 2030 vision includes ambitious targets such as achieving 500 GW of renewable energy capacity and

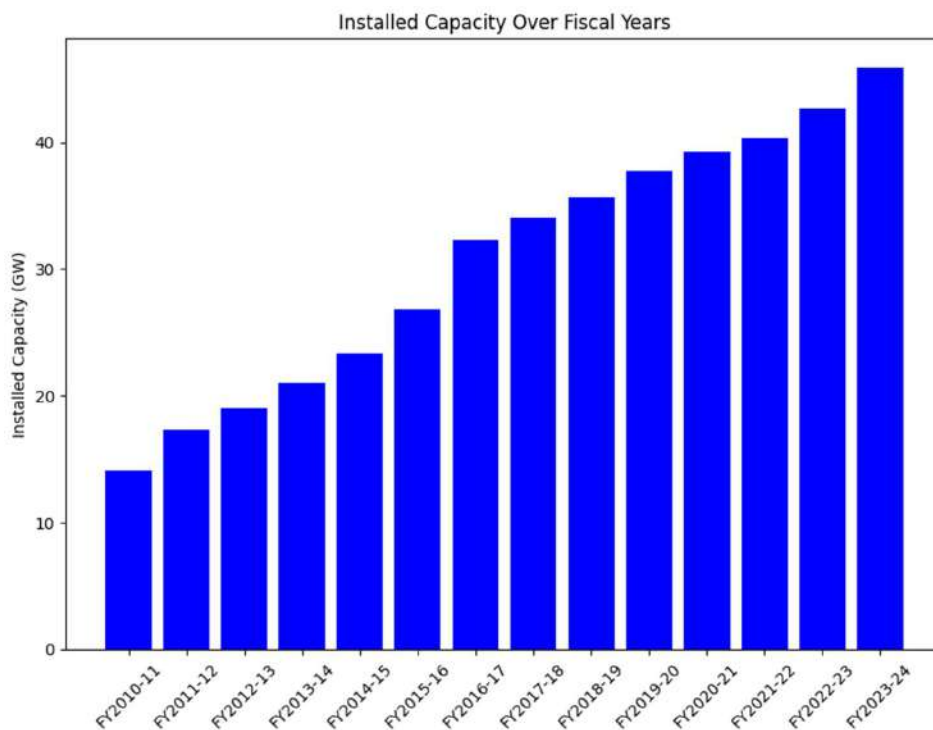
reducing carbon emissions by 1 billion tons. India aims for 90% of its energy to be from renewable sources by 2047.

Table 3 shows installed capacity in different states and union territories. Rajasthan, a northwest province of India, possesses significant solar energy potential and has already made strides by establishing expansive solar parks like the renowned Bhadla Solar Park, recognized as one of the world's largest (Ministry of New and Renewable Energy, GOI 2022). Rajasthan contributes nearly 25% (18089.21 MW) of India's total installed solar capacity. This significant contribution can be attributed to its vast arid land, high solar irradiance, and favorable government policies for large-scale solar parks. Gujarat ranks second with 10,417.56 MW, mainly due to strong government initiatives like the Gujarat Solar Policy and a robust rooftop solar program. The grid infrastructure and innovative models, such as solar-wind hybrid projects, support the solar capacity in the state of Gujarat. The state of Karnataka has the third-largest capacity (9,347.18 MW) owing to early investments in solar parks and rooftop solar installations. The northeastern states, such as Meghalaya (4.19 MW), Nagaland (3.04 MW), and Arunachal Pradesh (11.79 MW), have minimal contributions due to geographical and infrastructural challenges. The Union Territories like Lakshadweep (4.77 MW) and Andaman & Nicobar Islands (29.91 MW) rely on smaller, decentralized solar solutions tailored to their unique energy needs.

Wind Energy

The GOI established a National Offshore Wind Energy Policy in October 2015 that aims to harness offshore wind energy within the Indian Exclusive Economic Zone (EEZ) along its coastlines. As of FY 2023-24, India's wind energy capacity stood at 45.89 GW, placing it as the fourth largest in the world. A target of 140 GW of installed wind capacity by 2030 has been set by India. The National Institute of Wind Energy (NIWE), under the directive of the GOI, has set up over 900 monitoring stations nationwide and provided wind potential maps at various heights. Recent data shows a gross potential of 695.5 GW at 120 meters and 1163.9 GW at 150 meters above ground, predominantly across eight windy states. With a potential of 695.5 GW at 120 m height and 1163.9 GW at 150 m height, the target of 140 GW of installed wind capacity by 2030 is quite achievable. The progress in installed wind capacity over different years is shown in Fig. 3.

The distribution of total installed wind capacity across various states is shown in Table 4. It can be seen from Table 4 that the state of Gujarat has the highest installed capacity, followed closely by Tamil Nadu. The states of Gujarat and Tamil Nadu together account for nearly



(Source: NITI Aayog, 2024, India's Climate and Energy Dashboard).

Fig. 3: Wind energy capacity in India for FY 2010-11 to FY 2023-24.

half of India's total wind power capacity. The significant contributions from Karnataka, Maharashtra, Rajasthan, and Andhra Pradesh further underscore the widespread adoption of wind energy across the country. As of September 30, 2024, India's total installed wind power capacity reached approximately 47,362.92 MW. States such as Meghalaya (4.19 MW), Nagaland (3.04 MW), and Arunachal Pradesh (11.79 MW) have minimal contributions due to geographical and infrastructural challenges. Union Territories like

Lakshadweep (4.77 MW) and Andaman & Nicobar Islands (29.91 MW) rely on smaller, decentralized solar solutions due to their unique energy needs. The data shown in Table 4 indicates India's commitment to expanding its renewable energy infrastructure, aligning with national goals for sustainable development and climate change mitigation.

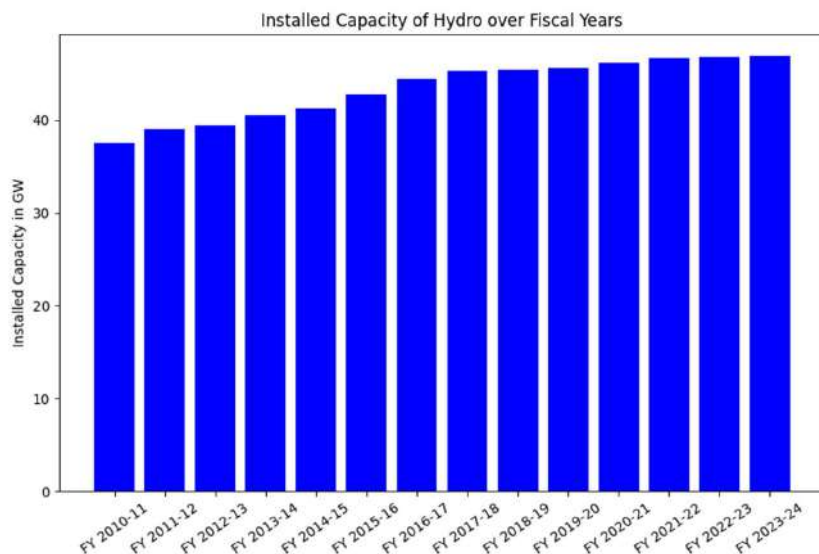
Hydropower

The transition from coal-based or other fossil fuel-based power generation to hydropower can drastically reduce carbon emissions, aiding India's efforts to meet its climate goals and international commitments. According to the Central Electricity Authority (CEA, 2023), India has harnessed only 29% of its vast 145320 MW hydropower potential. This is in sharp contrast with the United States and European Union, which have harnessed over 80% and 70% of their respective hydropower resources, according to the International Hydropower Association. As of 2023-24, the installed capacity for hydroelectric power in India is 46930 MW. It is evident from Fig. 4 that there is a steady increase in capacity from 2010 to 2024.

Table 5 clearly shows that the state of Himachal Pradesh has the highest installed hydropower capacity at 10,547 MW, accounting for approximately 22.5% of the total

Table 4: Installed wind capacity in various states.

State	Installed Capacity [MW]
Gujarat	12,209.18
Tamil Nadu	11,042.44
Karnataka	6,564.36
Maharashtra	5,214.28
Rajasthan	5,195.82
Andhra Pradesh	4,096.65
Madhya Pradesh	2,844.29
Telangana	128.10
Kerala	63.50
Others	4.30
Total	47,362.92



(Source: NITI Aayog, 2024, India's Climate and Energy Dashboard).

Fig. 4: Hydropower capacity in India from FY 2010-11 to FY 2023-24.

Table 5: State-wise distribution of installed hydropower capacity in India.

State	Installed Capacity, MW
Arunachal Pradesh	405.60
Assam	375.70
Bihar	60.20
Chhattisgarh	120.00
Gujarat	199.00
Himachal Pradesh	10,547.00
Jammu & Kashmir	3,359.00
Jharkhand	130.00
Karnataka	3,793.00
Kerala	2,078.00
Madhya Pradesh	2,435.00
Maharashtra	3,331.00
Manipur	105.00
Meghalaya	356.00
Mizoram	60.00
Nagaland	75.00
Odisha	2,085.00
Punjab	1,045.00
Rajasthan	411.00
Sikkim	610.00
Tamil Nadu	2,321.00
Tripura	16
Uttar Pradesh	547.00
Uttarakhand	3,866.00
West Bengal	1,469.00
Others	1,000.00
Total	46,850.00

capacity in India. The mountainous terrain and perennial rivers like the Sutlej and Beas provide ideal conditions for

hydropower generation in Himachal Pradesh. The other three major contributors are Uttarakhand (3,866 MW), Karnataka (3,793 MW), and Jammu & Kashmir (3,359 MW). Together, these states account for nearly 24% of the total hydropower capacity, reflecting their geographical advantages and focus on renewable energy. States in the northeastern parts of India, namely Tripura (16 MW), Nagaland (75 MW), and Mizoram (60 MW), contribute minimally due to either limited infrastructure or untapped potential.

Considerable progress has been made in developing renewable energy sources in India. However, reaching the ambitious target of 500 GW by 2030 remains a formidable challenge. To address this, various initiatives have been implemented to work toward the goal. Table 6 presents the capacity under development for the financial year 2023-24, reflecting an increasing acknowledgment of global environmental concerns and emphasizing the importance of sustainability and environmental preservation.

Table 6: Capacity under development of different energy sources.

Source	Installed Capacity [in GW]
Solar Under Construction	54.77
Solar Under Development	33.78
Hydro Under Construction	16.74
Hydro Under Construction but stalled	1.24
Wind Under Construction	18.17
Wind Under Development	13.00
Total	137.70448

Table 6 shows the capacity under development for different energy sources. The current installed solar energy capacity stands at approximately 82 GW, with an additional 88 GW in the pipeline. Wind power has an installed capacity of around 46 GW, with 32 GW under development. For hydropower, the installed capacity is 48 GW, while 18 GW is in the pipeline. This data demonstrates the dominant position of solar energy in the renewable energy sector, contributing over 50% of India's 2030 renewable energy target. This emphasis reflects India's strategic focus on utilizing its abundant solar potential. In contrast, wind energy is advancing at a more gradual pace and will require substantial policy support to reach its full potential, particularly in high-wind states such as Gujarat and Tamil Nadu. The growth of hydropower capacity development has been relatively limited due to challenges like long gestation periods, environmental concerns, and community displacement. Overcoming these hurdles is essential to achieving further progress in the hydropower sector.

The central core of sustainable development is the incorporation of economic, social, and environmental considerations into policy formulation. Approaching the issue of climate change through the lens of sustainable development may offer valuable insights and strategies for addressing this pressing challenge (Soltau 2006). The SDG-7 aims at cheap, reliable, sustainable, and modern energy access to all, which is a key component in climate change mitigation.

Challenges and Innovative Solutions

India aims to achieve a renewable energy capacity of 500 GW by 2030, but several challenges must be addressed to achieve this ambitious goal.

1. **Infrastructure and Grid Integration:** The generation of solar and wind energy is dependent on weather conditions, thereby creating challenges for grid stability and reliability. Therefore, transmission and distribution infrastructure require significant upgrades to handle the increased load from renewable sources. To mitigate intermittency, large-scale deployment of storage solutions like batteries and pumped hydro is essential. However, these technologies remain costly.
2. **Land Acquisition and Availability:** Solar farms and wind parks require vast areas, often competing with agricultural and forested lands. Acquiring land for large-scale renewable projects can lead to disputes, the displacement of communities, and delays.
3. **Financing and Investment:** Despite declining costs, renewable energy projects still require significant initial investments. Owing to inconsistent and frequent policy

revisions, small and medium developers often face challenges in accessing affordable financing.

4. **Technological Barriers:** Integrating high levels of renewable energy into the grid requires advanced forecasting, demand management, and smart grid technologies. Therefore, developing cost-effective, large-scale energy storage systems remains a significant technological challenge.
5. **Dependence on Imports:** India relies heavily on imports for critical components like solar cells and modules, primarily from China. Global supply chain issues caused by the pandemic or geopolitical tensions can delay project execution.

Some other countries that face similar challenges as India include China, Brazil, and South Africa, among others. China is a global leader in solar energy manufacturing, accounting for over 70% of the world's solar panel production. China has overcome the challenges of cost-effectiveness by scaling the production capacity of solar power equipment. The Chinese government introduced initiatives such as the Golden Sun Program and Top Runner Program to promote domestic solar adoption and innovation. Additionally, investment in research and development expenditure has led to improvements in solar cell efficiency and reduced manufacturing costs in the country. Brazil has successfully integrated bioenergy into its renewable energy portfolio, largely through its robust ethanol and biomass programs. Brazil is a global leader in ethanol production, primarily derived from sugar cane. The Proálcool Program, launched in the 1970s, promoted ethanol as a fuel alternative, reducing dependence on imported oil. Over 80% of Brazil's light vehicle fleet runs on a mixture of ethanol and gasoline. In India, less than 20% ethanol is blended with petrol. However, the National Policy on Biofuels–2018 has set a target of achieving 20% ethanol blending in petrol by 2030. South Africa has a model renewable energy promotion program known as the Renewable Energy Independent Power Producer Procurement Programme (REIPPPP). Under REIPPPP, independent power producers (IPPs) are encouraged to bid for renewable energy projects, creating a competitive and transparent procurement process. The program has attracted significant foreign investment, bringing over USD 14 billion into South Africa's renewable energy sector. As a result, thousands of jobs have been created in the renewable energy sector, leading to significant rural economic development.

To achieve the ambitious renewable energy target of 500 GW by 2030, India must adopt a range of innovative solutions that address technological, financial, and infrastructural challenges. The following key innovative solutions are recommended to achieve the ambitious target of 500 GW by 2030.

1. Advanced Energy Storage Technologies: Large-scale investment in lithium-ion, sodium-ion, and flow batteries is required to store surplus energy from solar and wind projects
2. Smart Grid Infrastructure: Smart power grids with advanced monitoring, forecasting, and demand-response technologies are urgently required to manage variable renewable energy efficiently
3. Offshore Wind and Hybrid Renewable Projects: India has a 7500 km coastline that can be utilized for developing offshore wind energy projects. To optimize land use and ensure a continuous power supply, policymakers should aim to develop combined solar and wind energy parks.
4. Innovations in Solar Energy: Deployment of bifacial solar panels that capture sunlight on both sides can enhance energy output. Additionally, canals and reservoirs can be utilized for floating solar projects.
5. Policy and Financing Innovations: To attract domestic and international investors, greater mobilization of funds for renewable energy projects through green bonds and crowdfunding platforms is required

CONCLUSIONS

India's immense potential in solar, wind, and hydropower offers significant opportunities to mitigate the impacts of climate change and drive sustainable development. Realizing this potential requires overcoming technological, financial, and policy barriers through strategies that minimize environmental impacts, address climate change, and enhance resilience against environmental disruptions. Over the past decade, notable progress has been achieved in enhancing installed capacities across solar, wind, biopower, and hydropower sectors. Initiatives such as the Solar Park Scheme and the PM-KUSUM scheme have led to significant growth, establishing India as a global leader in solar capacity. Wind energy, supported by the National Offshore Wind Energy Policy, has also seen significant advancements, with ambitious targets set for 2030. However, hydropower remains underutilized despite its potential, offering untapped opportunities for reducing emissions.

The installed solar capacity in India stands at 82 GW, with an additional 88 GW under development. Wind energy has an installed capacity of approximately 46 GW, with 32 GW in the pipeline. Hydropower contributes around 47 GW of installed capacity, and around 18 GW is under development. Collectively, India currently has about 175 GW of renewable energy capacity. Despite these successes, India faces considerable challenges in achieving its renewable

energy target of 500 GW by 2030. This is mainly due to the formulation of effective climate change mitigation policies that necessitate balancing international commitments with domestic developmental needs. Additionally, GHG emissions from fuel combustion and thermal power plants remain inevitable by-products of economic and infrastructure development. Therefore, innovative solutions, robust policy frameworks, and sustained investments in renewable energy are required to secure a sustainable and resilient energy future.

Renewable energy development offers a comprehensive and sustainable solution to address the dual challenge of mitigating climate change and meeting India's energy needs. By replacing fossil fuels with solar, wind, hydropower, and bioenergy, India can significantly lower its carbon footprint and move closer to achieving its commitments under the Paris Agreement. Additionally, renewable energy development would contribute to improved air quality by reducing harmful pollutants such as sulfur dioxide, nitrogen oxides, and particulate matter. This not only addresses public health concerns but also helps mitigate the economic costs associated with air pollution-related illnesses. The transition to renewable energy also supports Sustainable Development Goals (SDGs), particularly SDG 7 (Affordable and Clean Energy) and SDG 13 (Climate Action), fostering inclusive and sustainable growth. Renewable energy enables India to enhance its energy security by reducing dependence on imported fossil fuels. Moreover, the sector creates significant economic opportunities, including job creation across different sectors. To maximize these benefits, India must continue to invest strategically in renewable energy infrastructure, innovation, and research. By doing so, renewable energy can become the cornerstone of India's climate change mitigation strategy, ensuring a sustainable, resilient, and prosperous future.

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Equilibrium, Kinetic and Thermodynamic Studies of Methylene Blue Dye Adsorption from Wastewater Using Manila Tamarind Shell Copper Nanoparticles as Adsorbent

Dasari Kiran Kumar[†]  and Pulipati King 

Department of Chemical Engineering, Andhra University College of Engineering, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India

[†]Corresponding author: Dasari Kiran Kumar; dasarikirankumar018@gmail.com

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ABSTRACT

The current study's objective is to use Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as adsorbents in a batch process to remove Methylene Blue dye from their wastewater. Contact time, solution pH, initial Methylene blue dye concentration, adsorbent dosage, and temperature were the factors that were examined in batch research. The maximum removal efficiency was predicted to be 96.37% at a temperature of 318 K, a solution pH of 7, an initial dye concentration of 10 mg.L⁻¹, and an adsorbent dosage of 0.5 g.L⁻¹. The Freundlich isotherm model best describes Methylene Blue removal adsorption data at 303 K, with a correlation coefficient of 0.9975, while the Temkin and Langmuir isotherms have correlation coefficients of 0.8901 and 0.8656, respectively. The kinetics study analyzed adsorption data using pseudo-first order, pseudo-second order, Elovich, and intraparticle diffusion models. Pseudo-second-order kinetics for methylene blue showed close applicability, confirming chemisorption as the rate-limiting step. The pseudo-second-order kinetics model best describes Methylene Blue removal adsorption data at 10 mg.L⁻¹ with a correction coefficient of 0.9988, while the pseudo-first-order, Elovich model, and intra-particle diffusion model have correction coefficients of 0.9876, 0.9704, and 0.9174, respectively. The thermodynamic study found that the adsorption process is spontaneous and physisorption is dominant, with Gibbs free energy values ranging from -20 to 0 kJ.mol⁻¹. Endothermic adsorption and an activated complex formation are associated with the process. In this study, determine the ΔH^0 , ΔS^0 , and ΔG^0 at 303K with values of 25.766 kJ.mol⁻¹, 114.12 J.mol⁻¹, and -8.8003, respectively.

INTRODUCTION

Many times around the world, freshwater is a restricted resource. Population growth, urbanization, and climate change will all contribute to increased scarcity. The main reason for the scarcity of fresh water is not only the high water demand, but also contamination in freshwater ecosystems. Human-caused contamination in this ecosystem has significantly reduced usable water and increased the expense of purifying it. The primary sources of water pollution are point and nonpoint sources. Point sources include pollutants discharged through pipelines, such as home sewage, and industrial waste effluents from industries or plants into receiving waters. It differs from non-point pollution caused by storm runoff, which spreads contaminating elements diffusely across land.

Manila tamarind shells, which are commonly thrown as agricultural waste, are high in bioactive components such as tannins, flavonoids, and polyphenols. These chemicals can operate as natural reducing and stabilizing agents in the synthesis of copper nanoparticles, offering an environmentally benign and cost-effective method of nanoparticle production while supporting sustainable waste management (Prasad & Sasidhar 2023). Copper nanoparticles are known for their catalytic

effectiveness and high surface reactivity, making them excellent candidates for eliminating methylene blue dye from wastewater. Their capacity to break down dye molecules efficiently, along with their cost-effectiveness, highlights their viability for environmental cleanup. The removal of methylene blue dye from wastewater is crucial to minimizing environmental contamination. Traditional adsorbents, such as activated carbon and clays, are commonly used for dye removal, but they have considerable disadvantages, including high cost, limited reusability, and environmental problems during production. While several alternatives have been investigated, few focus on sustainable, green materials that provide both high efficiency and little environmental impact (Akintelu et al. 2021).

The goal of this study is to create a green synthesis technique for copper nanoparticles that uses manila tamarind shells as a natural precursor. The produced CuNPs will be studied and tested for their ability to remove methylene blue dye from wastewater. This work provides a long-term solution to water contamination by combining agricultural waste utilization and color remediation techniques. The purpose of this study is to investigate the efficacy, cost-effectiveness, and environmental sustainability of MTS-CuNPs for removing methylene blue dye from wastewater. By resolving the limits of existing adsorbents, this study paves the way for a more sustainable approach to wastewater treatment.

NaIO₄-CNP, a new adsorbent material derived from cotton fibers, effectively removes methylene blue dye from textile wastewater, demonstrating excellent reproducibility and potential for wastewater treatment (Melese & Tsade 2024). *Spirulina platensis* biosorbs lead, cadmium, and nickel ions from aqueous solutions, with endothermic sorption and selectivity towards Pb²⁺ ions. Its reusability and capacity towards these metal ions are studied (Şeker et al. 2008). Polyaniline with chitosan adsorbent for Acetaminophen removal achieved a high adsorption rate, best fit by pseudo-second-order and Langmuir models, and retains 69% capacity after five cycles (Daikh et al. 2022). The study investigated lead biosorption using *Tectona grandis* L.f. biomass, analyzing factors like contact time, initial metal ion concentration, pH, adsorbent dosage, temperature, and adsorbent size. Results showed good adsorption capacity and a spontaneous process (Naidu et al. 2013). This study investigates the use of genetically modified bacteria to address heavy metal pollution in water. Using *Escherichia coli* expressing human metallothioneins, the study found MT3 biosorbent as a promising method for Pb²⁺ biosorption, with a maximum capacity of 50 mg Pb²⁺.g⁻¹ and up to 74% recovery (Akkurt et al. 2024). The study explores the use of activated teak leaf powder as a biosorbent for Congo red dye

removal from wastewater, revealing its potential as a cost-effective, environmentally friendly, and efficient method (Gedam et al. 2019). Research on fungal decolorization of dye wastewater explores mechanisms, elution and regeneration methods, pretreatment methods, and factors affecting decolorization (Fu & Viraraghavan 2001). The study developed a Fe₃O₄/GO nanocomposite for efficient water treatment, demonstrating high adsorption capacity under optimal conditions like pH 6.0, 50.0 mg adsorbent weight, and 10 min contact time (Cao et al. 2024). The research evaluates the Langmuir Linear type 3 isotherm for optimal BG adsorption on MWCNTs, a low-cost, low-contact time adsorbent for dye removal (Mahdi et al. 2023). The study assessed adsorption and coagulation processes for removing reactive dyes Orange 16 and Black 5, using coconut-based PAC and alum chloride. Results showed Orange 16 had higher adsorption capacity (Lee et al. 2006). The study demonstrates the preparation of activated carbons from walnut shells using zinc chloride, with the optimum carbon having a BET surface area of 1800 m².g⁻¹ and a pore volume of 1.176 cm³.g⁻¹ (Yang & Qiu 2010). The study uses potato plant waste as an adsorbent to remove methylene blue and malachite green dyes from aqueous solutions, characterized using SEM and FTIR spectroscopy (Gupta et al. 2016). Mordenite and nanocrystals effectively adsorb new methylene blue from aqueous solutions with higher adsorption capacity. Adsorption kinetics follow pseudo-second-order kinetics, with higher pH resulting in higher adsorption capacity (Sohrabnezhad & Pourahmad 2010). A carbon-micro silica composite adsorbent was developed using microsilica, demonstrating effective cationic organic dye capture and methylene blue removal, with the Langmuir equation providing more accurate results (Zhang et al. 2012). Gambir Indonesia was modified to remove methylene blue from aqueous solutions, with an adsorption capacity of 149.3 mg.g⁻¹ and a well-fit pseudo-second-order equation (Tong et al. 2012). The study investigates the use of agricultural waste-based bioadsorbents as cost-effective, biodegradable, and environmentally friendly alternatives to conventional adsorbents for removing methylene blue dye from wastewater (Coşkun 2023). This review paper explores eco-friendly biosorbents for removing methylene blue from water bodies, highlighting their importance in green chemistry for wastewater treatment and environmental pollution mitigation (Saha & Debnath 2023). The study explores the use of biodegradable green adsorbents from natural and renewable sources for efficient methylene blue removal from water solutions, demonstrating cost-effective, environmentally friendly, and high-efficiency alternatives (Yardımcı & Kanmaz 2023). Using copper nanoparticles made from environmentally benign plant extracts, the study investigates green solutions for pollutant remediation,

demonstrating their exceptional dye adsorption properties and promise for wastewater treatment (Neeti et al. 2022).

MATERIALS AND METHODS

Preparing the Dye Solution

One g of 100% Methylene Blue dye was dissolved in 1000 mL of distilled water to make stock solutions with a concentration of 1000 mg.L⁻¹. The solution was produced in standard flasks. These stock solutions were then diluted to the desired dye concentration (10-100 mg.L⁻¹) by mixing with a suitable proportion of distilled water (Umoren et al. 2013)

Chemicals and Reagents

Copper Sulphate Pentahydrate (CuSO₄.5H₂O) was used without additional purification after being purchased from Sigma Aldrich. Manila tamarind shells were gathered in Medak District's Narsapur jungle. Double-distilled water was used to make the plant extract and copper sulfate solution. The UV spectrophotometer, beakers, Eppendorf tubes, funnels, and falcon tubes were all sterile, as was the work area (Bulut & Aydın 2006).

Preparation of Manila Tamarind Shell Extract

Manila Tamarind Shell samples were washed with distilled water and ground into fine pieces, then 20g of MTS powder was added to 100 mL of double distilled water, mixed thoroughly, and cooked in a hot water bath for 45 min. The extract was then filtered through Whatman No.41 filter paper to provide Manila Tamarind shell aqueous extract, which was refrigerated for later use (Ravikumar & King 2019).

Synthesis of Cu-NPs

Four g of copper sulfate pentahydrate and 100 cc of Manila Tamarind shell aqueous extract were magnetically stirred at room temperature (27°C) for 4 h. Within 10 min, the blue color of copper sulfate pentahydrate changed brown, indicating the formation of CuNPs due to the reduction of copper ions from Cu (II) ions to Cu metal. The samples were then centrifuged at 3000 rpm for 10 min, yielding a clear, room-temperature supernatant. Copper nanoparticles were dried in an oven at 80 to 90 degrees Celsius for four hours before being examined using FT-IR, SEM, and XRD (Akintelu et al. 2021). The size of particles is reported to be nano in size and is reported elsewhere (Dasari Kiran Kumar & Pulipati King 2025).

Batch Mode Adsorption Studies

Batch biosorption equilibrium studies were carried out in 250 mL conical flasks with a constant agitation speed (150 rpm).

Table 1: Range of parameters covered.

Parameters	Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) adsorbent	
	Methylene blue	
	Min	Max
Contact time(t), min	5	100
Solution, pH	4	9
Adsorbent dosage [w], [g.L ⁻¹]	0.1	0.8
Initial dye concentration(C ₀), [mg.L ⁻¹]	10	100
Temperature(T), (K)	303	318

All studies were conducted at room temperature (±300C). The concentrations of both dyes before and after sorption were determined with a UV-Vis spectrophotometer by measuring the absorbance of the dye employed (Aksu 2005, Mall et al. 2005, Kiran et al. 2006, Sreelatha & Padmaja 2008).The different parameters for removing Methylene Blue dye using Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as the adsorbent are given in Table 1.

Effect of Contact Time

By adding 0.5 g.L⁻¹ of 100 nm Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) to Erlenmeyer flasks holding 10 mg.L⁻¹ of Methylene Blue dye solution at pH 7 and temperature 303 K, the impact of contact length on dye biosorption was examined. This solution was centrifuged after being agitated for five minutes at 150 rpm. The supernatant was properly decanted after centrifugation, and the amount of remaining Methylene Blue dye was measured. At different intervals of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 min, the technique was repeated. This makes it possible to calculate the equilibrium time (Mall et al. 2005, Tsai et al. 2006).

Effect of Solution pH

A known quantity of Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) was added to 100 mg/L of Methylene Blue dye solutions at varying concentrations (10, 20, 30, and 50 mg.L⁻¹), each maintained at a different pH (4 to 9) and at 303K to ascertain the impact of solution pH on Methylene Blue dye biosorption. After agitating these solutions for an equilibrium period at 150 rpm, the concentrations of leftover dye were determined (Bulut & Aydın 2006, Salleh et al. 2011).

Effect of Initial Dye Concentration

By adjusting dye solution concentrations (Methylene Blue dye) between 10 mg.L⁻¹ and 100 mg.L⁻¹, the impact of starting dye concentration on dye biosorption was investigated. A known weight (0.5 g.L⁻¹) of Manila Tamarind

Shell Copper Nanoparticles (MTS-CuNPs) was added to solutions with varying Methylene Blue dye concentrations. After centrifuging and agitating these solutions for an equilibrium period at 303 K, the final dye concentrations were measured. Keeping all other factors equal, this process was carried out at various initial dye concentrations between 10 and 100 mg.L⁻¹ (Waranusantigul et al. 2003, Kavitha & Namasivayam 2007).

Effect of Adsorbent Dosage

By agitating 100ml of Methylene Blue solution with varying initial concentrations (10 to 50 mg.L⁻¹) using 0.1, 0.2, 0.3, 0.4, and up to 0.8 g.L⁻¹ of Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as an adsorbent dosage at room temperature for equilibrium contact time and optimal pH at a constant speed of agitation, the effect of adsorbent dosage on the amount of dye adsorbed was ascertained. The concentrations of unadsorbed dye were measured after these solutions were shaken for the equilibrium period. Keeping all other factors equal, this process was carried out with several concentrations of biosorbent (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 g.L⁻¹) (Chakraborty et al. 2012, Gouamid et al. 2013).

Effect of Temperature

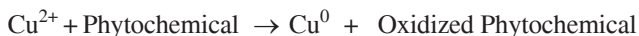
By stirring 0.5 g.L⁻¹ of Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) with varying quantities (10 mg.L⁻¹ to 50 mg.L⁻¹) of Methylene Blue dye at 303 K and the ideal pH, the impact of temperature on dye removal was investigated. Solutions were taken out, and their final dye concentrations were measured at the conclusion of the equilibrium period. This process was carried out again with comparable circumstances and at varying temperatures between 3030 and 3180 K (Anbia & Hariri 2010).

RESULTS AND DISCUSSION

Manila tamarind shells, derived from *Pithecellobium dulce*, serve as a crucial natural reducing and capping agent in the conversion of copper sulfate (CuSO₄) into copper nanoparticles (CuNPs). The mechanism typically involves the following:

Reduction of Copper Ions: Manila tamarind shells contain various phytochemicals, such as polyphenols, flavonoids, and tannins. These chemicals have hydroxyl and other functional groups that serve as reducing agents.

- The Cu²⁺ ions in CuSO₄ are reduced to Cu⁰ by the phytochemicals in an aqueous or suitable solvent medium.



Stabilization (Capping): Once the copper ions have been converted to metallic copper (Cu), the same phytochemicals serve as capping agents. The capping technique avoids nanoparticle aggregation and keeps them stable in the colloidal phase.

Mechanism Details: Electrochemical mechanism: The electron-donating characteristics of functional groups such as hydroxyls (-OH) and carbonyls (-C=O) aid in electron transfer to Cu²⁺, reducing it to Cu⁰. (Muthuvel & Jeyaraj 2020).

Effect of Contact Time

Fig. 1 displays the percentage of adsorption that was measured at various contact times. According to the figure, the percentage of Methylene Blue dye removal grew sharply as contact time increased from 5 to 60 min. After reaching equilibrium for 60 min, the proportion of dye removal

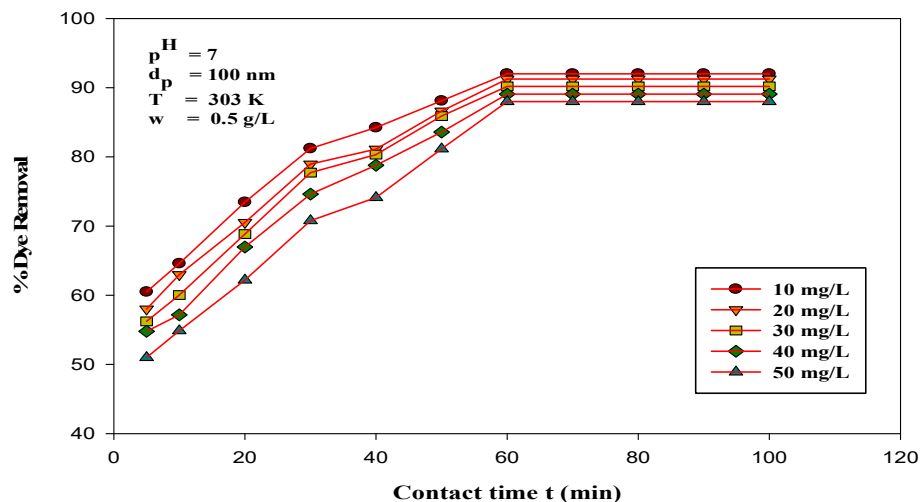


Fig. 1: Effect of contact time on % dye removal of methylene blue using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as adsorbent.

reached a plateau. Consequently, given the experimental setup employed in this investigation, a contact time of 60 min is adequate for the removal of Methylene Blue dye. For an initial dye concentration of 10 mg.L^{-1} , the percentage of Methylene Blue dye removed using Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as an adsorbent increased from 60.49 to 92.01%, and the contact time increased from 5 to 100 min. With an increase in contact time from 5 to 60 min, the percentage of dye removal for 50 mg.L^{-1} rose from 51 to 87.99 mg.g^{-1} , respectively.

Effect of Solution pH

One of the key regulating factors in the adsorption process is the pH of the solution. Using Fig. 2, the impact of solution pH on the percentage of dye removed for the removal of Methylene Blue dye was examined. At an initial concentration of 10 mg.L^{-1} , it was found that the percentage

of adsorption for Methylene Blue decreased from 44 to 64% as the pH of the solution increased from 2 to 6 and decreased when the pH of the solution increased from 6 to 9 for Methylene Blue. When the pH of the solution rose from 2 to 9, the percentage of adsorption for Methylene Blue increased from 36% to 58% at an initial concentration of 50 mg.L^{-1} . The highest percentage of dye clearance was found at pH 6.

Effect of Initial Concentration of Dye

Fig. 3 illustrates how the initial dye concentration affects the percentage of dye removal. The figure clearly shows that when the original dye concentration increased from 10 to 100 mg.L^{-1} , the percentage of dye removal declined. When the initial concentration of Methylene blue increased from 10 to 100 mg.L^{-1} at 303 K, the percentage of dye removal of Methylene blue dropped from 94.80 to 88.16 percent.

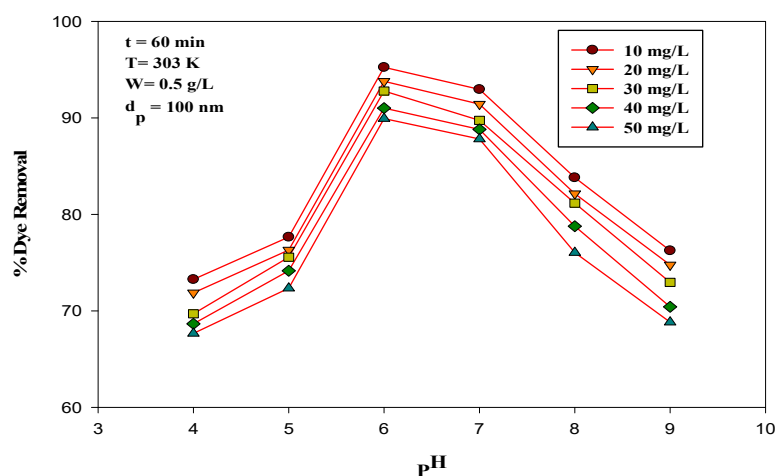


Fig. 2: Effect of solution pH on % dye removal of methylene blue using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as adsorbent.

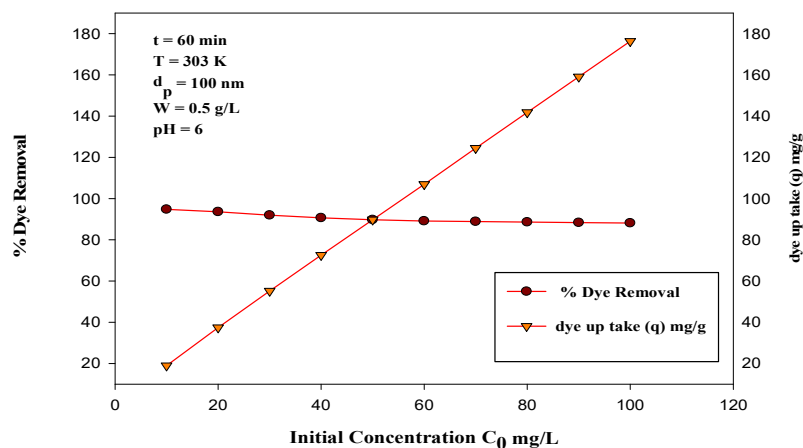


Fig. 3: Effect of initial concentration of METHylene blue on dye uptake and % dye removal using Manila tamarind shell copper nanoparticles (MTS-CuNPs) adsorbent.

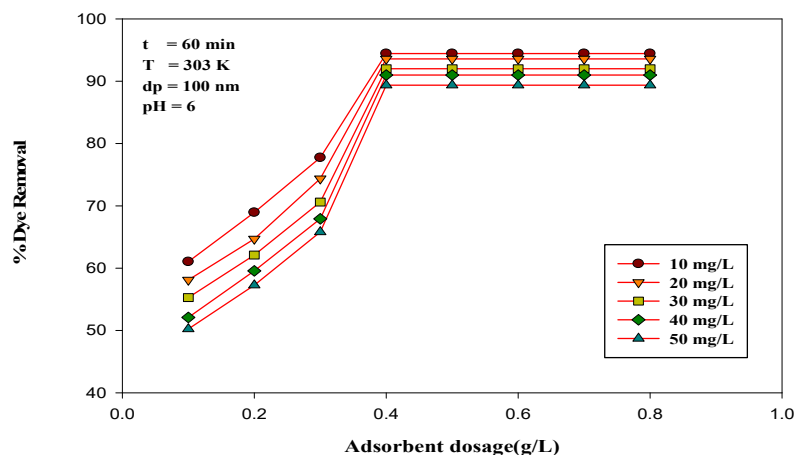


Fig. 4: Effect of adsorbent dosage on % Dye removal of METHYLENE BLUE Using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as adsorbent.

Effect of Adsorbent Dosage

The outcome, which is displayed in Fig. 4, shows that when the dosage of adsorbent was increased, so did the percentage of dye removal. At an initial concentration of 10 mg.L^{-1} of Methylene blue, the percentage of dye removal rose from 61.04 to 94.44% when the adsorbent dosage was raised from 0.1 to 0.8 g.L^{-1} . The percentage of dye removal rose from 50.25 to 89.36% for an initial concentration of Methylene blue of 50 mg.L^{-1} when the adsorbent dosage was raised from 0.1 to 0.8 g.L^{-1} .

Effect of Temperature

The outcome is displayed in Fig. 5. The graphic showed that as the solution's temperature rose, so did the percentage of dye elimination. This implies that the adsorption process is

endothermic. When the solution temperature was raised from 303 K to 318 K, the percentage of dye removal increased from 94.26 to 96.37% and 89.78 to 92.13% for initial concentrations of Methylene Blue dye of 10 mg.L^{-1} and 50 mg.L^{-1} , respectively.

Equilibrium Studies

Langmuir Adsorption Isotherm: For the adsorption of Methylene blue at 303 K, the Langmuir plot (C_e/q_e vs C_e) is displayed in Fig. 6. The adsorption of Methylene blue dye onto Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as adsorbent followed the Langmuir isotherm, correlation coefficient (R^2) determined to be 0.8656. The slope and intercept of the linear plots of C_e/q_e vs C_e for Methylene Blue were used to determine the parameters $q_{\max}$ and b . For Methylene blue, the highest adsorption capacity

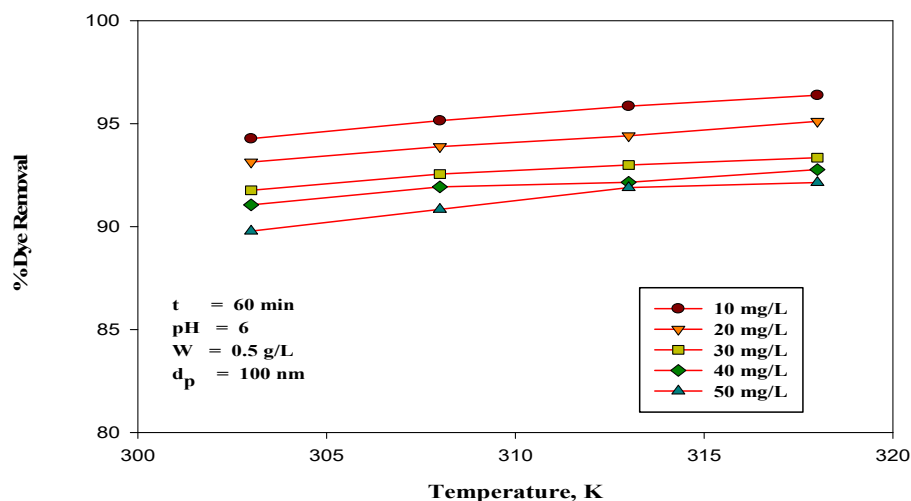


Fig. 5: Effect of temperature on % dye removal of methylene blue using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as adsorbent.

($q_{\max}$) and b values were 301.2 mg.g^{-1} and 0.097 L.mg^{-1} . Table 2 contains a tabulation of these values. A more accurate measure of adsorption is thought to be the R_L parameter. The separation factor R_L was computed at a concentration of 10 mg.L^{-1} based on the values of b . As seen in Table 2, it was discovered that the R_L for Methylene blue was 0.5. These findings showed that Methylene blue had a preferential adsorption onto the adsorbent.

Freundlich Isotherm: The slope and intercept of the graph of $\ln q_e$ vs $\ln C_e$ are used to determine the values of K_f and n . Fig. 7 displays the Freundlich curve ($\ln q_e$ against $\ln C_e$) for Methylene Blue adsorption at 303 K. The plot revealed that the $1/n$ value and the K_f value for Methylene blue were 0.698 & $29.964 \text{ (mg.g}^{-1})/(\text{L.g}^{-1})$. The findings showed that the $1/n$ value ranged from 0 to 1, indicating that Methylene blue's adsorption onto Manila Tamarind Shell Copper Nanoparticles (MTS-Cu-NPs) was advantageous under the conditions under investigation. This could be because of the chemical interactions between the adsorbent and the adsorbate. The correlation coefficient R^2 , which was used to examine the data, was determined to be 0.9975 for Methylene Blue. These findings showed that the Freundlich model fit the methylene blue adsorption data quite well.

Temkin Isotherm: A plot of q_e versus $\ln C_e$ enables the determination of the isotherm constants A_T and b_T . Fig. 8 shows the linear plot of q_e versus $\ln C_e$ for Methylene blue adsorption onto the Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as adsorbent, and the values of the parameters are given in Table 2. The correlation coefficient (R^2) value was determined as 0.8901. the determination of the isotherm constants A_T and b_T valves in Table 2.

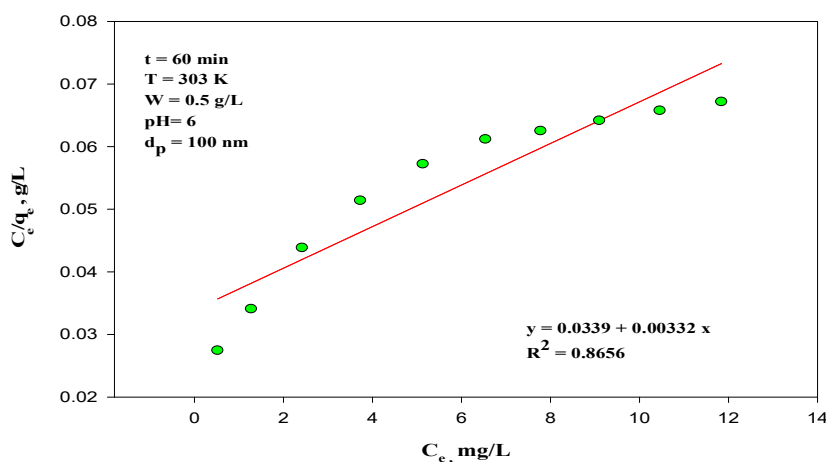


Fig. 6: Langmuir isotherm for adsorption of methylene blue dye using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as adsorbent.

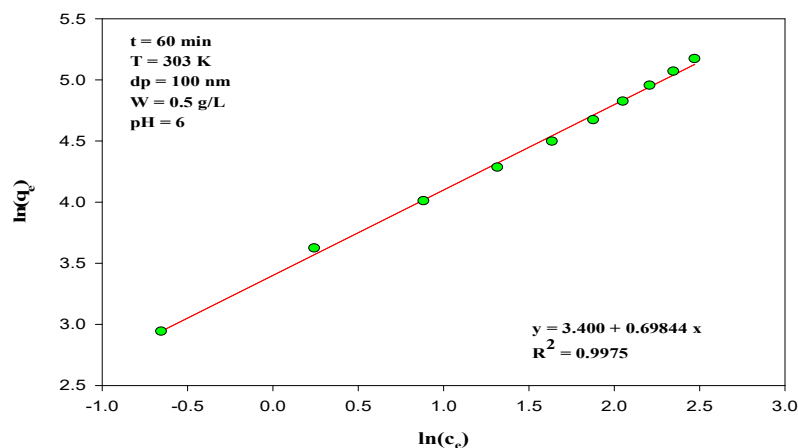


Fig. 7: Freundlich isotherm for adsorption of methylene blue using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as adsorbent.

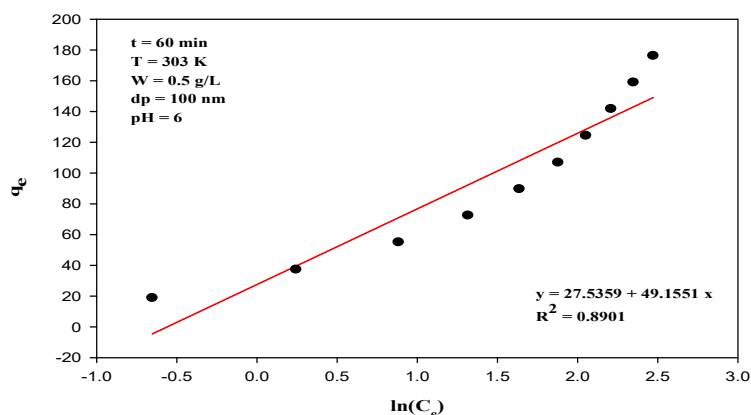


Fig. 8: Temkin Isotherm for Adsorption of Methylene blue using Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as adsorbent.

All three of the models under study examined the equilibrium data. The high correlation coefficient R^2 values shown in Table 2 were used to identify the best-fit isotherm model. Based on these R^2 values, it was determined that, at a solution temperature of 303 K, the Freundlich isotherm model, which had a correlation coefficient of 0.9975, best described the adsorption data for Methylene Blue Removal. Temkin and Langmuir isotherms, on the other hand, had correlation coefficients of 0.8901 and 0.8656, respectively.

The high correlation coefficient, R^2 , values suggest that the absorption process could be due to homogeneous surface coverage. This is in good agreement with the result of Freundlich Isotherm for Methylene blue dye.

Data comparing biosorption capabilities ($q_{\max}$) for Methylene blue:

Kinetic Studies

Pseudo-first-order kinetic model: The pseudo-first-order kinetic model for the adsorption of Methylene blue was evaluated at various initial concentrations (10 to 50 mg.L⁻¹)

Table 2: Adsorption isotherm constants for dye removal using Manila tamarind shell copper nanoparticles (MTS-CuNPs) adsorbent.

Name of the isotherm model	Constants	Methylene Blue
Langmuir	$B[L.mg^{-1}]$	0.097
	$q_{\max}[mg.g^{-1}]$	301.2
	R_L	0.5
	R^2	0.8656
Freundlich	$k_f[mg.L^{-1}.n.L^{-1}.g^{-1}]$	29.964
	N	1.4317
	R^2	0.9975
	R^2	0.8901
Temkin	$A_T[L.g^{-1}]$	1.7509
	$b_T[J.mol^{-1}]$	49.1551
	R^2	0.8901

Adsorbent	$q_{\max}[mg.g^{-1}]$	Reference
Wheat shells	16.56	Bulut and Aydin (2006)
Eggshell	16.43	Sharma et al. (2009)
Neem leaf powder	3.67	Bhattacharyya and Sharma (2005)
Indian rosewood sawdust	11.8	Garg et al. (2004)
Fly ash	1.91	Saha and Datta (2009)
Coconut coir	15.59	Tsai et al. (2006)
Orange peel	1.04	Zainol et al. (2022)
MTS-Cu-NPS	301.2	Present study

using Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) shown in Fig. 9. The linear plot of $\ln(q_e - q)$ versus t was used to calculate the equilibrium adsorption capacity and first-order rate constant values, which were then Table-3 with the correlation coefficient (R^2) values for Methylene blue. The correlation coefficient values of Methylene blue, despite being very high, did not align with the computed and experimental q_e values, indicating that the first-order kinetics of Methylene Blue's adsorption onto MTS-CuNPs were not followed.

Pseudo-second-order kinetic model: Plots of t/q vs. t were used to independently assess the validity of the pseudo-second-order kinetic model for the adsorption of Methylene Blue utilizing Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as an adsorbent. Fig. 10 displays these plots. The equilibrium adsorption capacity and second-order rate values are obtained from the slope and intercept of the linear plot and are listed in Table 3, along with the R^2 values. These findings imply that the pseudo-second-order model can be applied to the experimental data. Both physisorption and chemisorption are present, according to the pseudo-second-order kinetic model's applicability.

Elovich Kinetic model: The slope and intercept of the straight-line plot of q vs. $\ln t$ in Fig. 11 are used to determine the

constants. Table 3 provides a summary of the Elovich equation results acquired for the adsorption of Methylene Blue onto Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) in this investigation. For Methylene Blue dye, the correlation

coefficients for the Elvovich equation varied between 0.9517 and 0.9704, respectively. Low correlation coefficient values were discovered, indicating that the Elvovich model is not understandable with respect to the experimental data.

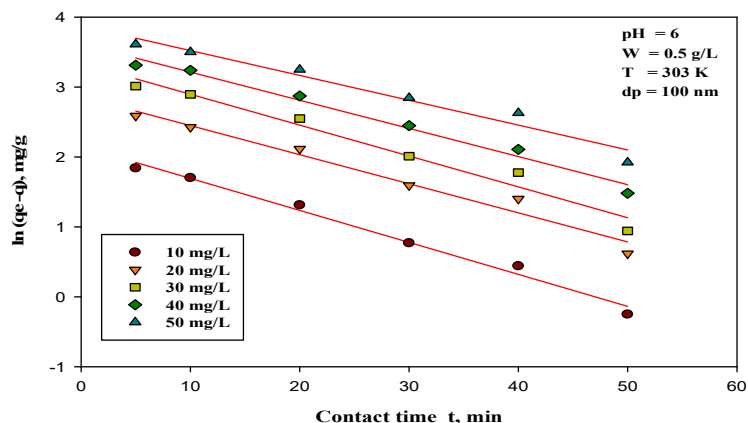


Fig. 9: Pseudo first-order kinetic model for methylene blue dye using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as Adsorbent.

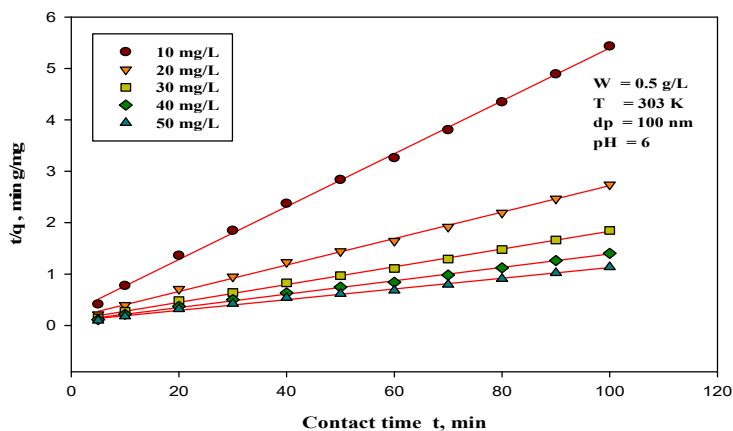


Fig. 10: Pseudo-second order kinetic model for methylene blue using Manila tamarind shell copper nanoparticles (MTS-CuNPs) adsorbent.

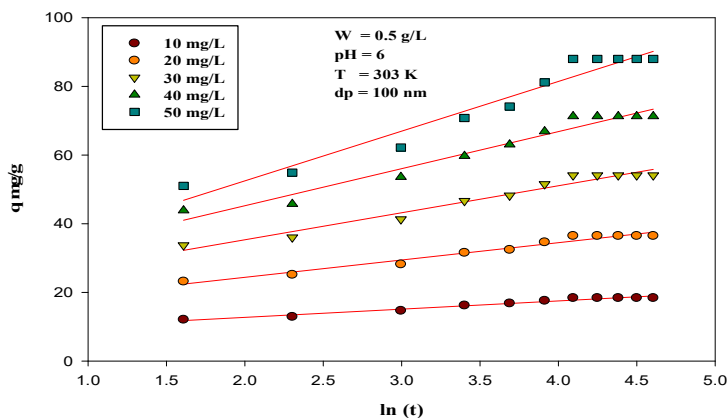


Fig. 11: Elovich model kinetic model for methylene blue using Manila tamarind shell copper nanoparticles (MTS-CuNPs) adsorbent.

Intra-particle diffusion kinetic model: The computed intra-particle diffusion rate constant K_{id} values for methylene blue are displayed in Table 3. Furthermore, the fact that the intra-particle diffusion region's straight line did not pass through the origin in Fig. 12 suggests that the intra-particle diffusion was not the only step that limited the rate. The substantial boundary layer effect on the adsorption process is indicated by the high intercept values. Therefore, the current adsorption process may be primarily regulated by the surface adsorption mechanism in conjunction with intraparticle diffusion.

The study analyzed adsorption data using pseudo-first order, pseudo-second order, Elovich, and Intra-particle diffusion models. The results showed that the theoretical q_e values from the first-order kinetic model and Elovich model were significantly different from experimental values, and the correlation coefficients were lower. The pseudo-first-order kinetic model, the Elovich model, and the Intra-particle diffusion model did not accurately describe the sorption system. However, the calculated q_e values were close to the experimental q_e values, and the correlation coefficients were close to unity for pseudo-second-order kinetics for Methylene blue. The applicability of the pseudo-second-order kinetic model results confirmed that the rate-limiting step in the process is chemisorption.

Thermodynamic Studies

Thermodynamic studies provide information about the feasibility of the process and the nature of the adsorption process. To estimate the thermodynamic parameters for the adsorption of Methylene blue dye using *Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs)* as adsorbent, the experiments were conducted, and data were analyzed.

The values of ΔH° and ΔS° were calculated from the slope and intercept of the linear Van't Hoff plot, i.e., $\ln(q_e/c_e)$ vs $(1/T)$. These plots are shown in Fig. 13 for Methylene blue dye. The estimated thermodynamic properties, along with the correlation coefficients (R^2), are tabulated in Table 4. The adsorption process of Methylene blue using Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) was studied using various parameters. The negative value of ΔG^0 at a given temperature confirmed the spontaneous nature of the adsorption process, while the decrease in ΔG^0 values with temperature indicated a decrease in adsorption feasibility. The physisorption mechanism was found to be dominant, with Gibbs free energy values ranging from -20 to 0 kJ.mol⁻¹. The positive value of ΔH^0 indicates endothermic adsorption, as the total energy absorbed in bond-breaking is more than the energy released in bond-making. The positive value of ΔS° suggests that the adsorption leads to order through the formation of an activated complex, suggesting an associated mechanism.

Van 't Hoff Equation: $\ln K_c = -\Delta H/R T + \Delta S/R$

Where: $\ln K_c$: Equilibrium constant at a given temperature.

T: Absolute temperature (K).

R: Universal gas constant (8.314 J mol⁻¹ K⁻¹)

$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

$$\Delta G^0 = -RT \ln K_c$$

CONCLUSIONS

The data obtained from the Adsorption studies showed that a contact time of 60 min was sufficient for the maximum removal of Methylene blue dye from aqueous solution using Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as adsorbent. The experimental data of adsorption of the

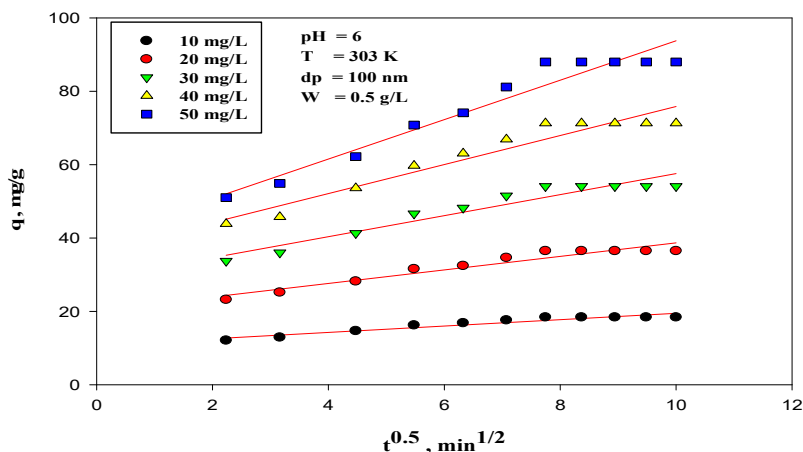


Fig.12: Intra-particle diffusion model Kinetis model for methylene blue dye using Manila tamarind shell copper nanoparticles (MTS-CuNPs).

Table 3: Kinetic model parameters for Methylene blue removal using MTS copper nanoparticles (MTS-Cu-NPs).

Concentration, mg.L ⁻¹	Pseudo-first order kinetics			Pseudo-second order kinetics		
	$K_{1,ad}$ [l.min ⁻¹]	q_e [mg.g ⁻¹]	R^2	$K_{2,ad}$ g/[mg.min ⁻¹]	q_e [mg.g ⁻¹]	R^2
10	0.0457	8.5906	0.9876	0.0103	19.4552	0.9988
20	0.0415	17.535	0.9703	0.0044688	38.9105	0.998
30	0.0441	28.2022	0.9685	0.002853	57.8703	0.99798
40	0.0402	37.1996	0.9831	0.001923	76.923	0.99737
50	0.0355	48.2984	0.9607	0.001247	96.1538	0.99483
Concentration, mg.L ⁻¹	Elovich model			Intra-particle diffusion model		
	γ [g.mg ⁻¹]	α mg/[g.min ⁻¹]	R^2	K_{id}	I	R^2
10	0.4165	64.7808	0.97044	0.8691062	10.79435	0.91736
20	0.19786	85.0536	0.96926	1.8441901	20.23917	0.931
30	0.1271	94.7596	0.96572	2.8672991	28.89434	0.92546
40	0.0926	96.8221	0.96144	3.9588287	36.26327	0.9339
50	0.0691	73.4276	0.9517	5.3714315	40.03729	0.94487

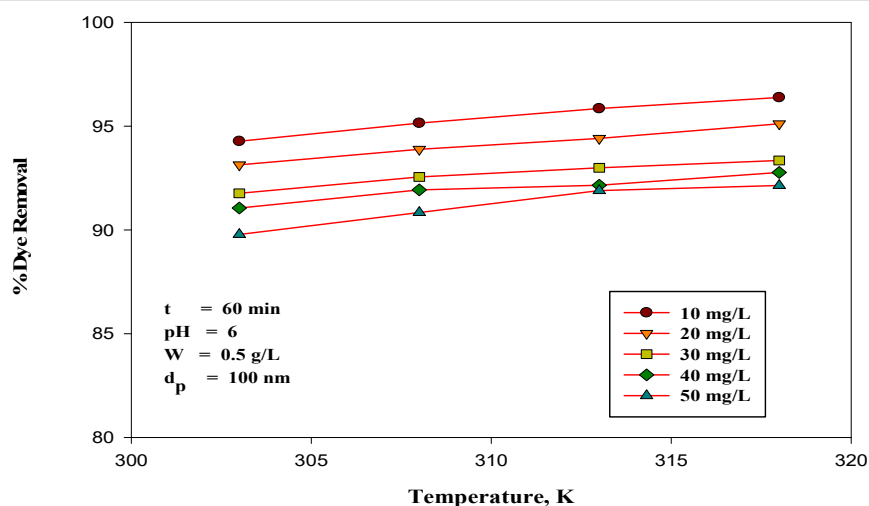


Fig. 13: Van't Hoff Plot for the determination of thermodynamic parameters for the adsorption of methylene blue dye by using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as adsorbent.

Table 4: Thermodynamic parameters for the adsorption of methylene blue dye using Manila tamarind shell copper nanoparticles (MTS-CuNPs) as adsorbent.

Temperature, K	Methylene Blue		
	ΔG^0 [kJ.mol ⁻¹]	ΔH^0 [kJ.mol ⁻¹]	ΔS^0 [J.mol ⁻¹]
303	-8.800313299	25.766	114.12
308	-9.395636191		
313	-9.974746926		
318	-10.50802723		

Methylene blue dye onto the Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) adsorbent fitted well with the Freundlich isotherm model. The isotherm reveals that the Adsorption of the dye onto Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) as adsorbent was favorable.

The maximum removal efficiency was predicted to be 96.37% at a temperature of 318 K, solution pH of 7, an initial dye concentration of 10 mg.L⁻¹, and an adsorbent dosage of 0.5 g.L⁻¹. The experimental data of adsorption of the Methylene blue dye onto the Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) adsorbent fitted well with the pseudo-second-order kinetic model results, confirming that the rate-limiting step in the process is chemisorption. The adsorption process of Methylene blue using Manila Tamarind Shell Copper Nanoparticles (MTS-CuNPs) was studied using various parameters. The negative value of ΔG^0 at a given temperature confirmed the spontaneous nature of the adsorption process, while the decrease in ΔG^0 values with temperature indicated a decrease in adsorption feasibility. The physisorption mechanism was found to be

dominant, with Gibbs free energy values ranging from -20 to 0 kJ.mol^{-1} . The positive value of ΔH^0 indicates endothermic adsorption, as the total energy absorbed in bond-breaking is more than the energy released in bond-making. The positive value of ΔS^0 suggests that the adsorption leads to order through the formation of an activated complex, suggesting an associated mechanism. MTS-CuNPs are useful materials in a variety of industries, including water treatment, catalysis, antimicrobials, and electronics. They are cost-effective because they use agricultural waste as a precursor and employ a green synthesis process. Their recyclability and low operating costs make them economically feasible. MTS-CuNPs are scalable for industrial applications because of their facile, low-energy synthesis technique and readily available raw ingredients. Their ease of integration into current systems, as well as the growing need for sustainable technology, increases their potential for large-scale production.

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Opportunities and Challenges in Plastic Waste Management Strategies in Boyolali, Indonesia

Kusmiyati¹, Hasan² and S. Isworo^{3†} 

¹Department of Industrial Engineering, Universitas Dian Nuswantoro, Semarang, Central Java, 50131, Indonesia

²Department of Environmental Health, Universitas Dian Nuswantoro, Semarang, Central Java, 50131, Indonesia

³Faculty of Economics, Universitas Wahid Hasyim, Semarang, 50232, Indonesia

†Corresponding author: S. Isworo; slamet.isworo@dsn.dinus.ac.id

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ABSTRACT

Plastic waste has become a complex issue in the Boyolali district, where much of it is either burned by the community or contributes to environmental pollution. Pyrolysis technology offers a solution by converting plastic waste into renewable and sustainable fuel. This research aims to evaluate the management system of plastic waste and its alternative utilization through pyrolysis technology. The research method for evaluating plastic waste is conducted using a descriptive qualitative approach, while the pyrolysis study is carried out experimentally. Renewable energy-based technologies, such as pyrolysis, are needed to convert plastic waste into high-calorific fuel. The lower heating value (LHV) of Pyrolysis Fuel Oil (PFO) is 9,240 Kcal/kg, with a density of 0.795, giving it high energy potential, making it a suitable candidate for renewable fuel. The pyrolysis process lasts for 7 hours per batch, resulting in a total monthly output of 1,625 L, which consists of 1,125 L of diesel, 250 L of kerosene, and 250 L of gasoline. To operate this process, four workers are required, with a monthly electricity consumption of 350 kWh. Pyrolysis technology offers a sustainable solution to reduce waste and decrease dependence on fossil fuels.

INTRODUCTION

Plastic waste consists of long carbon chains that are difficult to degrade naturally. This issue has become increasingly important due to the significant increase in plastic waste in various regions of Indonesia. Integrated waste management has become one of the greatest challenges in efforts to protect the environment from plastic waste pollution (Evode et al. 2021, de Graaf et al. 2003). Despite various recycling management strategies, single-use plastic regulations and eco-friendly design projects being quite effective in reducing plastic waste, challenges in implementing waste management regulations still exist, such as the lack of alignment between national and local regulations, insufficient public education on plastic waste, difficulty in finding suitable alternatives to plastics, and the complexity of transforming plastic waste into more useful materials (Cornago et al. 2021).

The Boyolali Environmental Service is the local government agency responsible for environmental management in the region, including waste management at the Final Disposal Site (TPA). The TPA Winong Technical Implementation Unit specifically handles operational tasks related to final waste processing in the Boyolali Regency (Wahid & Rizka 2024). According to the Ministry of Environment and Forestry, Indonesia generates more than 42 million tons of urban waste annually, including 7.8 million tons of plastic waste, of which about 4.9 million tons is not managed properly (Supriandi & Rahmawati 2023). Plastic waste comes from human activities in both urban and rural areas. According to data from BPS 2018, Indonesia has 75,436 villages and 8,444 sub-districts, where plastic



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waste management in rural areas is often inadequate due to the lack of waste management infrastructure (Wijaya 2020).

Boyolali Regency, which has experienced rapid economic growth, consists of 75 urban areas and 192 rural areas (Seruyanintyas 2019), with a population of 61,032 people generating 287.3 tons of waste per day, Boyolali faces significant waste management challenges, with 20.89% of the total waste being plastic waste (Pemda Boyolali 2021). Waste management practices in Boyolali show that 67% of residents still burn their waste, 7% dispose of it into water bodies, while the rest compost, stockpile, or take their waste to temporary collection sites (Sari et al. 2022). Waste management regulations in Boyolali are governed by Regent Regulation No. 2 of 2021, which includes strategies and policies for reducing household waste and improving its management (Pemda Boyolali 2021).

Waste management in Boyolali needs improvement, particularly in handling plastic waste and reducing the volume of unmanaged waste. While significant efforts have been made in waste reduction and handling, there are still major challenges in achieving fully effective waste management. The increasing amount of waste generated along with the growing population and consumption, coupled with the low percentage of waste management, indicates the need for innovation in waste management policies, improved recycling facilities, and public education to better reduce and manage waste (Raihan et al. 2024, Ishartomo et al. 2020).

The success of plastic waste management at TPA Winong heavily depends on the infrastructure, technology, and management practices in place. Proper management is essential to control plastic waste and minimize environmental impacts. Public participation in waste separation before disposal is crucial for effective management. Educational programs and awareness-raising can improve sorting practices and reduce the volume of plastic waste entering the final disposal site (Maskun et al. 2023).

One promising technology for managing plastic waste is pyrolysis, a recycling process that transforms plastic waste into renewable energy. Pyrolysis operates under atmospheric pressure and at around 500°C (Radhakrishnan et al. 2023). This process breaks down plastic waste into several by-products, such as gas (H_2 , CO, CO_2 , H_2O , and CH_4), pyrolytic oil, and charcoal (Armenise 2021). Pyrolysis technology has the potential to reduce waste volume, utilize by-products, and reduce environmental impacts, making it a valuable part of plastic waste management at TPA Winong. However, the success of this technology depends heavily on adequate investment, effective management, and strong legislative support (Eze et al. 2021).

This study aims to evaluate the plastic waste management system in Boyolali and assess the effectiveness of solutions designed to reduce the volume of plastic waste sent to the TPA Winong in Boyolali Regency.

MATERIALS AND METHODS

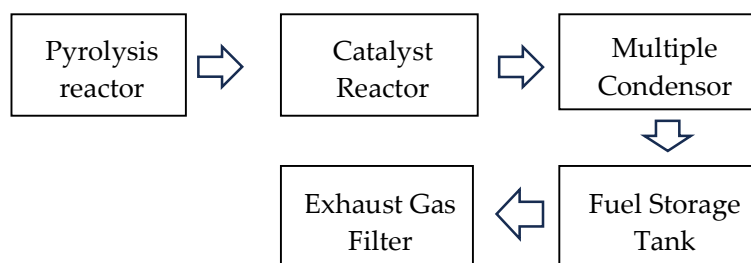
The research on the general description and condition of plastic waste in the Boyolali region was conducted using a qualitative descriptive approach, relying on secondary data. This approach involves utilizing data that has been previously collected by other researchers, such as reports, articles, statistics, and other relevant documents, to analyze and understand the state of plastic waste in the area. Secondary data enables a comprehensive examination of the phenomenon without the need for new primary data collection.

This method provides a clearer picture of the composition and management of plastic waste in Boyolali based on existing information. The article does not specify a particular sample size for waste analysis but focuses on discussing the composition of waste based on secondary data sources (Thorne 2013).

The problem of plastic waste requires effective management to mitigate its negative impacts on both the environment and public health. One potential solution to this issue is the adoption of pyrolysis technology. This technology allows for the conversion of plastic waste into liquid fuel that can be reused. By transforming plastic waste into an alternative energy source, pyrolysis technology can help reduce the volume of plastic waste while also decreasing reliance on fossil fuels, which are both depleting and environmentally harmful.

The research on the application of pyrolysis technology was carried out with an experimental approach using plastic waste as raw material. In the pyrolysis process, the plastic waste is heated in the absence of oxygen, resulting in a liquid product that can be used as an alternative fuel. This liquid product is then analyzed to determine its physical properties, such as density, viscosity, calorific value, and cetane/octane numbers. The analysis results are compared with conventional diesel fuel to assess its quality and feasibility as an energy source.

Furthermore, to ensure that the pyrolysis process does not have a negative environmental impact, gas emissions such as CO and CO_2 produced during the process are also tested. This emission testing aims to evaluate the potential environmental impact of the gases produced, thus helping to determine the extent to which pyrolysis technology is environmentally friendly and sustainable. In this way, pyrolysis technology



(Lubis et al. 2022).

Fig. 1: Pyrolysis reactor design.

not only provides a solution for managing plastic waste but also offers a more environmentally friendly energy alternative. All these steps are highly relevant to the efforts in managing plastic waste in Boyolali, contributing to the creation of sustainable solutions for both the environment and the local community (Ascher et al. 2022). The Prolysis reactor design is given in Fig. 1.

RESULTS AND DISCUSSION

Boyolali is a district located in Central Java Province, covering an area of 1,015.10 km² and having a population of approximately 1,090,131 people. Based on its geographical location, Boyolali is situated between 110°22' and 110°50' East Longitude, and between 7°7' and 7°36' South Latitude, with an elevation ranging from 75 to 1,500 meters above sea level (Fig. 2). The regency consists of 22 sub-districts, characterized by a topography that includes lowland areas as well as hills and mountains (BPS Boyolali 2023). The natural

conditions and strategic geographical location of Boyolali Regency support its economic development. The Gross Regional Domestic Product (GRDP) of Boyolali Regency exceeds the national economic growth target and realization, which is around 5-5.5%. (Raihan et al. 2024). Population plays a significant role in driving economic growth. As the population increases, it contributes to economic expansion by broadening the market, which enhances the level of specialization within the economy. Analyzing population density helps to determine how concentrated the population is in the Boyolali District. This information is essential for designing infrastructure, managing resources, and addressing the impacts of economic growth and development. Specifically, it aids in formulating policies related to waste management resulting from development activities. The population density in Boyolali Regency is 1,009 people per km² in 2023 and is projected to increase to 1,074 people per km² in 2024. This places the population density in the moderate category, indicating a relatively balanced

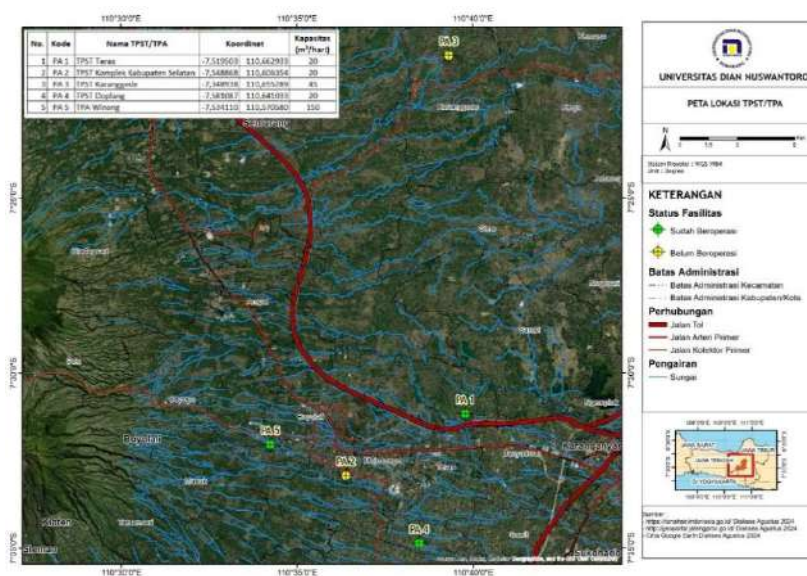


Fig. 2: Map of study area boundaries.

Table 1: Projected increase in waste generation in Boyolali District.

Period	Population	Growth Rate [%]	Waste Generation [ton.day ⁻¹]	Annual Waste Generation [tons]	Waste per Person [kg.day ⁻¹]	Plastic Waste [kg.day ⁻¹]	Percentage of Plastic [%]
th2019	984,807	1%	266	97,052	0.270	0.034	12.6%
th2020	989,619	1%	288	105,095	0.291	0.049	16.7%
th2021	1,070,247	1%	291	106,159	0.272	0.057	20.9%
th2022	1,079,952	1%	319	116,383	0.295	0.086	29.2%
th2023	1,090,131	1%	330	120,934	0.303	0.114	37.5%
th2024	1,133,245	1%	344	125,485	0.304	0.127	41.6%
Avg	1,058,000	1%	306	111,851	0.290	0.077	26.4%

Prediction of percentage composition of plastic waste $y = 16.58x + 4.31$

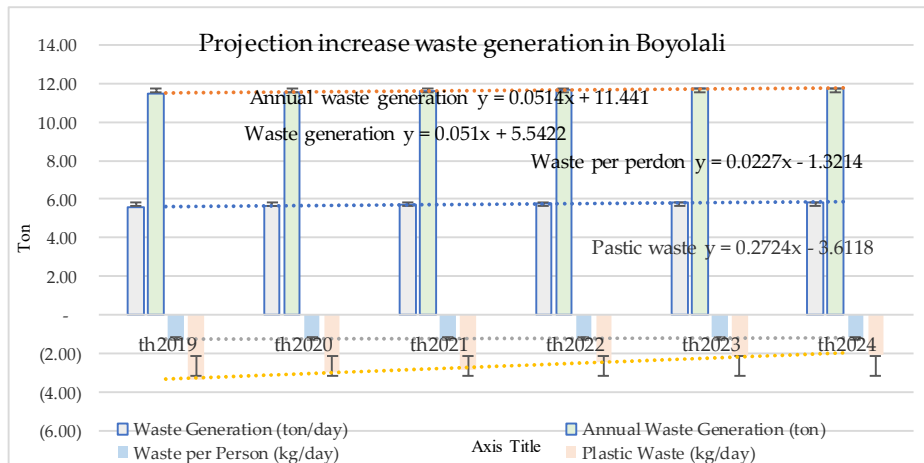


Fig. 3: Trends in the increasing generation of waste and plastic waste in Boyolali Regency.

population distribution. The population growth rate in Boyolali Regency remains stable at 1%, which falls within the stable growth rate range of 1% to 2%. (Cilluffo & Ruiz 2019) The continuous increase in population and economic growth will indirectly lead to an increase in the amount of waste generated as a result of anthropogenic activities.

Table 1 presents the population, population growth rate, and waste predictions for the period from 2019 to 2024. Fig. 3 illustrate that waste generation (tons.day⁻¹), annual waste generation (tons), waste production per person (kg.day⁻¹), and plastic waste production (kg.day⁻¹) in Boyolali Regency are projected to increase each year from 2019 to 2024.

The predicted increase for 2024 is 344 days per ton, or 125,485 years per ton, while the average waste production per person from 2019 to 2024 is 0.29 kg per day. In comparison, an average Indonesian citizen contributes 0.7 kg of waste per day. (Aprilia 2021) When compared to the waste produced per day in Boyolali District, it is still below the average waste production in Indonesia (0.29 kg.day⁻¹ is less than the average of 0.7 kg.day⁻¹). The average waste production per district in Indonesia is 883 days per ton, or 38,795,897

years per ton, which accounts for approximately 40% of the district data in Indonesia.

Boyolali Regency, the comparison of waste composition percentages in 2019 revealed that the highest contributors were food waste (53.84%) and plastic (20.89%). Other components included wood and branches (1.8%), paper and cardboard (13.3%), metal (1.1%), cloth (1.57%), rubber and leather (1.05%), glass (0.98%), and others (5.46%). By 2021, the composition had shifted, with food waste contributing 32.18% and plastic rising to 37.47%. The breakdown also included wood and branches (11.85%), paper and cardboard (9.37%), metal (5.39%), cloth (0.67%), rubber and leather (0.70%), glass (0.70%), and others (1.67%) (Table 2).

Based on projections, the percentage of plastic waste in Boyolali Regency has been increasing: 12.6% in 2019, 16.7% in 2020, 20.9% in 2021, 29.2% in 2022, 37.23% in 2023, and 37.5% projected for 2024. This increase in plastic waste is also occurring in all surrounding regencies of Boyolali.

In Boyolali Regency, waste management is regulated by Regional Regulation Number 13 of 2013 and Regent

Table 2: Waste composition percentages in Boyolali District and surrounding Districts.

District	Boyolali		Klaten		Sukoharjo		Wonogiri		Sragen	
Period	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023
Food Waste (%)	53.84	32.18	60.00	35.86	77.1	52.55	22.3	25.00	74.7	74.3
Wood-Twigs (%)	1.8	11.85	5.00	32.92	1.42	0.42	29.48	10.00	0.9	0.8
Paper-Cardboard (%)	13.31	9.37	5.00	3.52	3.12	13.33	19.74	20.00	8.4	8.9
Plastic (%)	20.89	37.47	20.00	35.87	14	23.79	11.81	25.00	11.1	11.2
Metals (%)	1.1	5.39	0.50	2.45	0.21	0.89	6.54	5.00	0.4	0.5
Cloth (%)	1.57	0.67	5.00	2.13	1.39	1.19	0	5.00	2.2	1.8
Rubber-Leather (%)	1.05	0.70	3.00	2.00	0.16	0.33	0	2.50	0.3	0.3
Glass (%)	0.98	0.70	0.50	0.36	0.16	1.03	6.07	5.00	1.7	1.8
Other (%)	5.46	1.67	1.00	0.31	2.4	6.47	4.06	2.50	0.3	0.4

Regulation Number 68 of 2018, which outline the strategies and policies of the local government regarding waste management. This framework emphasizes the reduction and proper handling of waste. Waste is a major environmental issue that significantly impacts society, as every household generates waste daily. Proper management of this waste is essential to ensure that it does not disrupt the comfort of the community.

The final disposal site in Boyolali Regency, with a controlled landfill system, is equipped with waste management facilities such as a waste weighing post, a methane gas utilization installation, a waste sorting area, a waste fermentation area (composting), and a liquid fertilizer manufacturing area. In addition to core waste management facilities, learning facilities are also provided for the community in the form of an educational park. The final disposal site in Boyolali Regency, namely the Kuncen Winong Final Disposal Site, covers an area of 3.7 ha, with the addition of 0.29 ha of land in 2018, so the total area of the Kuncen Winong Final Disposal Site is 3.99 ha. The area of the final disposal site that has been utilized as of 2020 is 3.8 ha.

The waste generated by the community in Boyolali Regency continues to increase each year. Table 3 presents the current conditions and forecasts for the Winong Final Disposal Site (TPA) in Boyolali Regency, including the potential for waste accumulation, the amount of waste reduction, the volume of waste processed, and both managed and unmanaged waste. Therefore, there is a need for alternative plastic waste management through sustainable technology.

The potential waste accumulation at Winong TPA indicates a significant increase in waste generation, necessitating effective handling strategies. This includes enhancing waste reduction practices, improving waste sorting and processing methods, and implementing sustainable

technologies to manage both managed and unmanaged waste efficiently. The key findings indicate that Boyolali is facing significant challenges in managing the increasing amount of waste, particularly plastic waste. Therefore, a more holistic and sustainable approach is needed to reduce and manage waste in the region, along with the implementation of technologies that can effectively address this issue.

On average, this amounts to approximately 0.08 tons per day per person. Divided by the number of residents, this translates to about 0.29 kg (Table 1) of plastic waste per person per day. This figure varies depending on the region and individuals' consumption habits.

Based on Table 4, the predicted average population from 2019 to 2024 is 1,058,000, with waste production amounting to 111,851 tons per year. Of this, 56,573 tons (51%) are managed in landfills, while 51,810 tons per year remain unmanaged (46%). Additionally, approximately 3.1% of waste, estimated at 3,468 tons per year, is lost, possibly due to transportation activities or being utilized by the community (scavengers) before reaching the landfill. The average daily production of plastic waste is 0.267 tons, with 0.137 tons per day managed and 0.127 tons per day being unmanaged. The percentage of unmanaged plastic waste in Boyolali Regency is estimated to be around 46% of the total plastic waste generated. This indicates that a significant amount of plastic waste accumulates and ends up in landfills or the environment, without going through proper recycling or management processes. This figure highlights the substantial challenges faced in plastic waste management and underscores the importance of improving recycling systems and raising community awareness about reducing plastic usage. (Dai et al. 2022). Therefore, more intensive management is needed, specifically through alternative technologies based on renewable and sustainable energy, as well as reducing dependence on fossil fuels by utilizing waste, particularly plastic waste (Achi et al. 2024). Pyrolysis

Table 3: Potential waste accumulation and its management at the Winong final disposal site.

		Year 2019	Year 2020	Year 2021	Year 2022	Year 2023	Year 2024						
I	Potential Waste Piles [tons. year ⁻¹]	96,262	97,052	104,044	105,095	105,097	106,159	115,219	116,383	119,772	120,934	124,280	125,485
(Population x 0.29 kg.person ⁻¹ .day ⁻¹)													
II	Waste reduction amount	24,417	25,080	26,973	28,319	29,329	29,409	29,543	29,623.44	29,758	29,840	29,976	30,058
	Waste reduction percentage	25	26	27	28	29	30	30	31.02	31	32	31	32
a	Waste generation limitation	18	19	21	22	23	24	22	23.12	21	22	21	22
	Adiwiyata school	18	19	21	22	23	24	22	23.12	21	22	21	22
b	Waste is utilized at source	7,073	7,635	8,489	8,816	9,034	9,159	8,678	8,776	8,378	8,459	8,118	8,155
	Waste bank unit	81	84	90	94	97	99	97	98.3	97	98	97	98
	TPS3R	225	352	491	510	457	531	304	352.97	202	235	135	156
	Scavengers of used goods	6,767	7,199	7,908	8,212	8,480	8,529	8,277	8,325	8,079	8,126	7,886	7,932
c	Amount of waste recycled at source	17,325	17,352	18,047	18,741	19,394	19,464	20,096	20,134	20,879	20,899	21,722	21,599
	Composter and Biopore	146	170	201	209	212	218	189	194.15	168	173	150	154
	Composting by TPS3R	76	152	234	243	187	253	97	131.59	51	68	26	36
	Biodigester	17,103	17,030	17,612	18,289	18,995	18,993	19,810	19,808	20,660	20,658	21,546	21,545
III	Waste handling amount	26	25,399	26,684	27,710	28,758	28,776	29,502	29,520	30,264	30,283	31,046	31,065
a	Waste handling percentage	26	26	27	28	29	30	30	31.46	31	33	33	34
b	Processing	0	80	164	170	175	177	180	181.56	185	187	190	192
	Waste processed into raw materials	0	80	164	170	175	177	180	181.56	185	187	190	192
	TPS3R is city-managed	0	80	164	170	175	177	180	181.56	185	187	190	192
c	Final processing	25,090	25,318	26,520	27,540	28,587	28,600	29,419	29,432	30,275	30,288	31,156	31,170
	Waste processed at TPA	25,090	25,319	26,520	27,540	26,943	28,602	27,897	28,405	29,767	29,954	32,048	32,166
	Landfilling TPA	24,411	23,530	23,553	24,459	25,400	25,401	27,365	27,365	29,482	29,482	31,763	31,763
	Composting TPA	460	1,643	2,888	2,999	1,477	3,115	429	905.72	125	263	36	77
	Recovery by scavengers	219	146	79	82	66	86	103	134.39	160	209	249	326
IV	Managed waste (ii + iii)	49,533	50,478	53,390	55,443	57,514	57,576	58,577	58,640.32	59,659	59,724	60,762	60,828
	Percentage of managed waste	51	52	55	57	59	60	60	61	62	63	64	65
V	Unmanaged waste (I - IV)	46,729	46,576	46,389	46,389	48,165	48,174	52,141	52,150.50	56,445	56,455	61,104	61,115

Description: TPA: Final Waste Disposal Site, TPS3R : Waste Management Place for Reuse, Reduce, and Recycle. Adiwiyata School is an environmental education program focused on promoting a healthy, clean, and beautiful environment (BPS Boyolali 2023).

Table 4: Annual trends in waste management and plastic waste composition in Boyolali (2019–2024).

A	B	C	D	E	F	G	H	I	J	K	L
Th2019	984,807	97,052	49,533	46,576	96,109	961	1.0%	0.13	0.067	0.063	0.130
Th2020	989,619	105,095	53,390	46,389	99,779	5,089	5.1%	0.17	0.091	0.079	0.170
Th2021	1,070,247	106,159	57,514	48,174	105,688	423	0.4%	0.21	0.114	0.096	0.210
Th2022	1,079,952	116,383	58,577	52,151	110,728	5,426	4.9%	0.29	0.153	0.137	0.290
Th2023	1,090,131	120,934	59,659	56,455	116,114	4,645	4.0%	0.38	0.195	0.185	0.380
Th2024	1,133,245	125,485	60,762	61,115	121,877	3,534	2.9%	0.42	0.209	0.211	0.420
Average	1,058,000	111,851	56,573	51,810	108,382	3,346	3.1%	0.27	0.139	0.127	0.267

Source: Boyolali Regency Environmental Service, 2019.

Information:

A	Period (Year)	I	Percentage composition of plastic waste (%)
B	Population (Number of inhabitants)	J	Managed plastic waste (tons/day)
C	Waste Production/year/ton	K	Unmanaged plastic waste (tons/day)
D	Amount of managed waste	L	Total amount of plastic waste (managed and unmanaged)
E	Amount of unmanaged waste		
F	Total waste is the sum of unmanaged + managed waste.		
G	Unknown/lost waste		
H	Percentage of unknown/lost waste		

technology is a process that breaks down polymer chains into simpler compounds through thermal processes (heating) while utilizing minimal oxygen. Plastic waste can be decomposed and converted into fuel with a high calorific value through pyrolysis. (Jahari and Saputra 2021).

Fast Catalytic Pyrolysis Technology

Catalytic pyrolysis technology is a process for recycling plastic by converting it into new plastic materials. The pyrolysis process yields three types of products: solids (char), gas (fuel gas), and bio-oil. The pyrolysis tool series consists of a pyrolysis reactor equipped with an induction heater and a condenser. The reactor generates steam, which is then transformed into liquid through the condenser, resulting in pyrolysis oil (Fink 2021). Fast pyrolysis is a sustainable method, as it can convert non-biodegradable plastic waste into useful energy sources, reducing dependence on fossil fuels. The resulting pyrolysis oil can be utilized as fuel for engines or as a substitute for fossil fuels in various industrial applications. The fast pyrolysis technology for plastic waste has the potential to become an innovative solution for waste management and renewable energy provision, while also reducing the environmental impact of plastic waste (Sonawane et al. 2024). In this study, pyrolysis technology was implemented on a small scale (less than 10 tons) to consider low initial investment, more efficient construction costs, flexibility, and simpler operations. According to Sakthipriya (2022), the design of a plastic processing system

using a pyrolysis device with a capacity of 5 tons per batch consists of 5 units, allowing for a plastic input of 25 tons per day. The estimated products produced from this system are 16.25 tons of oil per day, 6.25 tons of gas per day, and 2.5 tons of char per day (Sakthipriya 2022). The technical and economic analysis conducted on the feasibility of implementing a pyrolysis plant for producing fuel oil (FO) from plastic fractions of Municipal Solid Waste (MSW) indicates a promising opportunity for resource recovery. The catalytic pyrolysis process employs a fixed bed reactor with a capacity of 4 m³, which processes crushed and dried plastic waste at specific temperatures to achieve optimal yields. Additionally, the costs of managing MSW in the municipality can be reduced by up to 54.75% (Hauschild et al. 2022). The mass balance analysis of the pyrolysis process indicates that 35-40% of the output comprises liquid products (30-35% bio-oil and 5-10% water and other by-products), 40-45% consists of gas products, and 15-25% is char. The average yield of bio-oil from the studies ranges from 50-75%. This demonstrates that a small-scale biomass pyrolysis industry, with a capacity of 20-30 dm³ of bio-oil per day, can yield promising economic benefits (Jaroenkhasemmesuk & Tippayawong 2015). Table 5 presents the economic analysis of the pyrolysis plant using various types of fuels, including Liquefied Petroleum Gas (LPG), waste lube oil, and Refuse-Derived Fuel (RDF). (Mani et al. 2020).

Liquefied Petroleum Gas is a flammable mixture of hydrocarbons, primarily consisting of propane, butane, and

Table 5: Economic analysis of the pyrolysis plant based on fuel type (Rajca et al. 2020).

Fuel Type	LPG	Waste Lube Oil	RDF	Economic (Profit/Loss) IDR
Case - 1	100%	0	0%	+ 32.5 Million/Year
Case - 2	0%	100%	0%	+ 54.1 Million/Year
Case - 3	0%	0%	100%	+ 81.8 Million/Year

Table 6: Lower heating value (LHV) of various fuel types.

LHV - MSW (Municipal Solid Waste)	2.973 kcal.kg ⁻¹
LHV - LPG (Liquefied Petroleum Gas)	11.080 kcal.kg ⁻¹
LHV - PFO (Pyrolysis Fuel Oil)	9.240 kcal.kg ⁻¹
LHV - Gasoline	11.055 kcal.kg ⁻¹
LHV - Kerosene	10.270 kcal.kg ⁻¹
LHV - Diesel Oil	10.175 kcal/kg
LHV - NG (Natural Gas)	9.850 kcal.kg ⁻¹

propylene (Thompson et al. 2021). Waste lube oil is oil that has been used to lubricate engines or equipment and has lost its quality and ability to function effectively (Sharma et al. 2020). Refuse-derived fuel (RDF) is a fuel produced from waste or solid refuse after undergoing specific processing. (Shehata et al. 2022). Pyrolysis Fuel Oil (PFO) is a liquid product obtained from the pyrolysis of organic materials, such as biomass or plastic waste (Kumar & Kumar 2024). Table 5, case 3, indicates that RDF is the most profitable option, yielding the highest profit of 81.8 million per year, followed by waste lube oil and then LPG. RDF demonstrates strong performance in terms of calorific value and could be a better choice when considering applicable environmental policies (Sarquah et al. 2022).

Table 6 shows the Lower Heating Values (LHV) of various types of fuels. The Lower Heating Value (LHV) is

the amount of energy that can be obtained from a fuel during combustion, excluding the energy contained in the water vapor produced. LPG (Liquefied Petroleum Gas) has the highest LHV at 11,080 kcal.kg⁻¹, making it the best type of fuel. The LHV of Pyrolysis Fuel Oil (PFO) is 9,240 kcal.kg⁻¹ (Crespo et al. 2023), indicating that its energy efficiency as a fuel is quite good, comparable to commercial fuels such as gasoline, kerosene, and diesel oil. PFO serves as a sustainable energy source that can reduce dependence on fossil fuels and help address pollution caused by plastic waste.

Fig. 4 and Table 6 illustrate the process and schematic of plastic waste pyrolysis. Based on the results of the pyrolysis process using approximately 100 kg of plastic, a total fuel volume of 65 L can be produced, with a distribution of 45 L equivalent to diesel, 10 L equivalent to kerosene, and 10 L equivalent to gasoline. The pyrolysis process lasts for 7 h per batch, with an operating frequency of 1 batch per day, resulting in a total monthly production of 25 batches (assuming one day off, such as Sunday). The total monthly production is as follows: Plastic Diesel: 25 batches × 45 L = 1,125 L, Plastic Kerosene: 25 batches × 10 L = 250 L, and Plastic Gasoline: 25 batches × 10 L = 250 L. The overall total monthly yield is 1,625 L. The total workforce required is 4 people, consisting of 1 Operations Manager, 1 Supervisor, and 2 Operators. The electricity consumption for each batch is 2 kW over 7 h, resulting in a daily consumption of 2 kW × 7 h = 14 kWh. Therefore, the monthly consumption is 14 kWh × 25 batches = 350 kWh.

Table 7 presents the results of the analysis of the physical and thermal characteristics of various fuel samples obtained from the pyrolysis process. It highlights the variations and advantages of each sample in terms of sustainability and reducing dependence on fossil fuels.

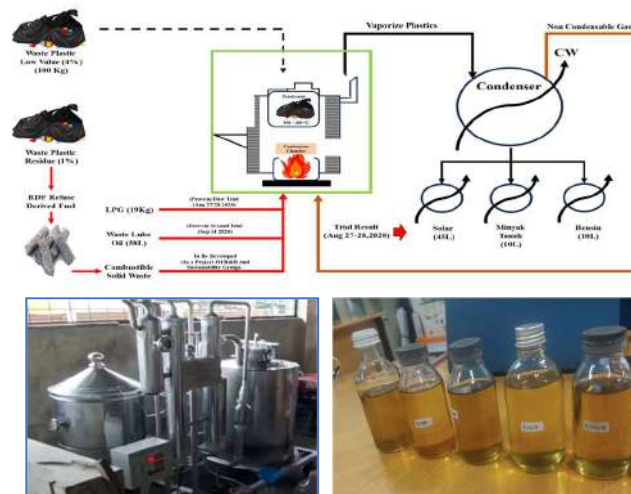


Fig. 4: Process and schematic of plastic waste pyrolysis.

Table 6: Product yield calculation and operating conditions for plastic pyrolysis.

1	Pyrolysis Capacity	= 100 kg/Batch
2	Pyrolysis Yield	
	Solar Plast (Diesel Oil)	= 45 L
	Minyak Tanah Plast (Carosene)	= 10 L
	Bensin Plast (Gasoline)	= 10 L
3	Running H Per Batch	= 7 H
4	Operation Day	One Batch/Day
5	Monthly Total Production (Every day Running, Except Sunday)	= 25 Batches
6	Total Manpower Requirement	= 4 Person
	Operation Manager	1
	Supervisor	1
	Operator	2
7	Electricity Consumption 2 Kw For 7 H Of the Pyrolysis Process	

The D-100 has a specific gravity of 0.795 and a density of 794 kg.m^{-3} , indicating that this sample can be considered a light liquid among fuels. Specific gravity is the ratio of a substance's density to the density of water at a specific temperature (4°C), allowing it to be categorized as a light liquid. Light liquids have the advantage of quick evaporation, which aids in the combustion process in engines, enhancing efficiency. With a high calorific value, they can produce more energy per unit volume, leading to a more efficient combustion process and lower emissions compared to heavier liquids. (Larionov et al. 2022). The kinematic viscosity of $2.25 \text{ mm}^2.\text{s}^{-1}$ at 40°C indicates that this sample has relatively good flow characteristics. With a boiling point and flash point below 25°C , this sample is classified as a flammable material. The heating value of 19,929 BTU/lb demonstrates that this fuel has a high energy potential, making it a suitable candidate for fuel applications. Overall, this sample possesses

characteristics that are well-suited for use as a fuel, though it has flammable properties. Despite its flammable nature, it has a flash point $< 250^\circ\text{C}$.

The D-100-1 data reveals several important characteristics. Its specific gravity at 60°F is 0.794, which is lower than the standard range of 0.860-0.880, indicating that this sample is lighter than other reference materials. The density at 15°C is 793 kg.m^{-3} , also below the standard range of 860-830 kg.m^{-3} , further confirming its lighter nature. The kinematic viscosity at 40°C is $2.43 \text{ mm}^2.\text{sec}^{-1}$, which is lower than the standard range of 4.0-6.1 $\text{mm}^2.\text{sec}^{-1}$. This lower viscosity suggests that D-100-1 flows more easily at this temperature. The flash point of 31°C , while higher than that of materials with lower flash points, is still below the standard range of $48\text{-}55^\circ\text{C}$, indicating that D-100-1 poses some fire risk. However, the higher heating value of 19,936 BTU/lb exceeds the standard value of 17,500-18,000 BTU/lb, suggesting good combustion efficiency and making it an effective energy source. D-100-1 is a lighter fuel with low viscosity and high combustion efficiency (Khan et al. 2016). While it offers increased safety compared to materials with lower flash points, it is important to remain aware of the fire risk due to its flash point being below the ideal standard (Gao 2010).

The D-100-1-50 data presents several key characteristics. Its specific gravity at 60°F is 0.827, which is lower than the standard range of 0.850-0.870, indicating that this sample is lighter than reference materials but heavier than both D-100 and D-100-1. The density at 15°C is 826 kg.m^{-3} , also below the standard range of 890 - 860 kg.m^{-3} . The kinematic viscosity at 40°C is $2.78 \text{ mm}^2.\text{sec}^{-1}$, which falls below the standard range of 3.5-5.5 $\text{mm}^2.\text{sec}^{-1}$. This suggests that while the viscosity is higher compared to D-100 and D-100-1, D-100-1-50 still has a lower viscosity than the ideal value. The flash point of 24°C indicates a higher fire risk, as it is

Table 7: Physical and thermal characteristics of various fuel samples (Capareda 2023) (Misna & BTBRD-BPPT n.d.).

SampeI	Specific gravity at 60°F	Standard specific gravity	Density at 15°C [kg.m^{-3}]	Standard density	Kinematic viscosity at 40°C [$\text{mm}^2.\text{sec}^{-1}$]	Standard kinematic viscosity	Flash point ($^\circ\text{C}$)	Standard flash point	Heating value (BTU/lb)	Standard heating value
D-100	0.795	0.880-0.895	794 [kg.m^{-3}]	830-860	2.25 [$\text{mm}^2.\text{sec}^{-1}$]	4.0-6.0	$<25^\circ\text{C}$	$48\text{-}55^\circ\text{C}$	19929	18,500
D-100-1	0.794	0.860-0.880	793 [kg.m^{-3}]	830-860	2.43 [$\text{mm}^2.\text{sec}^{-1}$]	4.0-6.1	31 ($^\circ\text{C}$)	$48\text{-}55^\circ\text{C}$	19936	17,500-18,000
D-100-1-50	0.827	0.850-0.870	826 [kg.m^{-3}]	860-890	2.78 [$\text{mm}^2.\text{sec}^{-1}$]	3.5-5.5	24 ($^\circ\text{C}$)	$48\text{-}55^\circ\text{C}$	19734	17,000-17,500
D-100-2-50	0.813	0.850-0.870	812 [kg.m^{-3}]	850-880	2.55 [$\text{mm}^2.\text{sec}^{-1}$]	3.5-5.5	24 ($^\circ\text{C}$)	$48\text{-}55^\circ\text{C}$	19817	17,000-7,500
Solar Fuel	0.831	0.820-0.870	837 [kg.m^{-3}]	820-860	4.55 [$\text{mm}^2.\text{sec}^{-1}$]	2.0-4.5	52 ($^\circ\text{C}$)	52 to 60°C	19300	18,500

Notes: Method American Standard Testing and Materials (ASTM)

significantly below the recommended standard range of 60-100°C, making D-100-1-50 quite flammable.

On the other hand, the heating value of 19,734 BTU/lb exceeds the standard value of 17,000-17,500 BTU/lb, indicating good energy efficiency despite the fire risk. In summary, D-100-1-50 exhibits characteristics that make it heavier and with higher viscosity compared to D-100 and D-100-1. While it has a favorable heating value, the low flash point signifies a heightened fire risk. Therefore, careful consideration of safety precautions is essential when using.

D-100-2-50 data highlights several important characteristics. Its specific gravity at 60°F is 0.813, which is below the standard range of 0.850-0.870, indicating that this sample is lighter than other reference materials. The density at 15°C is 812 kg.m⁻³, also lower than the standard range of 880 - 850 kg.m⁻³. The kinematic viscosity at 40°C is 2.55 mm².sec⁻¹, falling below the standard range of 3.5-5.5 mm².sec⁻¹. This low viscosity suggests that D-100-2-50 flows more easily. The flash point of 24°C indicates a high fire risk, as it is significantly below the recommended standard range of 60-100°C, making the sample highly flammable. Conversely, the heating value of 19,817 BTU/lb exceeds the standard value of 17,000-17,500 BTU/lb, indicating good combustion efficiency and making it an effective energy source despite the associated fire risk. D-100-2-50 exhibits characteristics that make it lighter with low viscosity and good energy efficiency. However, the high fire risk due to its low flash point is a significant concern. As indicated by the analysis of the flash point of fuel derived from the pyrolysis of plastic waste, it is crucial to exercise caution regarding workplace safety due to its flammability.

Solar Fuel has been Specific Gravity at 60°F: 0.831 (within the standard range of 0.820 - 0.870), Density at 15°C: 837 kg.m⁻³ (within the standard range of 860-820 kg.m³), Kinematic Viscosity at 40°C: 4.55 mm².sec⁻¹ (within the standard range of 2.0-4.5 mm².sec⁻¹), Flash Point: 52°C (at the upper end of the standard range of 52-60°C), Heating Value: 19,300 BTU/lb (above the standard value of 18,500 BTU/lb). The solar fuel sample meets or exceeds standard values for specific gravity, density, and heating values, while its kinematic viscosity is within acceptable limits. The flash point indicates a moderate fire risk, necessitating careful handling.

Low-density and low-viscosity fuels such as D-100 are more efficient in the combustion process, leading to reduced emissions and offering greater benefits as an environmentally friendly energy source. This results in cleaner and more efficient combustion, which, in turn, reduces emissions and pollution while maximizing the energy potential generated. However, fuels with a low flash point (like D-100) increase

the risk of fire, whereas fuels like Solar Fuel, with a higher flash point, are more stable and safer for storage and transportation. Fuels with high calorific value, such as D-100 and Solar Fuel, allow for more efficient combustion, reducing environmental impacts as they require less fuel to produce the same amount of energy (Praveenkumar et al. 2022). The use of tightly sealed containers for fuel, protected from high temperatures, is crucial to prevent fuel evaporation and avoid uncontrolled fires. Strict handling policies for fuels with a low flash point must be implemented by trained personnel, following stringent procedures to prevent fires and explosions. This includes the use of appropriate fire extinguishing equipment and personal protective gear (Nolan 2014, Santos et al. 2020).

Catalytic pyrolysis technology for plastics can have significant environmental impacts, with several key benefits and challenges as follows: a) Plastic Waste Reduction: Pyrolysis transforms plastic into valuable products such as fuel (Pyrolysis Fuel Oil/PFO), gas, and char, which helps reduce plastic waste in landfills and the environment, addressing plastic pollution. b) Fossil Fuel Replacement: The PFO produced has a calorific value nearly equivalent to fossil fuels like gasoline and diesel, potentially reducing dependence on fossil fuels and carbon emissions. c) Energy Efficiency: PFO and other fuels produced through pyrolysis have high energy efficiency, with a calorific value similar to conventional fuels, making them suitable for meeting energy needs in a more environmentally friendly manner. d) Air Pollution and Emissions: Pyrolysis can generate harmful gas emissions (VOCs, CO, hydrocarbons) if not properly managed. The treatment of pyrolysis gases is critical to reduce air pollution. Fire Risks: Pyrolysis products have a low flash point, increasing the risk of fire. Therefore, handling and storing these fuels must be done with strict safety procedures. e) Char Management: Char, a by-product of pyrolysis, can be used beneficially, but if not properly managed, it could become an environmental burden. f) Environmental Policies: This technology aligns with policies that support waste-to-energy conversion, but it requires regulations to monitor emissions and fuel quality..(Qureshi et al. 2020)(Saxena 2024). However, overall, catalytic pyrolysis of plastics can reduce the environmental impact of plastic waste, replace fossil fuels, and generate efficient energy, but it requires careful management to address emissions and potential fire risks. Bottom of Form

CONCLUSION

The average population from 2019 to 2024 is estimated to reach 1,058,000, with an annual waste production of 111,851 tons. Of this amount, 51% is managed in landfills, while

46% remains unmanaged, and approximately 3.1% is lost before reaching disposal sites. The average daily production of plastic waste is 0.267 tons, with 0.137 tons managed and 0.127 tons unmanaged, highlighting significant challenges in plastic waste management. There is a need for improved management through renewable energy-based technologies, such as pyrolysis, which converts plastic waste into fuel with a high calorific value. The LHV of Pyrolysis Fuel Oil (PFO) is 9,240 kcal.kg⁻¹, indicating good energy efficiency, comparable to commercial fuels like gasoline and diesel. The pyrolysis process lasts for 7 hours per batch, producing a total monthly output of 1,625 L, consisting of 1,125 L of diesel, 250 L of kerosene, and 250 L of gasoline. To operate this process, 4 workers are required, with a monthly electricity consumption of 350 kWh. Sample D-100 has a specific gravity of 0.795 and a high energy potential, making it a suitable candidate for fuel. Pyrolysis technology offers a sustainable solution to reduce waste and dependence on fossil fuels.

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Purification and Characterization of Epidermin from *Staphylococcus epidermidis* and the Impact of Heavy Metals on its Antibacterial Properties

M. K. Hameed†, Z. R. Abbas and S. H. Authman

Department of Biology, College of Science, University of Mustansiriyah, Baghdad, Iraq

†Corresponding author: M. K. Hameed: essamkarkh@gmail.com; maysam.khalil2021@gmail.com

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ABSTRACT

Epidermin, a bacteriocin produced by *Staphylococcus epidermidis*, possesses antimicrobial and anti-enzymatic characteristics. It has been speculated that heavy metals could enhance the inhibitory activities of epidermin against clinical pathogens. This study aimed to extract and characterize epidermin from *Staphylococcus epidermidis* and investigate the impact of heavy metals on its antibacterial action on *Pseudomonas* species. *Staphylococcus epidermidis* was collected and isolated from clinical and environmental samples in Baghdad, Iraq. The bacterial isolates were identified through phenotypic, microscopic, and VITEK 2 compact systems. Among the isolates, epidermin-producing strains were identified. Epidermin was extracted from the epidermin-producing isolates, purified, characterized, and fractionated. The effect of three heavy metal ions on the inhibitory activity of epidermin was tested against *Pseudomonas* species. One of the thirteen epidermin-producing isolates was selected for epidermin extraction. The epidermin crude concentration was determined to be 0.43 mg/ml, exhibiting 70% activity. The results also showed that 100 mM of cadmium increased epidermin activity, while 50 mM of cadmium showed less activity. Cobalt and copper demonstrated a similar inhibitory-enhancement activity. The present study found that the inhibitory activity of epidermin against *Pseudomonas* species could be enhanced with heavy metals. Further investigations are encouraged and recommended to explore the synergistic potential of epidermin and heavy metals as antimicrobial agents for controlling clinical pathogenic isolates.

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INTRODUCTION

Staphylococcus epidermidis is a type of bacteria that occurs in clusters and has a characteristic round shape (cocci). It is characterized as coagulase-negative and gram-positive. The organism is a facultative anaerobe that can survive without oxygen and has catalase activity. In their natural environment, such as human skin or mucosa, *Staphylococcus epidermidis* is typically harmless (Brown & Horswill 2020, Foster 2020). However, though harmless in their natural environment, these coagulase-negative *Staphylococcus* species can enter an individual's body through prosthetic devices, potentially introducing a small number of bacteria into the bloodstream. Once inside the body, these bacteria can form biofilms, which serve as protective structures against the host's immune defenses and antimicrobial treatments (Zheng et al. 2018).

The occurrence of bacteremia, caused by *Staphylococcus epidermidis* and other coagulase-negative staphylococci, is most commonly linked to medical device contamination (Severn & Horswill, 2023). When a prosthetic device is implanted in the human body, microorganisms from the skin can colonize the medical equipment. Some of these bacteria may enter the bloodstream, potentially leading to infection and health complications. *Staphylococcus epidermidis* is a major contributor to nosocomial (hospital-acquired) bloodstream infections (Liu et al. 2020). Additionally, *Staphylococcus epidermidis* infections are prevalent



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among infants (Cau et al. 2021). *Staphylococcus epidermidis* can produce and release epidermin, a tetracyclic peptide that belongs to the family of antibiotics.

Epidermin exhibits inhibitory effects on numerous Gram-positive bacteria, especially sensitive cells. Bacteriocin targets various cellular processes simultaneously, including RNA, DNA, polysaccharides, and protein synthesis. This leads to energy depletion and biosynthetic disruption in treated cells. Epidermin's primary biochemical target is the energy-transducing cytoplasmic membrane (Said-Salman et al. 2019). Through a barrel-stave mechanism, epidermin appears to impact membrane permeability by creating water-filled channels or gaps in the membrane structure (Alinaghi et al. 2022). Epidermin's classification as either an antibiotic or a bacteriocin sparked extensive debate. It was ultimately identified as a type-AI lantibiotic, satisfying the criteria for both antibiotics and bacteriocins, leading to it being categorized as neither (Alkhawaja et al. 2020).

Heavy metals are elements with a high atomic weight and density, typically greater than 5 g/cm³, and are characterized by physical and chemical properties (Nriagu 1996). While these elements are present in the Earth's crust, human activities such as mining, industrial processes, and agriculture have significantly increased their concentrations in the environment, leading to widespread contamination. Heavy metals, such as lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and chromium (Cr), are naturally occurring elements with high atomic weights and densities. Excess of heavy metals can pose significant environmental (Alloway 2013) and human health risks (Grandjean & Landrigan 2014). In the environment, they can contaminate soil and water. Furthermore, heavy metals have been linked to neurological disorders, cardiovascular diseases, carcinogenesis, and reproductive and developmental consequences. However, some of these heavy metals are essential in trace amounts for biological functions. Heavy metals like cadmium, cobalt, and copper are essential for the physiological functioning of living organisms (Madhu & Sadagopan 2020). Heavy metal pollution exerts selective pressure on microbial communities, driving the evolution of metal-tolerant and antibiotic-resistant microorganisms through shared genetic mechanisms like efflux pumps (Seiler & Berendonk 2012). Certain metals, such as silver and copper, exhibit natural antimicrobial properties, inspiring their use in medical applications like coatings and wound dressings (Lemire et al. 2013). Additionally, heavy metals can synergize with antibiotics, enhancing their effectiveness against resistant pathogens (Baker-Austin et al. 2006). Heavy metals can enhance the activity of antimicrobial peptides (AMPs) like epidermin through various mechanisms. Metal ions such as zinc and copper can bind to AMPs, stabilizing their

structure and increasing their charge, which strengthens their interaction with microbial membranes. Additionally, metal ions can facilitate the production of reactive oxygen species (ROS), further damaging microbial cells. These interactions make AMPs more potent against pathogens, even in resistant strains, offering therapeutic potential in combating infections (RSC Publishing, 2018).

The present study aimed to purify and characterize epidermin, a bacteriocin produced by *Staphylococcus epidermidis*, and to investigate the impact of various heavy metals (cadmium, cobalt, and copper) on its antibacterial efficacy, focusing on understanding the mechanisms by which heavy metals may enhance or inhibit its activity against *Pseudomonas* species.

MATERIALS AND METHODS

Sample Collection

A total of one hundred (100) samples, consisting of blood, skin, wound, and burn specimens, were obtained from Ibn Al-Baladi Hospital in Baghdad. Twenty (20) environmental samples were also collected from sewage water.

Isolation of Bacteria

Selective media (Himedia Company, India) were prepared and autoclaved for 15 min at 121°C. The samples were then inoculated on selective media, and the cultures were incubated for 24 hours at 37°C to isolate *Pseudomonas* species and *S. epidermidis*. The experiment was replicated three times. Subsequently, the mixed cultures were subcultured onto freshly prepared nutrient agar plates through streaking, and the cultures were incubated at 37°C for another 24 hours. This process allowed the growth of distinct, isolated colonies, which were identified (Tille 2015).

Identification of Bacterial Isolates

The characteristics of the bacterial isolates were determined using various phenotypic traits, such as form, odor, color, diameter, and pigmentation on culture media. Microscopic examination was also conducted for this purpose. In addition, biochemical tests were performed using the VITEK 2 compact system.

Detection of Epidermin-Producing Isolates

The epidermin-producing isolates were identified using the diffusion method according to Gupta (1996). Cultures of *S. epidermidis* isolates, aged 18-24 hours in brain heart infusion agar (BHIA), were prepared. These bacterial cultures were then centrifuged at 6,000 rpm for 30 min. The resulting filtrate was collected for each isolate, while the precipitates

were discarded. The collected filtrate was heated to 70°C for 3 min. To perform the test, 100 µL of a suspension of *Pseudomonas* species, aged 18-24 hours, was transferred to the center of the solid BHIA plates. The bacterial suspension was adjusted using a 0.5 MacFarland solution. Using a sterile cork borer, wells with a diameter of 5 mm were made in the BHIA culture plates. Each well was then filled with 100 µL of each *S. epidermidis* suspension. The experiment was performed in triplicate for each isolate, with a negative control (PBS) and a positive control (100 µL of 6% acetic acid). The cultures were left at room temperature for 15 min, incubating at 37°C for 18-24 h. After the incubation period, the diameters of the *Pseudomonas* species growth inhibition zones around the wells were observed as evidence of a positive result, indicating epidermin production.

Extraction of Epidermin from the Epidermin-Producing *Staphylococcus epidermidis*

Epidermin extraction was conducted following the modified procedure outlined in Mirhosseini et al. (2010). An aliquot of 250 mL of BHIB medium was prepared and inoculated with *S. epidermidis* isolate No. 10 (the isolate with the most effective anti-growth activity against *Pseudomonas* species). The culture was allowed to grow for 18-24 h at 37°C. Following the incubation period, the bacterial culture was centrifuged at 10,000 rpm for 30 min. This process separated the bacterial cells, forming sediment, which was discarded. The filtrate, containing the desired epidermin, was collected. The collected filtrate was then heated to 70°C for 3 min. Once the heated solution cooled, the supernatant, containing the crude epidermin, was carefully collected.

Purification of Epidermin Using the Ammonium Sulfate Precipitation Method

To further purify the extracted crude epidermin, the ammonium sulfate precipitation method was used as described by Al-Soufi et al. (2005). Ammonium sulfate was slowly added to the extracted crude epidermin at 4°C while stirring. This was done until it reached the desired saturation level of 70%. The solution was then kept overnight at 4°C to facilitate protein precipitation. Subsequently, the solution was centrifuged at 10,000 rpm at 4°C for 15 min to separate the protein pellets from the remaining solution. To dissolve the protein pellets and remove most of the contaminated proteins and water, a small amount of 0.1 M phosphate buffer at pH 7 was used. This process resulted in the production of a partly purified epidermin. Several trials with gradual saturation of ammonium sulfate were performed to determine the optimal saturation ratio for epidermin precipitation. After obtaining the precipitated fraction, the number of

proteins and epidermin activity in the dissolved precipitate was measured. Ammonium sulfate precipitate was filtered via an active dialysis membrane and then added to a buffer solution (0.01 M phosphate buffer) in a test tube.

Fractionation of Epidermin Using Ion Exchange and Gel Filtration Chromatography

In the present study, ion exchange and gel filtration chromatography were used to fractionate epidermin. Both methods are effective for purifying epidermin due to their ability to separate based on charge and size, respectively, offering high resolution and reproducibility. Ion-exchange chromatography selectively isolates proteins like epidermin by utilizing charge interactions, while gel filtration separates based on molecular size, reducing contaminants. These methods are less costly and simpler to implement compared to High-Performance Liquid Chromatography (HPLC), which may require more complex equipment and is typically reserved for smaller-scale or high-precision purifications (Qin et al. 2020, Sahu et al. 2019).

Epidermin was fractionated using the ion exchange chromatographic method described by Whitaker & Bernhard (1972). The DEAE-cellulose column was prepared by suspending 20 g of resin in 1 L of distilled water. After allowing the suspension to settle, it was washed multiple times with distilled water until clear. The suspension was filtered through a Whatman No. 1 filter paper using a Buchner funnel. The resin was resuspended in a solution containing 0.25 M sodium chloride and sodium hydroxide. After another round of filtration and rinsing with a 0.25 M hydrochloric acid solution followed by distilled water, the resin was equilibrated in a 0.05 M phosphate buffer at pH 7. The column was washed with an equal volume of the same buffer to perform chromatography. The bound proteins were gradually eluted using increasing concentrations of sodium chloride (ranging from 0.1 to 1 M). The flow rate across the column was set at 30 mL.h⁻¹, and each fraction's absorbance was measured using an ultraviolet-visible (UV-VIS) spectrophotometer at 280 nm. The activity of each fraction of the epidermin was evaluated.

Sephadex G-150 (Pharmacia Fine Chemicals Company) was prepared following the manufacturer's instructions. First, Sephadex G-150 was suspended in a 0.05 M phosphate buffer at pH 7. Then, it was heated at 90°C for 5 hours to achieve bead swelling. After the beads swelled, the suspension was degassed to remove any trapped air bubbles. Next, the swollen Sephadex G150- was packed into a glass column measuring 2x40 cm. The column was then equilibrated with the same 0.05 M phosphate buffer. The column was loaded with the concentrated sample obtained from the previous

ion exchange chromatography stage. The elution flow rate was set at $30 \text{ mL} \cdot \text{h}^{-1}$, and the same 0.05 M phosphate buffer was used for equilibration and elution. As the samples passed through the column, the absorbance of each fraction was measured at 280 nm using a spectrophotometer. The protein concentration in each fraction was assessed using the Bradford method (Bradford 1976). Additionally, epidermin activity was evaluated in each fraction to determine the presence and activity of purified epidermin.

Molecular Weight Determination

To determine the molecular weight of epidermin, gel filtration chromatography was employed using a Sephadex G-150 column measuring $2 \times 40 \text{ cm}$. The column was equilibrated and eluted with 0.05 M phosphate buffer at $\text{pH } 7$. For calibration, several standard proteins with known molecular weights were used. These proteins included alcohol dehydrogenase, albumin, carbonic anhydrase, and lysozyme. Blue dextran was applied and measured at 600 nm to determine the volume of the void. The elution volume for each standard protein was then measured using a UV-Vis Bio-Rad spectrophotometer at 280 nm . By comparing the elution volume of epidermin with that of the known benchmark proteins, the molecular weight of epidermin was determined. This allowed researchers to determine the approximate size of the epidermin molecules compared to the standard proteins used as references.

Thermal Stability Evaluation

The optimal temperature for maximum epidermin production was identified by incubating cultures at various temperature ranges ($30, 37, 42, 47$, and 53°C) for 30 min . After that, the epidermis was transferred immediately to an ice bath, according to the method described by Makky et al. (2013). The activity was determined, and the remaining activity (%) was plotted against temperature.

Determination of the Effect of pH on Purified Epidermin Stability

The purified epidermin was pre-incubated in a buffer solution of various pH ($5, 6, 7, 8$, and 9) for 30 min at 37°C using 1 M NaOH and 1 M HCl . After that, the tubes were cooled in an ice bath, according to the method described by Makky et al. (2013). The activity was determined, and the remaining activity (%) was plotted against pH .

Evaluation of the Effect of Heavy Metals on Epidermin Activity

The effects of different concentrations of heavy metal ions, such as Co (CoCl_2), Cu , and Cd ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$) on epidermin activity were evaluated. Epidermin was pre-incubated alone

at two different concentrations (50 and 100 mM). These concentrations were selected based on the results from preliminary experiments, which identified 50 and 100 mM as the optimal concentrations for the study. Heavy metal ions (Co , Cu , and Cd) at the test concentrations were added to the culture of *P. aeruginosa*. Then, epidermin activity and relative activity (the ratio of epidermin activity in the presence and absence of heavy metal ions) were calculated. The activity was assayed without metal ions and taken as 100% (Gandhi et al. 2019).

Statistical Analysis

The Statistical Packages of Social Sciences (SPSS; 2019) program was used to detect the effect of different factors on study parameters. The least significant difference (LSD) and analysis of variance (ANOVA) were used to compare means. $P \leq 0.5$ was considered significant.

RESULTS AND DISCUSSION

Heavy metals have a variety of medical applications due to their unique chemical and physical properties (Prasad 2008). Some heavy metals, such as zinc, barium, gadolinium, and technetium, have been utilized for various diagnostic purposes. Some cancer treatment procedures have used platinum and gold to suppress and inhibit the growth of cancerous cells (Kelland 2007). This study examined the development and clinical use of platinum-based chemotherapy medicines like cisplatin, as well as their efficacy in treating a variety of malignancies. Some investigations reported that copper, silver, cadmium, and cobalt possess some antimicrobial properties. Valko et al. (2005) reported the biological effects of various metals, including cobalt, and discussed their antimicrobial properties concerning toxicity and oxidative stress. Therefore, the present study extracted epidermin from *Staphylococcus epidermidis* and purified it. More so, the extracted epidermin was further characterized. The influence of three heavy metals (cobalt, cadmium, and copper) on the enhancement of the antibacterial activity of epidermin on a test pathogen, *Pseudomonas* species, was studied.

Epidermin Producing Isolates

Thirteen isolates were first identified (Figs. 1A and B) as *Staphylococcus epidermidis* based on their morphological, cultural, and biochemical characteristics, as well as the Vitek2 system confirmation. These isolates were obtained from various clinical sources. Simultaneously, thirty isolates were identified as *Pseudomonas aeruginosa*, originating from clinical sources, along with five isolates from the environment (sewage water). Out of the thirteen

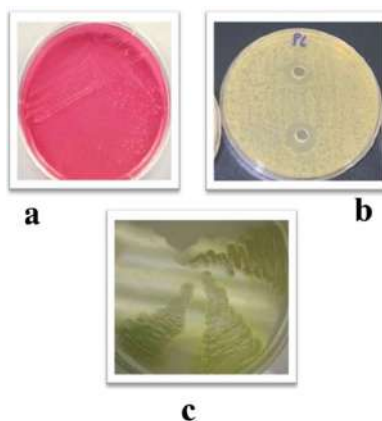


Fig. 1: (a) *Staphylococcus epidermidis* on mannitol salt agar; (b) Colonies of *Pseudomonas* species on *Pseudomonas* agar; (c) Epidermin production from *Staphylococcus epidermidis*.

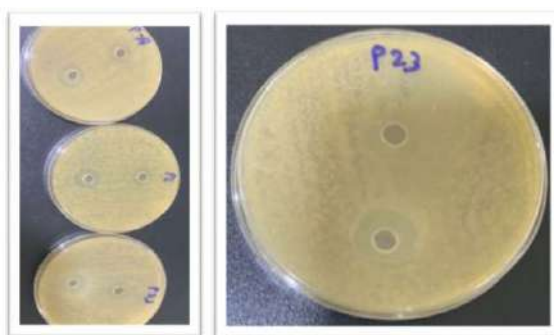


Fig. 2: Inhibitory effect of epidermin on the growth of *Pseudomonas aeruginosa*'s epidermin fractions.

S. epidermidis isolates, three isolates (23.08%) produced epidermin. These particular isolates demonstrated a clear inhibitory zone against *Pseudomonas aeruginosa* isolates (Fig. 2). This result indicated that these epidermin-producing isolates of *S. epidermidis* possess antimicrobial properties, specifically inhibiting the growth of *Pseudomonas aeruginosa* (Fig. 1C).

Purified Epidermin

Ion exchange chromatography was employed to purify epidermin (Fig. 3A). During the washing stage, a single protein peak was obtained at varying sodium chloride concentrations, and similarly, another protein peak was obtained during the subsequent elution step. These protein peaks were further analyzed to assess their epidermin activity. Based on the results, the proteins that eluted within fractions 45 to 61 showed the highest concentration of epidermin activity. These fractions were combined, and, upon testing, exhibited an activity of 45 U/ml, a specific activity of 187.5 U/mg, a fold purification of 2.6, and a protein yield of 36%. The fractions containing epidermin were combined and subsequently further purified using a

Sephadex G-150 column after ion exchange chromatography. The Sephadex G-150 column has separation limits ranging from 5,000 to 600,000 Da, ensuring efficient separation and purification. This column is advantageous for protein separations due to its low maintenance requirements, ease of preparation, rapidity, and excellent recovery (Stellwagen 1990). The results after using Tris-HCl buffer to wash the sample demonstrated that epidermin activity appeared as a single peak. The separate parts were then merged to form one protein peak. The analysis of this peak (fractionation tubes 13-31) revealed an epidermin activity of 43 U/ml, a specific activity of 430 U/mg, and a purification fold of 6.1, with a protein yield of 34.4%.

Antibacterial Potential of Epidermin

Thirteen filtrates from *S. epidermidis* were evaluated for their inhibitory activity using a diffusion assay against an indicator isolate of *Pseudomonas* species. The results indicated significant inhibition of these isolates. However, there was a noticeable variation in the inhibition effectiveness, as evidenced by the differences in the diameters of the inhibition zones compared to the control. One of the isolates displayed

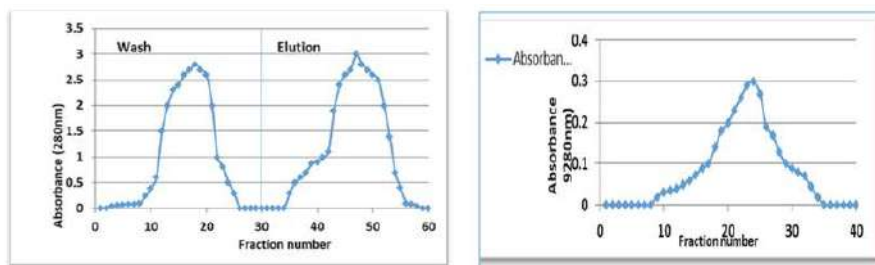


Fig. 3: (A) Ion exchange chromatography on DEAE cellulose; (B) Gel filtration chromatography on Sephadex.

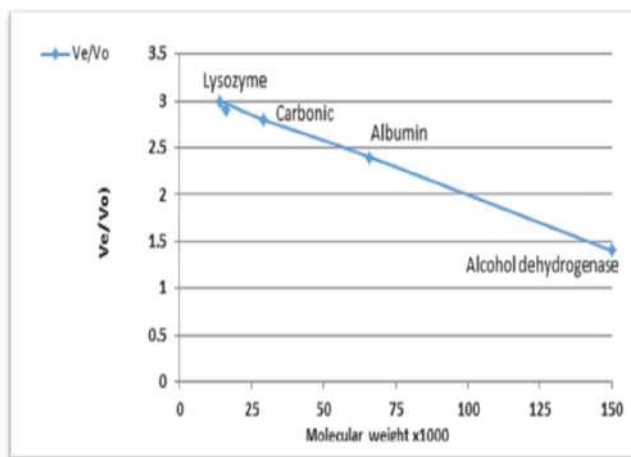


Fig. 4: Molecular weight determination of epidermin by Sephadex G-150 column.

moderate anti-bacterial activity, with inhibition zones measuring 10, 12, and 15 mm, respectively, as presented in Fig. 2. On the other hand, other isolates did not exhibit any activity against *Pseudomonas* species. A crude extract derived from *S. epidermidis* isolates inhibited the growth of *Pseudomonas* species. Unlike lysostaphin, epidermin does not possess lytic properties and has a narrow spectrum of action. It does not inhibit Gram-negative bacteria, like other bacteriocins produced by Gram-positive microorganisms. However, epidermin exhibits inhibitory activity against other Gram-positive bacterial species, as reported by Maisnier-Patin & Richard (1995).

Molecular Weight of Purified Epidermin

The inhibitory activity of epidermin on *Pseudomonas* species provided valuable insights into the biological properties and potential applications of this molecule. The molecular weight of epidermin was determined by the Sephadex G-150 column (Fig. 4).

Thermal Stability of Purified Epidermin

As indicated in Fig. 5, the epidermin maintained 89% of its activity when incubated at temperatures ranging from 30 to 37, 42, and 47°C. However, above this temperature,



Fig. 5: Effect of temperature on epidermin activity as an antibacterial agent against *Pseudomonas* species.

the epidermin's activity decreased, with residual activity reaching 30-40% at 53°C ($P \leq 0.05$). The activity of epidermin, as an antibacterial agent against *Pseudomonas* spp. It could be affected by temperature. Generally, higher temperatures enhance epidermin's antimicrobial efficacy by less than 45%, while lower temperatures may reduce its activity by more than 30%. The epidermin's inhibitory activity rose at 37°C (Fig. 5), and this property decreased with higher temperatures until it lost its antibacterial potential at 53°C without an inhibition zone in the medium (Fig. 6A-C).

Pseudomonas species.

Elevated temperatures, within a certain range, can increase epidermin activity against *Pseudomonas* species. This is because higher temperatures promote epidermin diffusion into the bacterial cells, facilitating its interaction with the microbial membrane. The increased fluidity of the bacterial

cell membrane at higher temperatures also enhances the binding and disruption of the membrane by epidermin, leading to more effective antimicrobial action (Yao et al. 2022). Lower temperatures can diminish epidermin's antimicrobial activity. At colder temperatures, epidermin mobility and diffusion may be reduced, limiting its ability to effectively reach and interact with bacterial cells. This reduced diffusion can impede the binding and disruption of the bacterial cell membrane, leading to decreased antibacterial efficacy against *Pseudomonas* species (Guo et al. 2022). It is imperative to note that the specific temperature range at which epidermin exhibits optimal antimicrobial activity against *Pseudomonas* species may vary and can be influenced by factors such as the specific strain of *Pseudomonas* species, the concentration of epidermin, and the duration of exposure. Additionally, extreme temperatures (either too high or too low) can denature or degrade the epidermin, rendering it less effective or inactive (Darbandi et al. 2022).

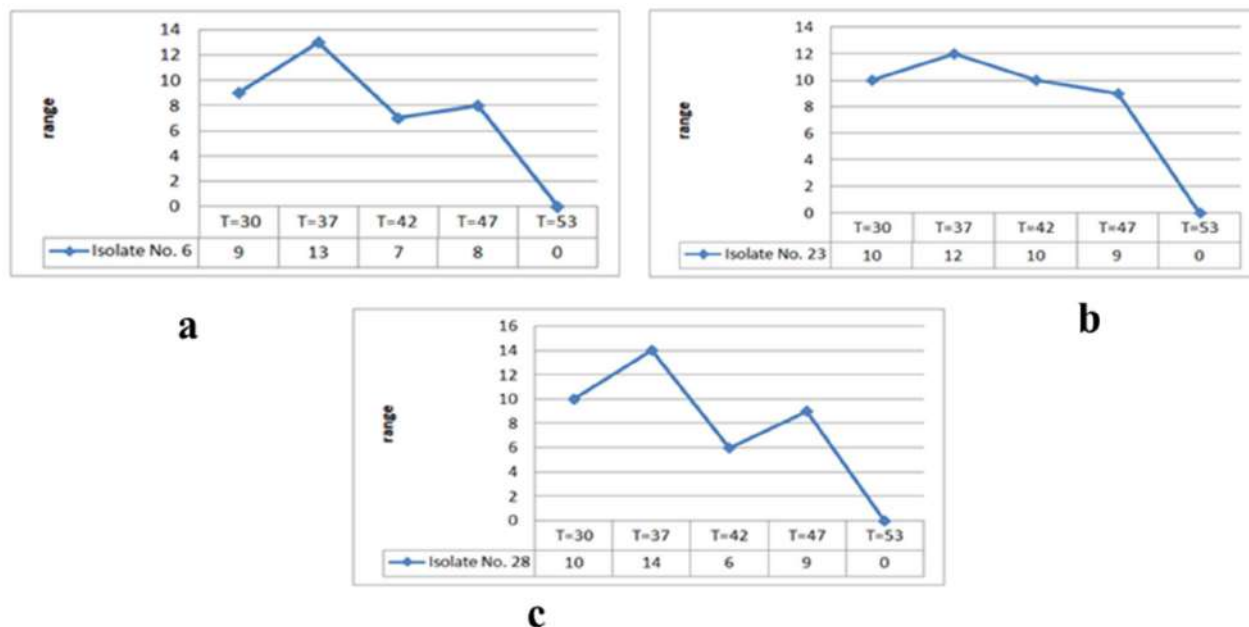


Fig. 6: Effect of temperature on the inhibitory effect of epidermin against *Pseudomonas* species. a: Isolate No. 6; b: Isolate No. 23; c: Isolate No. 28.

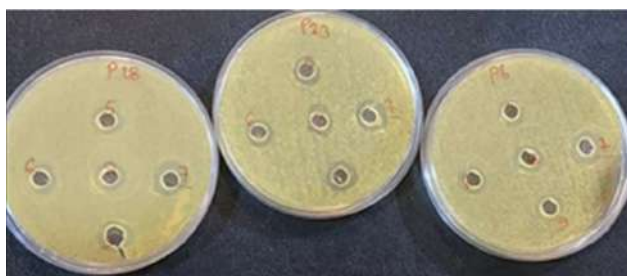


Fig. 7: Effect of pH on epidermin stability as an antibacterial agent against *Pseudomonas* species.

Optimal pH for Epidermin Stability

The optimal pH for the stability of epidermin, an antimicrobial peptide, can vary depending on the specific characteristics of the peptide and the experimental conditions. However, studies have shown that epidermin exhibits increased stability and activity in a slightly acidic to neutral pH range. The pH ranges from 5 to 9 affect epidermin stability, with the highest inhibition ($P \leq 0.05$) observed at pH 7 for three isolates. The lowest inhibition was observed at pH 6, with no resistance isolates identified (Table 1; Figs. 7 and 8A-C).

Epidermin demonstrated high stability and activity at neutral pH values, around pH 7. At this pH range, the peptide's structural integrity and antibacterial potential are generally maintained. The neutral pH range is relevant for applications where maintaining epidermin stability and activity are crucial (de Szalay & Wertz 2023). It is critical to note that extreme pH values (extremely acidic or highly alkaline) can negatively impact epidermin stability and

activity. Very low pH values can lead to peptide denaturation or degradation, while highly alkaline conditions can cause structural changes that reduce epidermin's effectiveness as an antimicrobial agent (Kuo et al. 2020). The optimal pH for epidermin may also depend on the specific bacterial target or the physiological conditions of the environment where the peptide is employed.

Effects of Heavy Metal Ions on Epidermin Activity

The impact of heavy metals on bacterial resistance and public health is a growing concern worldwide, particularly as it relates to the spread of antibiotic resistance and its implications for human health. Heavy metals, such as mercury, lead, cadmium, arsenic, and chromium, are toxic elements that persist in the environment due to industrial activities, agricultural practices, and improper waste disposal. These metals can accumulate in water, soil, and food chains, posing a significant risk to both ecosystems and human health (Pal et al. 2017). The results (Table 2) showed that in the

Table 1: Effect of pH on epidermin stability as an antibacterial agent against *Pseudomonas* species.

Isolate No.	Diameter of inhibition zone (mm)					L.S.D. value
	pH 5	pH 6	pH 7	pH 8	pH 9	
6	10 $\pm$ 0.52	8 $\pm$ 0.37	12 $\pm$ 0.64	9 $\pm$ 0.51	10 $\pm$ 0.57	1.749 *
23	8 $\pm$ 0.37	6 $\pm$ 0.22	11 $\pm$ 0.58	8 $\pm$ 0.45	9 $\pm$ 0.51	2.057 *
28	6 $\pm$ 0.21	7 $\pm$ 0.29	12 $\pm$ 0.61	10 $\pm$ 0.52	8 $\pm$ 0.37	2.166 *
L.S.D. value	2.071 *	1.894 *	1.447 NS	1.791 *	1.791 *	---

* ($P \leq 0.05$).

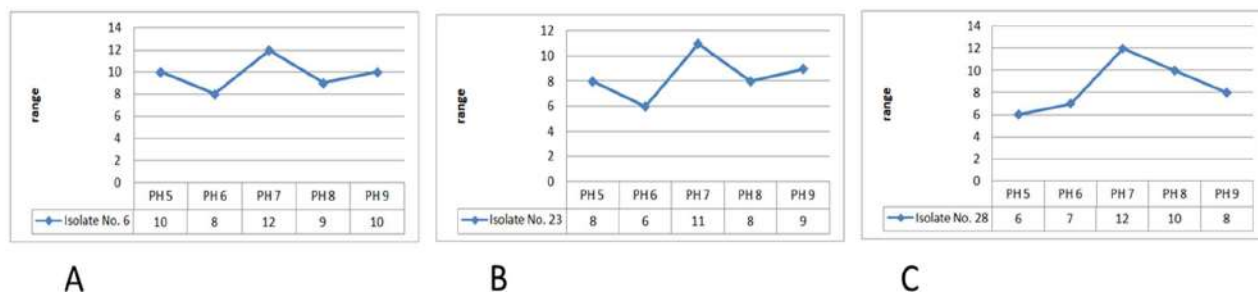


Fig. 8: Effect of pH on epidermin stability as an antibacterial agent against *Pseudomonas* species A: Isolate No. 6; B: Isolate No. 23; C: Isolate No. 28.

Table 2: Effect of various metal ions on epidermin activity as an antibacterial agent against *Pseudomonas* species.

Isolate No.	Diameter of inhibition zone (mm)					
	Cu (50 mM)	Cu (100 mM)	Cd (50 mM)	Cd (100 mM)	Co (50 mM)	Co (100 mM)
6	11 $\pm$ 0.47	16 $\pm$ 0.59	22 $\pm$ 1.06	17 $\pm$ 0.72	15 $\pm$ 0.67	19 $\pm$ 0.85
23	R	14 $\pm$ 0.71	16 $\pm$ 0.82	13 $\pm$ 0.56	12 $\pm$ 0.55	R
28	13 $\pm$ 0.61	11 $\pm$ 0.54	18 $\pm$ 0.89	16 $\pm$ 0.82	R	14 $\pm$ 0.71
L.S.D. value	1.945 *	2.702 *	2.510 *	1.966 *	1.718 *	2.052 *

* ($P \leq 0.05$).

presence of 50 mM Cu, isolate 23 demonstrated resistance against the test pathogen, *Pseudomonas* species, while all isolates at 100 mM Cu had reduced activity. A concentration of 100 mM Cd increased epidermin antimicrobial activity against the test isolates of *Pseudomonas* species, while less activity was observed at 50 mM Cd. There was resistance of isolates 28 and 23 when 50 and 100 mM of Co were used to supplement epidermin, respectively. The results of the present study are in agreement with the findings of Ahmed et al. (2015), who reported that Co and Cu inhibited epidermin activity. Copper ions (Cu^{2+}) have antimicrobial properties and are often used in various applications to control bacterial growth. When it comes to copper's effects on epidermin, it is expected that copper ions can enhance epidermin's antimicrobial activity in several ways. Epidermin exerts its antimicrobial effects by interacting with bacterial cell membranes and disrupting their integrity. Copper ions can synergize with epidermin by binding to bacterial membranes and destabilizing them further. This enhanced membrane disruption can increase epidermin efficacy against bacteria.

Copper ions can also bind to the epidermis peptide chain, promoting structural stability. This stabilization may improve epidermin's overall activity and effectiveness as an antimicrobial agent (Tomić & Vuković 2022). Copper ions can bind to the peptide and enhance its membrane-disrupting properties, resulting in improved antibacterial action, which is concentration-dependent (Libardo et al. 2014). While lower concentrations of copper can enhance the antimicrobial activity of epidermin, higher concentrations may become toxic to the peptide itself or the host cells. Therefore, careful consideration of copper concentrations is essential for optimizing epidermin's antimicrobial efficacy in the presence of copper ions (Rajput et al. 2021). Generally, copper ions can play a beneficial role in enhancing the antimicrobial activity of epidermin, making it a promising combination for antimicrobial applications.

Cadmium ions (Cd^{2+}) are heavy metallic ions with toxic effects on biological systems. The specific effects of cadmium on epidermin have not been extensively studied. Cadmium can inhibit enzymatic activities within bacterial cells, which may indirectly affect bacteria's susceptibility to antimicrobial peptides like epidermin. Cadmium ions can enter bacterial cells and disrupt various cellular processes. It is possible that cadmium accumulation within bacterial cells could alter their response to epidermin, making them more resistant to its antimicrobial effects. It should be taken into consideration that the specific effects of cadmium on the epidermin can vary depending on the concentration of cadmium, exposure duration, and other factors (Li et al. 2021). Given the toxic nature of cadmium, high concentrations are likely to have

a more pronounced negative impact on the antimicrobial activity of epidermin. However, further research is needed to fully understand the effects of cadmium on epidermin and its implications for antimicrobial efficacy.

Cobalt's specific effects on epidermin have not been extensively studied, and the information available on its direct impact on epidermin is limited. However, considering the characteristics of cobalt ions and their potential interactions with antimicrobial peptides, the possible effects of cobalt on epidermin can be speculated. Cobalt ions could bind to epidermin's peptide chain and induce structural changes. This interaction might affect the peptide's stability and conformation, potentially compromising its antimicrobial activity (El Ali et al. 2020). Cobalt exhibits cytotoxic effects, particularly at high concentrations. This toxicity could affect both the bacteria and potentially the host cells involved (Claudel et al. 2020). The exact effects of metal ions on epidermin can depend on various factors, including the concentrations of the metal ions, pH, and the specific bacterial strains being targeted. Moreover, epidermin's mechanism of action may involve multiple factors beyond metal ions alone. Further research is needed to understand the intricate interactions between metal ions and epidermin's antimicrobial activity.

The findings of the present study demonstrated a significant antibacterial activity, particularly against Gram-negative pathogens like *Pseudomonas* species. Similar research on bacteriocins shows comparable findings, as several bacteriocins (like nisin and mutacin) also displayed potent activity against multidrug-resistant (MDR) and biofilm-forming bacteria (Gänzle & Hammes 2009). Combining bacteriocins with antibiotics, such as chloramphenicol or ciprofloxacin, has been shown to enhance efficacy, suggesting a potential synergy (Kumar & Bhattacharya 2014). Furthermore, heavy metals' influence on these antibacterial properties is notable, as certain metals can either enhance or inhibit bacteriocin function, much like their impact on antibiotic resistance (Matias 2013). Heavy metals drive bacterial resistance through co-selection, stress-induced mutations, and horizontal gene transfer (HGT). Co-selection occurs when resistance genes for heavy metals and antibiotics coexist on mobile genetic elements, enabling bacteria to acquire multidrug resistance. Heavy metal exposure triggers stress responses, increasing mutation rates and activating efflux pumps that expel both metals and antibiotics, enhancing resistance. Additionally, oxidative stress damages bacterial DNA, fostering resistance evolution. Horizontal gene transfer further spreads resistance traits across bacterial populations, complicating the control of infections with standard antibiotics (Hobman & Crossman 2015, Fashola et al. 2016).

Combining bacteriocins like epidermin from *Staphylococcus epidermidis* with heavy metals could have significant clinical and environmental applications. In clinical settings, bacteriocins are known for their antibacterial activity, and heavy metals can enhance this effect by disrupting bacterial cell walls and increasing the permeability of bacterial membranes. This combination could be particularly useful in overcoming antibiotic resistance by synergizing bacteriocins with metals to target resistant pathogens, a potential new treatment for resistant infections in hospital settings. In environmental applications, this combination could be used in wastewater treatment or soil remediation to control microbial contamination, especially in environments polluted with heavy metals.

The combination of heavy metals like cadmium with bacteriocins, such as epidermin, enhances antibacterial activity by disrupting bacterial membranes and boosting bacteriocin efficacy. However, cadmium's toxic effects, including oxidative stress, DNA damage, and chronic health risks like kidney damage and cancer, pose significant concerns. Prolonged cadmium exposure and tissue accumulation amplify these risks, raising questions about its safety in clinical applications (Klaassen & Liu 2008). While cadmium may broaden bacteriocin effectiveness, its potential toxicity necessitates careful evaluation to balance therapeutic benefits against environmental and health hazards. To mitigate the risks associated with cadmium toxicity, several strategies could be explored. For instance, using lower concentrations of cadmium in combination with bacteriocins, or targeting specific delivery mechanisms that reduce systemic exposure, could help balance the therapeutic benefits and toxic risks. Additionally, bioremediation techniques, which use microbes or plants to detoxify environments contaminated with cadmium, could potentially help reduce the environmental impact of such combinations (Foley 2019). Overall, while the combination of bacteriocins with heavy metals offers exciting possibilities, further research into their safety profiles, especially concerning heavy metal toxicity, is essential to ensure their safe and effective use in both medical and environmental applications.

CONCLUSIONS

The purification and characterization of epidermin from *Staphylococcus epidermidis* confirm its potential as a natural antibacterial agent, particularly against gram-positive bacteria. Also, heavy metals enhanced the inhibitory activities of epidermin. Understanding the anti-enzymatic activity of purified epidermin and heavy metal interaction will help to develop novel strategies for combating

Pseudomonas infections in clinical and environmental situations. Further investigations are required to explore the potential applications of the synergistic effects of epidermin and heavy metals as antimicrobial agents in the future.

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An Analysis of Stormwater Management with the Internet of Things (IoT)

Kusum Choudhary[†]  and Ravish Kumar 

Department of Architecture and Planning, NIT, Panta-800005, Bihar, India

[†]Corresponding author: Kusum Choudhary; kusumc.ph21.ar@nitp.ac.in

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ABSTRACT

The study offers a comprehensive analysis of stormwater management durability in Chandigarh, India, primarily focusing on employing the Internet of Things (IoT). Eleven key stormwater management indices were examined, and their data sources and methodologies were detailed. The investigation independently evaluates the urban areas' durability, emphasizing the singular indicators' worth, and their consequences. Moreover, a consolidated comprehensive system durability indicator was computed utilizing the Analytical Hierarchy Method (AHM), offering a holistic viewpoint on stormwater administration. Chandigarh's stormwater management uses a 100% weighted approach, this 100% weighted approach allows stakeholders to make informed decisions about stormwater management sustainability, ensuring the city's water quality and effectiveness in mitigating flooding. Their findings showed a moderate overall sustainability index of 0.761, indicating challenges like waterlogging and limited drainage coverage. The outcomes provide perspectives into Chandigarh's tenacity and durability in metropolitan water administration over a decade, highlighting the ever-changing character of urban growth and the requirement for flexible resolutions.

INTRODUCTION

Stormwater control is a key element of urban design and ecological durability as urbanization continues to expand swiftly globally. Along with it is the intricacy of overseeing stormwater drainage. Conventional stormwater management systems frequently face challenges in managing the growing magnitude and force of storm occurrences, resulting in inundation attrition and water purity concerns. Traditional stormwater management systems often struggle with issues such as overflow, pollution, and inefficient resource use. This study addresses these challenges by demonstrating the effectiveness of IoT in real-time data collection, predictive analytics, and remote control of infrastructure, leading to more efficient and cost-effective operations. This research contributes to the existing body of knowledge by providing a comprehensive analysis of how IoT can revolutionize stormwater management. The study highlights the practical implications and advantages of integrating IoT into urban water management systems by focusing on real-world applications and empirical data. Additionally, it emphasizes the need for flexible solutions to cope with the dynamic nature of urban growth and environmental challenges. To tackle these obstacles, cities and municipalities are embracing cutting-edge technologies. One of the most encouraging resolutions is the Internet of Things (IoT) (Ress et al. 2020, Ramovha et al. 2024).

The World Wide Web of Things, frequently mentioned as IoT, is a revolutionary technology that links everyday objects and devices to the Internet, enabling them to gather and interchange data. IoT has acquired substantial momentum in diverse sectors, encompassing healthcare, transportation, and fabrication. In the milieu of stormwater management, IoT presents a groundbreaking approach to overseeing, regulating, and enhancing how urban areas manage

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precipitation (Sheng & Nawari 2016, Teshome 2020, Sharma 2008).

This examination plunges into the convergence of stormwater administration and IoT, investigating how IoT technologies are transforming the way we tackle this crucial facet of urban planning. It explores the difficulties of conventional stormwater management, the advantages and possibilities of IoT in tackling these difficulties, and offers practical instances of IoT implementations in stormwater management (Benzerra et al. 2012, Andimuthu et al. 2019). Traditional stormwater management systems face challenges like overflow pollution and a lack of data, leading to inefficient resource use and environmental damage. IoT revolutionizes stormwater management by providing real-time data collection, predictive analytics for better planning, remote control for infrastructure, and cost-efficient operations. Real-world applications show how cities leverage IoT to enhance urban resilience, sustainability, and quality of life, promising a smarter, eco-friendly future for urban water management (Channi et al. 2022, Emama et al. 2022).

Smart Urban Water Management uses modern technologies and uniform methods to make effective use of water resources in urban areas. Smart water management ensures equitable access to water, safeguards the environment, and facilitates adaptation to climate change. This facilitates sustained urban expansion. The current study evaluates various sustainability indicators for stormwater management in Chandigarh. The results indicate that Chandigarh has a relatively strong sustainability status with high scores in several indices such as EDSI, DSI, and PI. This

study on stormwater management can help urban planners design more effective and sustainable systems. Sustainable Urban Drainage Systems (SUDS) mimic natural processes, improving water quality and aesthetics. Artificial Intelligence modeling tools can predict stormwater behavior and optimize system design. Understanding conduit geometry and physical parameters in karst systems can also improve stormwater management models.

The Author (Sheng & Nawari 2016) analyzed stormwater management systems in Wuhan, China, emphasizing sustainable approaches similar to those evaluated in Chandigarh. However, Wuhan's strategies focus more on green infrastructure and permeable surfaces, which might address the pooling issues identified in Chandigarh. The study done by Swathi et al. (2018) applied a stormwater management model to an urban catchment, highlighting the importance of predictive models in managing urban stormwater. The use of such models could potentially help Chandigarh better predict and manage pooling issues. Teshome (2020) presented a review of urban stormwater drainage systems for flood management, underscoring the importance of integrating smart technologies with traditional drainage systems. This integration could enhance Chandigarh's stormwater management by reducing water stagnation and improving overall system efficiency. The review done by Webber et al. (2022) on future perspectives of smart stormwater management suggests incorporating real-time data analytics and AI tools to optimize drainage systems. Implementing such technologies in Chandigarh could address the identified shortcomings in pooling

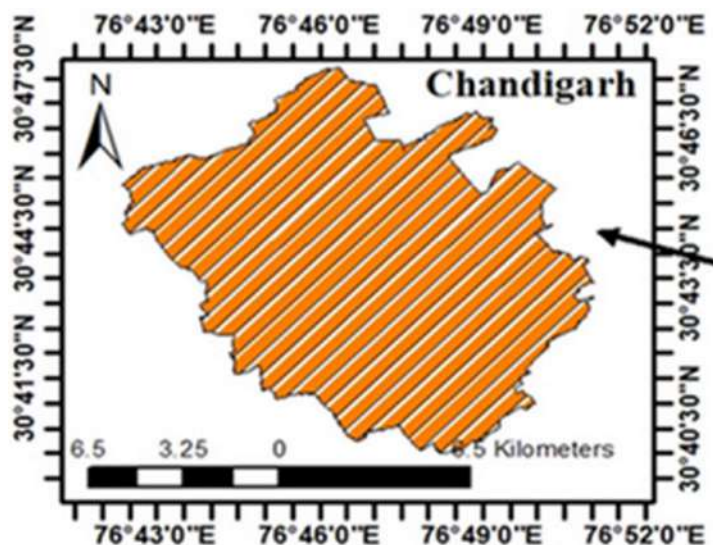


Fig. 1: Map of the study area (Chandigarh).

Table 1: Catalog of diverse stormwater control measures.

"Sr. No.	Indicator	Ideal/Minimum Desirable	Definition	Included/Excluded with Reason
1	Index for Drainage Cleaning	1.0/0.65	Important drain maintenance and cleaning parameters.	Eliminated. Intricacy and data deficiency: Management of technical status evaluation
2	Index of Water Logging	0	Waterlogging occurs in four-hour-plus, six-inch-deep floods.	Incorporated
3	Vulnerability Index for Water Bodies	1.0/0.8	Current encroachment on the total water body area at datum.	Incorporated
4	Index of Complaints Redressal	1.0/0.7	The ratio of effectively resolved drainage complaints to total complaints.	Excluded. Complexity/data lack; Management over technical status assessment
5	Drainage Coverage (Construct) Index	1.0/0.8	Effectively resolved drainage complaints/ total complaints.	Incorporated
6	Index for the Recharge of Artificial Groundwater and Rainwater Harvesting	0.3	Relative rainfall volume stored/harvested/ measured.	Eliminated. Lacking of data/information
7	Sewage Mixing Index	0	Stormwater drainage receives a percentage of sewage flows.	Eliminated. Lacking of data/information
8	Water Bodies Rejuvenation Index	1.0/0.6	Comparing planned water body rejuvenation to the total water body area, including encroached areas.	Incorporated
9	System Robustness Index	<1	Storm inflow/pumping rate ratio.	Eliminated. Lacking of data/information
10	Natural Drainage System Index	1.0/0.7	The percentage of operational natural drainage systems as of a certain date.	Incorporated
11	Area Vulnerability Index	0	The area prone to floods is now both inside cities	Incorporated
12	Vulnerability Index of People	0	The residents of vulnerable areas, whether they have drainage or not, are divided.	Incorporated
13	Flood Moderation Index	1.0/0.6	Moderated versus unmoderated areas prevented flooding.	Incorporated
14	Permeability Index	0.4/0.7	The percentage of the catchment that is impermeable.	Incorporated
15	Index of Master Plans	1.0/0.6	If a city lacks a basic master drainage plan, one will be created and indices included.	Eliminated. Absence of data/information
16	Tidal Index	<1.0	The city's maximum water level for current protection.	Excluded. Data and information are unavailable; there is a lack of relevance for multiple cities.
17	Meteorological Index for Rainfall	Variable	Proportion of measured rainfall intensity to flooding-causing rainfall in that region	Incorporated
18	Index for Early Warning and Readiness	1	Point-specific lead time-flow duration ratio.	Excluded. No data or information is available.
19	Stormwater Discharge Quality Index	1	Calculate the storm drain water's TSS/ BOD ratio to the recommended ranges.	Incorporated
20	Index of Climate Change Stress	1.4	The ratio of a city's expected rainfall intensity to its designed rainfall intensity.	Eliminated. Challenges in enumeration complexity; Lack of data availability

and improve adaptive responses to varying precipitation patterns.

MATERIALS AND METHODS

Description of Cities

Chandigarh, located near the Shivalik Hills at 30°43' N latitude and 76°46' E longitude, saw its population increase

from 1,055,450 in 2011 to 1,169,000 in 2021, marking a 19.65% rise (Fig. 1). Urban drainage systems can have significant impacts on stormwater hydrology. In recent years, managing urban stormwater has become a major issue around the world. Climate change and increased urbanization have adversely impacted water flow in metropolitan areas (Lee et al. 2023).

Traditional solutions like installing additional stormwater

pipelines would be costly and only move the flooding downstream (Broekhuizen et al. 2019).

SUDS are technological and policy interventions designed to combat diffuse pollution and manage stormwater sustainably. That's why blue-green infrastructure (BGI), which is also called nature-based solutions (NBS), best management practices (BMP), sustainable urban drainage systems (SuDS), and low-impact development (LID), is important. That's why it has become a more popular way to handle rainwater over the last few decades. Moving towards smart stormwater management involves integrating advanced technologies and frameworks for better control and management of urban runoff. By focusing on these hydrological and urban characteristics, the study can provide a comprehensive understanding of the factors influencing stormwater management and potential mitigation strategies (Fletcher et al. 2013).

Chandigarh is encompassed on the northern, western, eastern, and southern sides by the states of Punjab and Haryana, respectively. The customary yearly precipitation is approximately 105 millimeters, and the mean temperature fluctuates between 0 to 44 degrees Celsius. Furthermore, there is intermittent snowfall in the winter due to the western disturbance. Based on the 24-hour data, Chandigarh experienced an unprecedented torrential downpour of 302.2 mm within the timeframe leading up to 8:30 a.m. on October 20, 2023.

Choice of Indicators for the Investigation

Eleven of the twenty plausible indices were contemplated for this investigation (Table 1) as they were intimately connected to our study's paramount aim of executing comprehensive technical scrutiny of stormwater systems with an emphasis on their utmost pivotal components (Dos Santos et al. 2017). The justifications for obliterating indicators from this investigation may be classified as follows, even though all indicators provide valuable insights into numerous interconnected facets of sustainability. A dearth of data/information, b the intricacy of enumerating all of the variables, and c a concentration on management and administration rather than technical status evaluation (Masaki et al. 2014, Zafar et al. 2024). The inclusionary status of indicators and the rationale for their exclusion are both displayed in Table 1.

Data Accessibility

Geospatial data sets taken from many sources helped to derive the necessary spatial inputs. The spatial and other data sets have these specifics:

- ArcGIS 10.4 is used to analyze Landsat 7 OLI satellite images with 1 to 7 bands.

- ArcGIS 10.4 can be used to analyze Landsat 8 OLI satellite images with 1 to 7 bands.
- A digital elevation model (DEM) with a resolution of 30 meters from the Shuttle Radar Topographic Mission (SRTM).
- Information about the population was gathered from old census records.

Processing of Data

Acknowledging and delineating the principal aqueduct network: The initial phase entailed discerning and charting the indigenous watercourses within the designated study zone. The primary innate hydrological network was ascertained utilizing SRTM DEM (30 m x 30 m), and its perimeters were delineated employing ArcGIS 10.1's hydrographic chart. To evaluate the topographical attributes of the investigation area, we employed the state-of-the-art Electronic Altitude Blueprint (EAB).

Quantification of Drainage Profiles

By utilizing the advanced capabilities of ArcGIS 10.1's cutting-edge hydrological mapping, we successfully discerned and quantified the intricate network of conduits within the designated research vicinity, thereby effectively demarcating their extensive longitudinal profiles.

Identification and Demarcation of Aqueous-Saturated Regions

Using the 2020 Digital Elevation Model (DEM) from ASTER and Landsat 7 TM imagery, we analyzed the spatial and temporal dynamics of how individuals interact with their flood-prone environments. Maps were created in ArcGIS 10.1 to detail the region's topography, including elevation variations, gradients, watercourses, and embankment distribution. Further analysis using the GLOVIS ASTER dataset refined the DEM, and ArcGIS 10.1, along with ERDAS Imagine 9.1, was used to convert spectral data into surface brightness values. Finally, the Landsat imagery was used to classify and identify waterlogged areas within the study region.

Establishment of Delineation for the Research Domain

The DEM was precisely delineated with the aid of the cutting-edge ArcGIS 10.1 software.

Cartographic Representation of the Research Region's Terrain Features

A cartographic diagram was generated utilizing ArcGIS 10.1.

Calculation of Despondency Repositories

The customary catchment vicinity water profundity is a cutting-edge indicator of the hollow retention's magnitude, which fluctuates with land cover, innate topography, and slope. Based on extensive research, the typical profundity of despondency harbored by paved surfaces ranges from 1.0 to 3.0 mm, while it can escalate to a maximum of 7.5 mm in sylvan regions. Nevertheless, the storage capacity may be significantly amplified on level, undulating terrain and in areas with concealed drainage, such as colossal hollows. Lawns possess an impressive capacity for moisture retention, with a range of 3.0–5.0 millimeters. Linsley postulated a groundbreaking formula for the volumetric capacity (V_s) of aqueous content in permeable substrates:

$$V_s = S_d \left(1 - e^{-\frac{P_e}{S_d}} \right) \quad \dots(1)$$

Where,

V_s = depression storage per unit area, mm;

S_d = depression storage capacity, mm;

P_e = depth of precipitation in excess of interception and infiltration.

Evaporation (P_e) is considered to be zero in the model, and depression storage is taken to be at full capacity.

Experiments show that on a 1% gradient, impermeable surfaces retain an average of 3 mm of water, which decreases to 1.3 mm at a 3% gradient. Natural basin storage ranges from 10–50 mm, and grassy surfaces can hold around 5 mm. The rate at which depression storage fills during storms can be further explained by-

$$V_s = e^{-\frac{P_e}{S_d}} (I - f) \quad \dots(2)$$

where,

I = rainfall intensity, mm.h⁻¹

f = infiltration rate, mm.h⁻¹

Listing of Singular Indicators in Independent Mode

Natural drainage system indicator: Delineating the research area and identifying the natural waterways within it allowed us to calculate the index shown in Fig. 2.

Inclusion in the drainage ratio: This quantifiable parameter was computed by juxtaposing the degree of affluence at a momentous juncture in chronology with the exigency for comprehensive efflux at the current juncture.

An index of penetration: The penetrable region was ascertained by amalgamating the agrarian and blossoming zones and deducting the metropolitan and desolate zones. Land utilization and land envelopment diagrams of the exploration domains are exhibited.

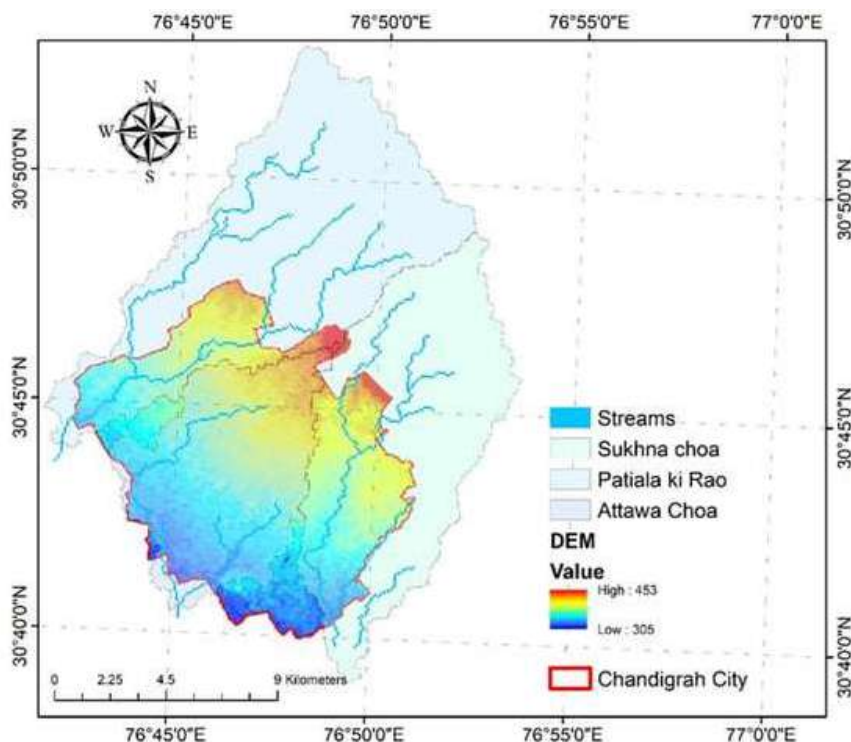


Fig. 2: Runoff diagram of Chandigarh.

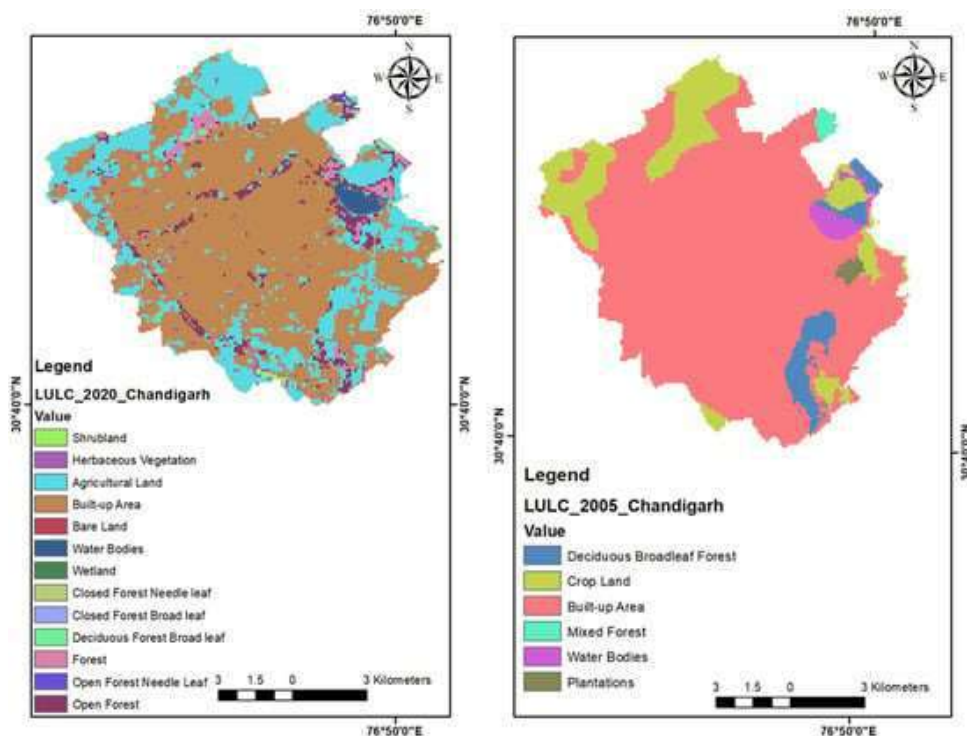


Fig. 3: LULC map of Chandigarh.

Rejuvenation of water bodies index: The necessity for rejuvenation of an aqueous entity may be assessed utilizing the Aquatic Organism Revitalization Indicator. It was meticulously calculated by juxtaposing the entirety of aquatic formations at a historical baseline with the current state, precisely identifying encroached regions, and subsequently approximating the overall expanse necessitating restoration.

Wetness measurement index: Waterlogged areas were pinpointed using Indian Meteorological Department rainfall data, with the benchmark being areas inundated for over four hours and a water depth of over 6 inches. The study suggests reducing this time to one hour to improve effectiveness, based on past events in cities like Delhi, Mumbai, and Chennai, aiming to build cities with disaster resilience in mind from the start.

Area vulnerability index: The Region Susceptibility Indicator provides a detailed evaluation of a city's flood risk by analyzing elevation data, topography, and surface characteristics (Kourtis & Tsihrintzis 2021, Lashford et al. 2020, Gimenez-Maranges et al. 2020, Zahmatkesh et al. 2015, Peterson & Wicks, 2006). Through formulas, the study calculated potential flood basins within urban areas, estimating each basin's capacity based on average water depth. The total area at risk of flooding was determined by summing up these basins. Finally, the indicator was obtained by comparing the flood-prone area to the total urban area,

offering a measure of the city's vulnerability to flooding.

Waterbody vulnerability index: The Aquatic Entity Vulnerability Metric was computed utilizing an iterative methodology. Acquiring a LULC (Land Utilization and Terrain Coverage), cartography of the exploration vicinity was the inaugural measure (Fig. 3). This cartographic representation greatly assisted us in discerning the specific land cover that corresponded harmoniously with the aqueous characteristics. ArcGIS 10.1 was employed to compute the aggregate extent beneath the encroaching hydrophilic organisms by amalgamating the dimensions of all pixels that are categorized as hydrophilic organisms at the current moment. Simultaneously, the aggregate expanse engulfed by aqueous characteristics on the designated date was computed by amalgamating the dimensions of every pixel denoted as water features during the said period.

People vulnerability index: This quantitative measure was computed utilizing data from the Census of India and other sources elucidating the demographics of metropolitan regions, by industry sector. The approach entailed discerning and tallying the populace inhabiting perilous regions proximate to aqueous reservoirs (such as rivers or streams), that could potentially be engulfed amidst inclement climatic conditions. Upon gathering this valuable data, diligent researchers employed it to ascertain the precise proportion of the entire population residing within these flood-prone

regions, whether equipped with sufficient drainage systems or not, that fell victim to the calamity.

Stormwater discharge quality index: In the nonexistence of guidelines for the excellence of metropolitan surface runoff, information on overall suspended solids (TSS) and biochemical oxygen demand (BOD) were collected from recent scientific publications and suggested thresholds were established under standards for releasing treated effluent into surface water (30 mg.L⁻¹ for TSS and 20 mg.L⁻¹ for BOD). Although certain TSS thresholds may be quite stringent owing to the accumulation of loosened surface soil and dispersed substances by the runoff, the directory furnishes an assessment of adherence to TSS/BOD guidelines. Nevertheless, the compendium accentuates regions that might necessitate enhanced stormwater governance and filtration techniques in the forthcoming years.

Flood moderation index: The primary stage in computing this measurement (Webber et al. 2016) was identifying areas near aquatic characteristics that are exceedingly susceptible to inundation owing to an insufficiency of flood-mitigation measures such as dikes, reservoirs, or canal systems. Regional governmental archives may potentially offer valuable perspectives into flood-vulnerable regions and may serve as the foundation for this type of evaluation (Cuppens et al. 2013, Singh et al. 2012, Jones & Macdonald 2007, Swathi et al. 2018, Sirishantha & Rathnayake 2017). Following that, regions that were spared from inundation or experienced diminished inundation as a consequence of the executed mitigation strategies were ascertained (De Almeida et al. 2018). The Flood Moderation Index offers a comprehensive depiction of the effectiveness of flood prevention endeavors by quantifying the proportion of the area that remained unharmed as a result of moderation initiatives in the vicinity that would have otherwise been submerged without such interventions (Census of India 2011, Roy et al. 2023).

Rainfall intensity index: Intensity-Duration-Frequency (IDF) diagrams were employed to augment the correlation among precipitation patterns utilized in catchment hydrology examination. These undulations were crafted across diverse temporal spans employing frequency analysis of information on yearly zenith rainfall. This investigation endeavored to

fabricate IDF curves for the ensuing return periods: 5, 10, 25, 50, and 100 years. These curvatures were formulated utilizing diurnal precipitation data from both antiquated and projected climatic scenarios. Utilizing IMD's empirical reduction methodology, hourly precipitation data were derived from the daily records.

By employing the continuity equation and logical methodology, an approximation of the precipitation volume that instigated the inundation within the storm drain system was computed. Based on the historical archives for Chandigarh, Table 2 exhibits the precipitation magnitude (mm.h⁻¹) for diverse storm recurrence periods. “

Analytical Hierarchy Process (AHP): Concept and Function in the Research Aiming at Weight Coefficient Estimation

The Analytical Hierarchy Process (AHP) is a widely used technique for multi-criteria decision-making. It has been applied in various fields such as budget-conscious stormwater management, alternative energy selection, air pollution analysis, urban water systems evaluation, surface water quality, and wastewater treatment analysis. While AHP is known for its flexibility and intuitive approach, it is not without drawbacks, including its reliance on subjective judgments and the need for numerous pairwise comparisons.

Equation (3) illustrates the comprehensive assessment lattice diagram as well as the lattice portrayed with magnitude coefficients.

$$A = \begin{bmatrix} 1 & a_{12} & \cdots & a_{1n} \\ 1/a_{12} & 1 & \cdots & a_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ 1/a_{1n} & 1/a_{2n} & \cdots & 1 \end{bmatrix}$$

$$\begin{bmatrix} w1/w1 & w1/w2 & \cdots & w1/wn \\ w2/w1 & w2/w2 & \cdots & w2/wn \\ \vdots & \vdots & \ddots & \vdots \\ wn/w1 & wn/w2 & \cdots & wn/wn \end{bmatrix} \quad \dots(3)$$

Table 2: Chandigarh's rainfall intensity, duration, and frequency analysis.

Return Period	Intensity [mm.h ⁻¹]	Intensity [mm.h ⁻¹]	Intensity [mm.h ⁻¹]	Intensity [mm.h ⁻¹]	Intensity [mm.h ⁻¹]	Intensity [mm.h ⁻¹]
	20 min	40 min	60 min	80 min	100 min	120min
5 yr	115	95	75	65	45	35
10 yr	120	100	90	80	55	40
25 yr	160	120	110	90	65	50
50 yr	170	135	115	105	75	55
100 yr	195	155	135	115	80	60

The subsequent measure is to compute the magnitudes for every constituent, indicator, and facet once the verdict grid A has been formulated, where a_{ij} represents the assessments between elements i and j for all $i, j = 1, 2, \dots, n$. Subsequently, it is imperative to authenticate the coherence of the acquired outcomes.

$$CR = \frac{CI}{RI} \quad \dots(4)$$

The coherence coefficient (CC), ascertained utilizing Formula (5), can be utilized to compute the arbitrary coefficient (AC), which is derived from the average of the coherence coefficient (CC) that is generated and depends on the configuration of the matrix provided by Saaty.

$$CI = \frac{\lambda_{max} - n}{n - 1} \quad \dots(5)$$

The utmost eigenvalue is indicated by the foremost eigenvalue, maximum, and the tally of rows or columns in the square assessment matrix is denoted by n . The matrix consistency is evaluated to be satisfactory if the coherence ratio (CR) is less than or equal to 0.1. Nevertheless, if the CR surpasses 0.1, it implies that the evaluations might necessitate fine-tuning to mitigate disparities.

Aggregation of Indices Process

The comprehensive gauge of system sustainability was computed utilizing a weighted linear summation of eleven distinct metrics, as articulated in the subsequent equation.

$$SSIA = \sum_{i=1}^n w_i I_i \quad \dots(6)$$

RESULTS AND DISCUSSION

The results and discussion are displayed in the two subsequent sections, highlighting comprehension of enumerated indicators in the autonomous mode and in the amalgamated mode, respectively.

Sustainability Indices in a Standalone Mode

Table 3 exhibits the outcomes of the solitary stormwater drainage indicators computed for Chandigarh. For every indicator, the specified values must be examined against their corresponding optimal condition and minimal desired intervals. These furnish significant perspectives on the

condition of stormwater governance and urban adaptability in these regions. Disparities in indicator values manifest the diverse ramifications of urbanization, hydrological variables, and the extent to which cities are equipped to handle precipitation.

Table 3 presents a detailed comparison of sustainability indicators across study sites, highlighting Chandigarh's stormwater management performance. It lists various indices, such as Ecological Drainage, Drainage State, Pooling, and Aquifer Susceptibility, among others, and compares them to ideal and minimum desired states. Chandigarh's scores, such as 0.86 on the EDSI and 0.76 on the DSI, are relatively high, indicating a strong sustainability status. However, a higher Pooling Index score of 0.43 compared to the ideal 0.3 suggests potential issues with water stagnation. This comprehensive analysis provides insight into the effectiveness and areas for improvement in the city's stormwater management practices. Certain indicators performed better or worse because of the strong ecological drainage state, reflecting effective natural water flow and minimal ecological disruption. Some suggest that the city's drainage systems are functioning well but could still benefit from improvements to reach optimal performance. Some suggest that certain areas may be prone to pooling, which can lead to problems such as mosquito breeding and waterborne diseases.

The Internet of Things (IoT) can play a significant role in addressing the issues highlighted by these indicators by providing real-time data on water levels, flow rates, and precipitation, enabling more responsive and adaptive management of stormwater systems. By using predictive analytics, IoT systems can forecast potential pooling events and allow for pre-emptive measures to be taken, reducing the likelihood of water stagnation and associated issues. IoT enables remote control of stormwater infrastructure, such as automated gates and pumps, which can be adjusted based on real-time data to optimize drainage and prevent pooling. The integration of IoT in stormwater management can lead to more efficient use of resources and cost savings by reducing the need for manual monitoring and intervention. By leveraging IoT technologies, cities like Chandigarh can improve their stormwater management systems, addressing the specific issues indicated by performance metrics and enhancing overall urban water resilience.

Table 3: Calculated sustainability indices for study sites.

S.N	Cities	NDSI	DCI	PI	WBRI	WLI	AVI	WBVI	PVI	SWDQI	FMI	RII
Ideal/Mini. Desirable Condition		1.0/.7	1/.7	.3/.7	1.0/.8	0	0	1.0/.6	0	1	1/.7	Variable
1.	Chandigarh	0.86	0.76	0.43	0.82	0.12	0.1	0.82	0.1	0.56	0.84	0.64"

Table 4: Priority ranking for technology selection based on criteria for Chandigarh.

Indicators	Class	Sustainability Class Ranges and Ratings	Sustainability Class Ratings	Weight %
Natural	0.7–1	Very High	4	16
Drainage System Index	0.7–0.78	High	3	
	0.5–0.49	Moderate	2	
	0.3–0.48	Low	1	
	0–0.18	Very low	1	
Drainage	0.7–2	Very High	4	16
Coverage Index	0.5–0.88	High	3	
	0.3–0.48	Moderate	2	
	0.1–0.8	Low	1	
	0–0.18	Very low	1	
Permeability	0–0.17	Very High	4	12
Index	0.3–0.28	High	3	
	0.3–0.58	Moderate	2	
	0.7–0.89	Low	1	
	0.9–1	Very low	1	
Water Body	0.9–1	Very High	4	12
Rejuvenation Index	0.5–0.89	High	3	
	0.5–0.68	Moderate	2	
	0.3–0.48	Low	3	
	0–0.18	Very low	1	
Water Logging Index	0–0.18	Very High	4	6
	0.2–0.48	High	3	
	0.3–0.68	Moderate	2	
	0.7–0.78	Low	3	
	0.9–1	Very low	1	
Area	0–0.18	Very High	4	2
Vulnerability Index	0.1–0.29	High	3	
	0.3–0.68	Moderate	2	
	0.5–0.89	Low	1	
	0.5–1	Very low	1	
People	0–0.19	Very High	4	2
Vulnerability	0.2–0.29	High	3	
Index	0.3–0.89	Moderate	2	
	0.5–0.78	Low	1	
	0.8–1	Very low	1	
Stormwater	0.8–1	Very High	4	9
Discharge	0.6–0.79	High	3	
Quality Index	0.4–0.49	Moderate	2	
	0.2–0.49	Low	1	
	0–0.18	Very low	1	
Rainfall Intensity Index	0.7–1	Very High	4	11
	0.5–0.68	High	3	
	0.5–0.48	Moderate	2	
	0.2–0.29	Low	1	
	0–0.18	Very low	1	
Water Body	0.7–1	Very High	4	11
Vulnerability	0.5–0.68	High	5	
Index	0.5–0.48	Moderate	2	
	0.1–0.38	Low	3	
	0–0.18	Very low	1	
Flood	0.7–1	Very High	4	6
Moderation	0.5–0.68	High	5	
Index	0.3–0.48	Moderate	2	
	0.2–0.28	Low	3	
	0–0.18	Very low	1	
		Total		100

Recent stormwater management studies can help urban planners develop more efficient and sustainable systems. IoT can improve stormwater management systems' efficiency and efficacy. IoT allows real-time data collecting, predictive analytics, and remote infrastructure control, which can help predict and mitigate excessive rains and flooding. Sustainable Urban Drainage Systems (SUDS) replicate natural processes to manage stormwater holistically. These systems reduce runoff, improve water quality, and beautify and entertain cities. AI modeling can anticipate stormwater behavior and optimize stormwater system design and operation. These tools improve water quality and decrease flooding by delivering precise and timely decision-making information. Understanding conduit shape and physical factors in karst environments can improve stormwater management models. Complex subterranean drainage systems require this understanding. Urban planners may design stormwater systems that are more resilient to harsh weather and improve urban sustainability and livability by using these tactics.

Aggregated Overall System Sustainability Index

The input parameters utilized to ascertain the relative significance of diverse stormwater drainage indicators differed among municipalities. Carefully chosen for their pertinence to stormwater management, these parameters encompassed elevation, slope, precipitation, land use/land cover, stream and streamflow density, NDVI, TSS, and BOD measurements. The Analytical Hierarchy Process (AHP) methodology, as elaborated in Section 2.5, offered a systematic approach to allocating importance to the indicators through paired evaluations and mathematical calculations. For every municipality, Table 4 exhibits the magnitude values allocated to diverse stormwater drainage indicators. The Homogeneity Quotient (HQ) was computed to assess the consistency of the findings. If the CR is less than or equal to 0.1, the matrix is sufficiently homogeneous, whereas a value exceeding 0.1 implies that modifications are necessary to diminish disparities. A congruity proportion of less than or equal to 0.1 was necessitated as the sole criterion for ascertaining the weightage magnitudes in every urban matrix.

Table 4 outlines a method to prioritize technology choices for Chandigarh's stormwater management using sustainability indicators across various categories, each weighted by importance. Key indicators include the Organic Drainage System Index (15% weight) assessing natural drainage effectiveness, the Drainage Coverage Index (17%) for infrastructure extent, and the Percolation Quotient (11%) for ground absorption capabilities. The Aquatic Rejuvenation Metric (13%) and Aquatic Inundation Metric (5%) gauge

water body recovery and flooding issues, respectively. Additionally, the susceptibility of the area and population to water-related issues is covered by the Region and Individuals Susceptibility Indexes (1% each), while the Stormwater Emission Excellence Indicator (8%) evaluates stormwater quality. Precipitation Intensity (12%) and Aquatic System Susceptibility Index (12%) measure weather severity and water system fragility, and the Deluge Moderation Index (5%) considers flood mitigation effectiveness. This comprehensive weighting system, totaling 100%, helps stakeholders make informed decisions for enhancing the city's stormwater management sustainability.

Table 5 combines various sustainability indicators to measure Chandigarh's stormwater management performance. Each indicator is given a weight reflecting its importance, and the overall score is the sum of each indicator's value multiplied by its weight. For instance, the Ecological Drainage System Indicator (EDSI) with a weight of 0.15 has a weighted score of 0.129, while the Drainage Inclusion Indicator (DII) with a higher weight of 0.17 scores 0.044. After evaluating all indicators, including the Percolation Quotient, Aquatic Habitat Restoration, and Hydrological Saturation, the resulting Comprehensive Sustainability Indicator (CSI) totals 0.761. This metric synthesizes Chandigarh's approach to sustainable stormwater management and helps identify strengths and areas for improvement.

CONCLUSIONS

Ecological Drainage, Drainage State, Pooling, and Aquifer Susceptibility were used to evaluate Chandigarh's stormwater management. EDSI scores of 0.86 and DSI

Table 5: Aggregated overall sustainability indicators.

Sr. No.	Chandigarh			
	Indices	Ii	Wi	Ii Wi
1	NDSI	0.86	0.15	0.129
2	DCI	0.26	0.17	0.044
3	PI	0.53	0.11	0.057
4	WBRI	0.82	0.13	0.107
5	WLI	0.32	0.05	0.076
6	AVI	0.58	0.01	0.058
7	WBVI	0.82	0.01	0.028
8	PVI	0.6	0.08	0.048
9	SWDQI	0.56	0.12	0.067
10	FMI	0.84	0.12	0.101
11	RII	0.74	0.05	0.042
12	OSSI			0.761"

values of 0.76 indicated high sustainability. Water stagnation may occur with a higher Pooling Index score. The study also prioritised stormwater drainage indicators using the Analytical Hierarchy Process (AHP). The Organic Drainage System Index, Drainage Coverage Index, Percolation Quotient, Aquatic Rejuvenation Metric, Region and individual susceptibility Indexes, Stormwater Emission Excellence Indicator, Precipitation Intensity, Aquatic System Susceptibility Index, and Deluge Moderation Index scored high for technology selection in Chandigarh. The Comprehensive Sustainability Indicator (CSI) determines Chandigarh's stormwater management strengths and weaknesses using multiple sustainability metrics. The Comprehensive Sustainability Indicator (CSI) evaluates Chandigarh's sustainable stormwater management and identifies strengths and weaknesses. This comprehensive research illuminates the city's stormwater management efficacy and areas for improvement. The findings offer valuable insights for urban planners and policymakers aiming to advance sustainable stormwater infrastructures in Chandigarh and cities with similar challenges.

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A Compendium on the Eco-Sustainable Biosynthesis of Palladium Nanoparticles (PdNPs) and Their New Avenues Towards Environmental Applications

Geetanjali Basak† 

Department of Microbiology, Raniganj Girls' College, Raniganj, West Bengal, India

†Corresponding author: Geetanjali Basak; geetanjalisbasak@gmail.com

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ABSTRACT

Palladium Nanoparticles (PdNPs) are considered significant catalytic agents, along with a wide range of applications, mainly hydrogen storage and sensing, biomedical imaging, and remediation strategies. Extensive studies are being carried out on the formation of PdNPs (PdNPs) worldwide, showing the predominance of the chemical approach among various technologies for the synthesis. The traditional chemical method employed in the formulation of PdNPs contains certain limitations that have been overcome by the use of the alternative biological method, as they are meek, low-cost, and benign to ecosystems. Therefore, the present review provides an overview of the modernized techniques involved in the biological approach for PdNPs formation by utilizing various natural origins, for example, plants as well as microbes, for their enhanced stability and applications in fields of the environment. Various mechanisms and parameters involved, along with approaches utilized for the characterization of bioPdNPs, are described, with an insight being delivered on the utility of biologically synthesized palladium nanoparticles. Recovery of PdNPs to achieve a circular economy is also being focused. In addition, the future prospectus on palladium nanoparticle research is also summarized.

INTRODUCTION

Palladium is considered one of the most extensively utilized precious, switching metals that displays typical physico-chemical, optical, and thermodynamic characteristics at the nanoscale margin (Watanabe et al. 2006, Cheong et al. 2010). Although the metal palladium has been the interest of researchers since ancient times, its revitalization as PdNPs has resulted in a substantial enhancement in the context of various biotechnological applications through emerging nanoscience and nanotechnological aspects. Moreover, PdNPs imparts great attention because of their utility as a catalyst in coupling reactions (Karimi & Enders 2006), Heck reaction (Heck & Nolley 1972), Kumada reaction (Jana et al. 2011), Sonogashira reaction (Sonogashira 2002), Negishi reaction (Astruc 2007), Stille reaction (Milstien & Stille 1978), Buchwald-Hartwig reaction (Widenhoefer & Buchwald, 1996), hydrogenation reactions (Wilson et al. 2006), and fuel cell reactions (Zhang et al. 2011). They are also considered good catalysts in both homogeneous and heterogeneous reactions as they possess enhanced surface-to-volume ratios with great surface energy (Narayanan & El-Sayed 2005). Moreover, PdNPs performs a vital part in several biotechnological fields, such as the production of pharmaceutical compounds, degradation of harmful environmental pollutants (Kora & Rastogi 2016, Martins et al. 2017, Safavi & Momeni 2012), and preparation of non-enzymatic biosensor to detect hydrogen peroxide in milk (Baccar et al. 2013).

An analysis of PdNp's applications through region-wise market study depends on various factors. They are mainly consumption, its growth significance, market share between competitive nations, and their adopted technologies. The top 5 competitive regions that are involved in its manufacture and applications are North America, Europe, Asia Pacific, Latin America, and the Middle East and Africa, according to data provided by the Worldwide "PdNPs market" Research Report 2024. Their major trends involved the use of PdNPs in medical diagnostics, sensors, fuel cells, and catalysis (environmental usage). They are globally demanded as vehicle exhaust catalysts in the automobile sector. They also act as catalysts for degrading hydrocarbons in petroleum sites. U.S. plays the dominant role, succeeded by Germany and the U.K. North America and Europe contribute about 45% of the market share, followed by China and Japan, around 30 %. Finally, Latin America, and Middle East & Africa follow the path by accounting for around 15 % of the market share, and the remaining is contributed by Brazil and Saudi Arabia.

An extensive variety of wet chemical methods, *viz.*, polyol (Xiong et al. 2005a), sonochemical (Nemamcha et al. 2006), electrochemical (Cha et al. 2007), as well as chemical reduction (Nguyen et al. 2010), have been explored for the morphologically regulated formation of PdNPs. The catalytic function of palladium is determined by its structure at the nano level (Xiong et al. 2005b). Hence, the formation of uniform-sized palladium colloidal particles has been considered an essential matter in research. Several approaches, particularly uniform-shaped palladium particles in aqueous solutions by a wide variety of stabilizing agents, have been used by researchers (Ganesan et al. 2007). Fig. 1 shows the traditional methods for materializing PdNPs.

Among various techniques, the chemical method is widely used, where the salts of metallic precursors are reduced to metal atoms by utilizing various reducing agents, *viz.* citrate, hydrides, ethylene glycol, and hydrazine, all of which pose serious risks to the environment and health of living beings. Hence, there is an increasing demand aimed at the development of a clean, nontoxic, and ecological synthetic approach. The expansion of enhanced production and inexpensive technology for nanoparticle creation is a crucial task. To date, the enormous variety of microorganisms, involving prokaryotes and eukaryotes, has been accounted for the formation of nanobiomaterials (Basak et al. 2014, Basak et al. 2020). Although reports are there on its biosynthesis and applications, reports are still scanty on its mechanism of synthesis through microbes and the 'RRR' concept. The author has tried to portray this information about PdNPs from its cradle to grave. Probably this is the first report to go through a compendium including the aforementioned aspects, challenges, and benefits of environmental cleanup as well.

Therefore, the current study envisages the overall technology applied for the sustainable preparation of PdNPs throughout the last decade. The objective of the refurbished report is also to consolidate various literature about PdNPs' utilities in several fields involved in environmental applications. Moreover, the review tries to envisage prospects, challenges, and research facilities regarding the particular area of interest. Furthermore, the recovery, recycling, and reuse ('RRR') are also discussed in the particular comprehensive review.

MATERIALS AND METHODS

The compendious review of literature involves the critical

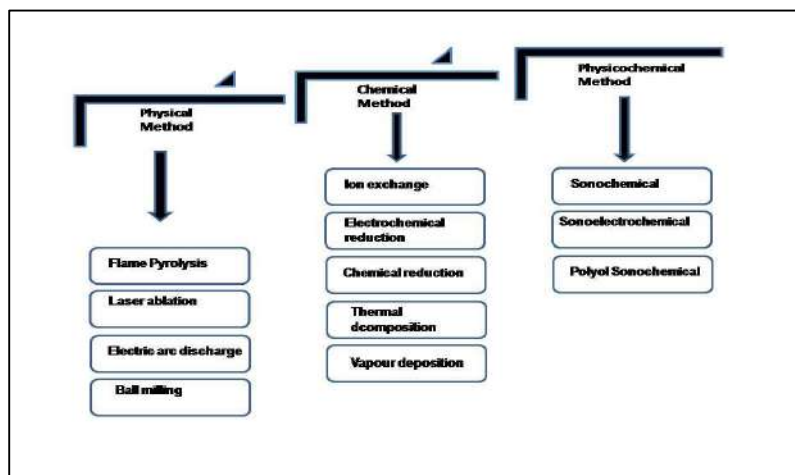


Fig. 1: Traditional methods for materializing PdNPs.

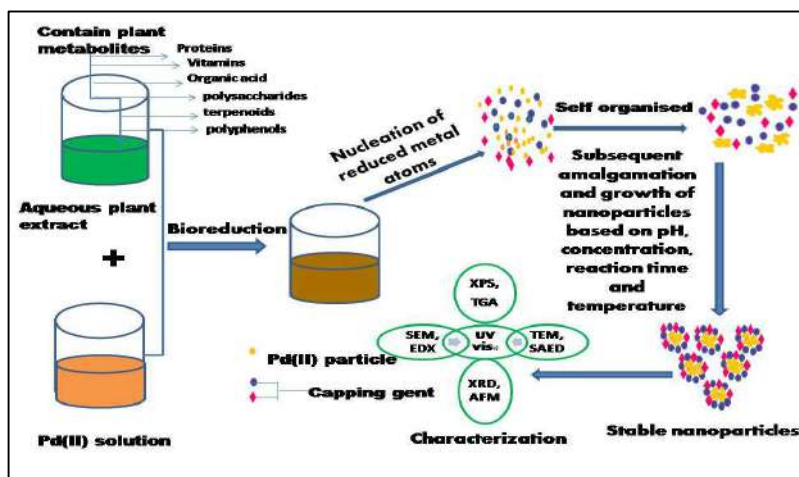


Fig. 2: General route for the biological formulation of nano-biopalladium through plant metabolites.

exploration of the extensive literature review and research papers existing over the last two decades with respect to palladium nanoparticle biosynthesis and its utilities. The comprehensive study used the exploration of different websites like JSTOR, INFLIBNET, Scopus, Google Scholar, and Science Direct through combined keywords such as “palladium biosynthesis”, “characterization, properties”, “environmental application,” and “recovery, recycle and reuse”.

Biosynthesis of PdNPs

Owing to the drawbacks in the physico-chemical synthesis of the PdNPs with respect to their maintenance, the discharge of perilous waste or the formation of Pd nanomaterials by using chemical agents pose hindrances in their application in the clinical fields (Manjare et al. 2021). The biological formation of PdNPs has proven to be an alternative advantageous path. This has contributed to the attainment of sustainability with better results in the formation of products along with their advanced properties. Various biological agents, *viz.*, plant extracts, microorganisms, and biological products of the microbes, are used for the synthesis of PdNPs

Biological Formation of Nano-Palladium by Using Plant Extracts

The biological process involving plant extract is found to be an uncomplicated and adaptable technique followed during the formation of PdNPs (Fig. 2).

Owing to its advantage over the traditional process, researchers have shifted their interest towards the effective utilization of diverse compounds obtained from plants. An overview of PdNPs synthesized biologically is given in Table 1.

For the biosynthesis of nano palladium, the chosen extract of the plant will be mixed with palladium salt solution at a specified concentration under ambient conditions in a single-step experiment (Fig. 2). In the absence of any stabilizer or capping agent from outsource, these active biological compounds obtained from plants acts both as reducer as well as stabilizer. Furthermore, the reaction mixture will be carried out for the reduction of metal salt to a visual color alteration. The phytochemicals/ metabolites extracted from various plants used for PdNPs formation are polyols, polyphenols, flavonoids, vitamins, organic acids, polysaccharides, terpenoids, and proteins. These particular plant extracts are reported to biogenically synthesize nanosized Palladium particles (Qazi et al. 2016).

Microbe-Mediated Synthesis of Nano-Palladium Particles

Nano-sized palladium particles formed by microbial factories have come out as a promising complementary method that demands achieving environmental sustainability with enhanced strength and controlled morphology of nanoparticles (Shedbalkar et al. 2014). Several experiments using various bacteria (a palladium-compatible agent) were conducted to elucidate the mode of action implicated in the materialization of nano-palladium particles (Abbasi et al. 2023, Sharma et al. 2023). Therefore, the possible way for the formation of these nano-sized Palladium particles through microbes can be elucidated in two ways, *viz.* (i) intracellular mode of action and (ii) extracellular mode of action (as shown in Fig. 3).

Intracellular mode: Here, the PdNPs remain concentrated within the cell cytoplasm so that they can sustain their reliability while the process is being carried out. There

Table 1: Biological formation of nano-biopalladium through different bioreductants/ biostabilizers from plants.

Plant as bioreductant/biostabilizer	Size and shape	References
<i>Dioscorea bulbifera</i>	2.0-5.0 nm, globular	Ghosh et al. 2015
<i>Cinnamom zeylanicum</i>	15.0-20.0 nm, spherical	Satishkumar et al. 2009a
<i>Cinnamon camphor</i>	3.2-6.0 nm, quasi-spherical	Yang et al. 2010
<i>Curcuma longa</i>	10.0-15.0 nm, spherical	Satishkumar et al. 2009b
<i>Musa sp. (banana)</i>	50.0 nm, distorted, crystalline	Bankar et al. 2010
<i>Glycine max</i>	15.0 nm, spherical	Vivekanandhan et al. 2009
<i>Hippophae rhamnoides</i>	2.5-14.0 nm, spherical	Nasrollahzadeh et al. 2015
<i>Camellia sinensis</i>	5.0-8.0 nm, spherical	Lebaschi et al. 2017
<i>Terminalia bellirica</i>	30.0-45.0 nm, triangular	Viswadevarayalu et al. 2016
<i>Prunus X yedoensis</i>	50.0-150.0 nm, spherical	Manikandan et al. 2016
<i>Catharanthus roseus</i>	38.0 nm, spherical	Kalaiselvi et al. 2015
<i>Moringa oleifera</i>	10.0-50.0 nm, spherical	Anand et al. 2016
<i>Anacardium occidentale</i>	2.5-4.5 nm, spherical	Sheny et al. 2012
<i>Artemisia annua</i>	20.0- 30.0 nm	Naushad et al. 2015
<i>Stachys lavandulifolia</i>	5.0-7.0 nm, spherical	Veisi et al. 2015
<i>Pulicaria glutinosa</i>	20.0-25.0 nm, globular, crystalline	Khan et al. 2014
<i>Anogeissus latifolia</i>	4.8 nm, cubic crystal	Kora & Rastogi, 2018
<i>Eclipta prostrate</i>	18.0-64.0 nm, spherical	Rajakumar et al. 2015
<i>Annona squamosa</i>	80.0-100.0 nm, spherical	Roopan et al. 2011
<i>Gardenia jasminoides</i>	3.0-5.0 nm, polyhedral	Jia et al. 2009
<i>Euphorbia granulate</i>	25.0-35.0 nm	Nasrollahzadeh & Sajadi, 2016
<i>Asparagus racemosus</i>	1.0-6.0 nm, cubic	Raut et al. 2013
<i>Coffea arabica</i>	20-60 nm, cubic symmetry	Nadagouda & Varma, 2008
<i>Azadirachta indica</i>	5.0-50 nm, small and large spheres	Joseph et al. 2014
<i>Phoenix dactylifera</i>	5-21 nm, small and spherical	Tahir et al. 2016
<i>Ananas comosus</i>	1-16 nm, spherical	Olajire & Mohammed, 2019
<i>Salvia hispanica</i>	9.0-20.0 nm Semi-Spherical	Kiani et al. 2020
<i>Urtica</i>	3.0-12.0 nm Irregular	Gulbagca et al. 2021
<i>Cissus quadrangularis</i>	12.0-26.0 nm Spherical	Anjana et al. 2019
<i>Peganum harmala</i>	16.0-32.0 nm Spherical	Fahmy et al. 2021
<i>Fritillaria imperialis</i>	20.0-30.0 nm Quasi-spherical	

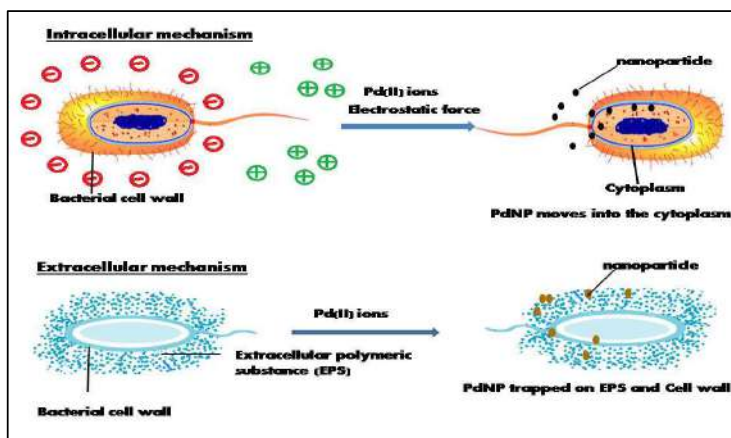


Fig. 3: Mode of action involved in the microbe-mediated nano-palladium particle formation.

are reports on bacteria (*B. benzeovorans* NCIMB 12555, *D. desulfuricans* NCIMB 8307, and *D. desulfuricans* NCIMB 8326) for the residence of approximately 8 nm sized icosahedral PdNP particles (Omajali et al. 2015). The possible stepwise mechanism for the intracellular method might be through trapping electrostatically, bioreduction, nucleation, and capping (Nasreen & Taranath 2014). Recently, scientific reports have demonstrated the intracellular microbial-mediated formulation of nanoparticles from various non-precious metals *viz.* iron (Peng et al. 2010); copper (Navarrete et al. 2011); Chromium (Polti et al. 2011, Ravindranath et al. 2011); uranium (Sousa et al. 2013). However, to date, information about the formation of intracellular palladium is very limited. E.g., intracellular palladium is situated within the inner membrane (Foulkes et al. 2011). Although microbes act as the biogenic bearers of palladium, whose valency is converted from 2^+ to 0, they lose their viability, but still, *E. coli* could remain physiologically activated with the inability to proliferate (Joudeh et al. 2021).

Extracellular mode: Here, the palladium colloidal suspension is present over the outer wall of the periplasm, plasmalemma, or surface of the cell wall, especially on the extracellular polymeric substance can be observed. Several reports have been conveyed on the use of a wide range of bacteria, *viz.* *Shewanella sp.* (Windt et al. 2005), *Desulfovibrio sp.* (Yong et al. 2010), *B. Megatarium* (Chen et al. 2023), and for shifting the oxidation phase from +2 to 0 of PdNPs lying over the periphery of the cell. Thus, it makes the metallic ion nontoxic, along with suitable retrieval of nanoparticles without cell destruction. The basic mechanism for the extracellular formation of PdNPs is through the microbial hydrogenase enzyme (Yang et al. 2020). The formation of bio-nano-palladium particles by members belonging to the groups of fungi (Tarver et al. 2019) has been reported so far. Few algae, *viz.* *Chlorella vulgaris* (Arsiya et al. 2017) and *P.boryanum* UTEX 485

(Maggy et al. 2007) are being used to make nano-palladium particles. Table 2 shows the collection of microbes for PdNPs formation.

PdNPs Synthesis Through Biological Products

Apart from the microbial synthesis of PdNPs, the usage of secondary metabolites regarding the development of PdNPs gives budget-friendly solutions for the attainment of a safer Earth. Among biomolecules, nucleic acids such as RNA serve as great biogenic catalysts for the formation of palladium nano-bio-conjugates with controlled morphology (Liu et al. 2006). DNA in double-stranded form, compared to single-stranded one, proves to be an efficient biocatalyst. They make minimum-sized nanoparticles with greater reducing capability of H_2O_2 and oxidizing capacity of ascorbic acid (Fang et al. 2007).

The utilization of high molecular weight biomolecules, *viz.* protein, polypeptide, and amino acid varied sequences for stabilization of peptide chains has been reported to ensure a substantial influence over nanomaterial structure. An amino acid residue can perform a dual role in increasing stability while reducing it simultaneously. Zhou et al. (2016) showed the potential of biofilm-producing natural polymer externally bearing functional groups that would attach or stabilize PdNPs. Continuous production of PdNPs by exploiting a denitrifying biofilm encouraged the creation of zerovalent Pd from divalent Pd. Both enzymatic as well as autocatalytic conversion of the ion is carried on top of the outer layer of concave-fiber membranes so as to carry (H_2) for electron donation. Several exopolysaccharides, like xanthan gum, obtained by fermentation of the bacterium *Xanthomonas campestris*, act both as a reducer and stabilizer for materializing PdNPs of 10 nm size (Kumari et al. 2015). This nanomaterial stabilized by the aforementioned polysaccharide served as an excellent hydrogenating property in converting 4-nitrophenol to 4-aminophenol using borohydride. Hormones and vitamins, *viz.* ascorbic

Table 2: Synthesis of nano-palladium using microbes.

Bacteria	Size/shape	Reference
<i>Pseudomonas sp.</i>	4.0-20.0 nm	Schluter et al. 2014
<i>Shewanella oneidensis</i>	irregular	Windt et al. 2005
<i>Clostridium butyricum</i> , <i>Citrobacter braakii</i> , <i>Enterococcus faecium</i> , <i>Bacteroides vulgates</i>	irregular	Hennebel et al. 2011, Ghosh 2018
<i>Bacillus benzeovorans</i> , <i>Bacillus sphaericus</i> , <i>Desulfovibrio desulfuricans</i>	9.0-12 nm, icosahedral	Omajali et al. 2015
<i>Geobacter sulfurreducens</i>	14.0-25.0 nm	Matthew et al. 2013
Microalgae		
<i>Chlorella vulgaris</i>	5.0-20 nm, crystalline	Arsiya et al. 2017
<i>Plectonema organum</i>	≤ 30 nm, spherical and elongated	Maggy et al. 2007

acid (Ameri et al. 2020) and oxytocin + amino acid (Bendre et al. 2020), have recently acted as potential agents for the synthesis of the target material. Clergeaud et al. (2013) depicted that lipid glycerol monooleate, along with the tetrachloropalladate solution, synthesized 4 nm-sized PdNPs. This is mainly accompanied by a polyol-type reaction where the palladium is formed from the reduction of palladium through a hydroxyl group present in glycerol at 25°C.

Significant Parameters for PdNPs Synthesis

Important parameters considered for the regulated synthesis of PdNPs are as follows: (i) pH, (ii) the type and amount of the stabilizing agent used, (iii) the relative concentrations of both metallic precursor and reducing agent, (iv) the variation of reaction temperature and (v) using of foreign ions (Cheong et al. 2010).

The optimized pH for the development of PdNPs using 45.0 ml of *Prunus xyedoensis* leaf extract with 0.1 M palladium (II) chloride solution is 7.0 (Manikandan et al. 2016). The appearance of palladium nanomaterials varies based on the nature of different stabilizing agents that cap on the same-shaped palladium nuclei. The precursor PdCl₂ was reacted with ethane 1,2-diol in an argon atmosphere using polyvinylpyrrolidone as stabilizer synthesized round-shaped, 7.5 nm apparent-sized PdNPs. Approximately 8.4 nm sized and polyhedral-shaped PdNPs in the atmosphere, along with 6.3 nm sized particles in the presence of argon, were obtained in a mixture of amphiphilic compounds (PVP and CTAB), respectively. The ratios of metallic precursor along with the reducing agent have been found to play a crucial role. A higher reductant concentration might result in an enhanced reduction rate of change in the morphology of nanomaterials (Xiong et al. 2007). The size of the nano-palladium can be transformed based on the different preliminary concentrations of PdCl₂ over amine. The reaction temperature is one of the main factors that not only affect the reduction rate of metal precursor but also bring about morphological change in Palladium nanomaterials. Guisbier et al. (2011) explained the consequences of various temperatures triggering changes in the geometrical shape of PdNPs. Several works have emphasized the influence of foreign species (trace metallic ions) on the control of the shape formation of PdNPs. The addition of trace metal ions mainly facilitated the self-assembly, in addition growth of the nanoscale materials too (Cheong et al. 2010). Manikandan et al. (2016) mixed leaf extract and Pd(II) at a ratio of 40:5 to obtain green nano palladium particles. Parameters have been depicted in Fig. 4.

Properties and Characterization of BioPalladium Nanomaterials

Properties: PdNPs have interesting properties compared to the metal ion itself, which contributes to immense medicinal applications. The properties are (i) efficient catalytic activity (Prashant et al. 2006), (ii) physicochemical properties (Yang et al. 2006), and (iii) optical and electronic properties. Recently, a wide number of investigations were carried out to find novel approaches for PdNPs synthesis with improved properties. Due to their catalytic and/or optical properties, they have several environmental applications that will be discussed in the review. Though PdNPs impart excellent mechanical and chemical stability, they are still thermodynamically unstable because of the accumulation of nanoparticles in a reaction mixture. This might be a general process that might lead to complications while studying its properties and usage. A wide range of chemicals, like polymers, ligands, dendrimers, and tensides, are being reported to reduce and stabilize PdNPs (Cookson 2012). Electrostatic force of attraction or a combination of the two, i.e., oppositely charged forces, might be the reason for stabilization.

Characterization: Biologically synthesized PdNPs can be observed through various instrumentation techniques. A series of analytical techniques must be used to determine the character of the material. Starting with the colloidal suspension, the color change of the synthetic solution to brownish back from orange confirms the formation of PdNPs. These biopalladium nanomaterials are initially detected by a UV-Vis spectrophotometer. FT-IR spectral analysis is being used in various biological agents to spot the charged groups occurring over the superficial material required for the addition of electrons to metallic Pd. Further, the crystal structures of the formed PdNPs are investigated by XRD-spectroscopy (Bankar et al. 2010). Nanomaterial has a different structure (different phase) than their bulk version (crystal). A detailed diffraction study illustrates that the most significant peak is due to the crystal unit cell structure of PdNPs. The elements present and characterization of a nanoparticle are being analyzed using the instrumentation technique of energy dispersive X-ray (Omajali et al. 2015). Both elemental analysis and surface characterization can be analyzed by X-ray photoelectron spectral (XPS) techniques. The difference in kinetic energy from the similar dispersed states of the particle size, derived from the varying magnitude of experiments between different core levels, determines the morphology of particles. Optionally, the average size of the particle can also be analyzed from a fraction of monolayer and crystallite samples on a concentration basis. In the case of the formation of nanoparticles located intracellularly, the dimension of the nanoparticle is very minimal. This can be voluntarily visualized in Transmission

electron microscopy. Therefore, it is necessary to analyze electron backscattering and confirmatory analysis via EDX analysis. In addition, it may also show the existence of carbon and oxygen peaks, inferring the occurrence of these elements on the exterior of PdNPs. Furthermore, the capping effort of organic molecules can also be confirmed during the biosynthesis of PdNPs through EDX analysis (Tahir et al. 2016). Identification of palladium, phosphorus, and sulfur within *D. desulfuricans* and *B. benzeovorans* cells was illustrated by EDX (Omajali et al. 2015). Further existence of these particular elements was established by Scanning transmission electron microscopy (STEM) that visibly revealed the entrapped miniature-sized particles within the bacterial cells. The morphological appearance and dispersion of nanopalladium are visualized by SEM and TEM. The internal structural elucidation of the particle, mainly crystallinity and lattice structure, is noted by TEM by observing electrons transmitted from the sample. The surface topography and its organization, depending on its functionality, are determined by SEM. The crystalline structure and size of PdNPs are further investigated through high-resolution TEM (HRTEM) - selected area electron diffraction (SAED). A schematic diagram presenting the factors, properties, and characterization of PdNPs is highlighted in Fig. 4.

Environmental Applications of PdNPs

The rapid expansion of the concentration of the contaminants released into the commercialized world poses a threat to living beings. Hence, the researchers are trying to combat these problems through several modern attempts, like the use of external stabilizing agents, as various biological constituents extracted from floras and microorganisms can act solely as stabilizing as well as capping agents.

Several works have reported that bacteria-attached catalytic nanoparticles may show equivalent or better effective catalysts compared to other conventional catalysts involved in photodegradation procedures (Behera et al. 2019) to remove nitro compounds and organic dyes (Edal et al. 2024). Environmental application of biologically synthesized PdNPs mainly focuses on the environmental clean-up of various contaminants. These have been discussed in the following sub-sections.

Removal of dyes: Enhanced modernization and industrial activity have generated the discharge of dyes that have hazardous effects on the ecological balance of the Earth. Therefore, several efforts are being made to eliminate these dyes. The synthesized biopalladium nanomaterials using exudates of *C. roseus* leaves in broth suspension showed an enhanced photocatalytic role in degrading phenol red dye at an optimized pH of 8.0 (Kalaiselvi et al. 2015). In one of the reports, hollow PdNPs-activated biofilm showed better elimination of azo dyes and methyl orange dye present in wastewater by obstructing the kinetic barrier (Kalathil & Chaudhuri, 2016). Decolorization of dyes such as methyl orange, acid blue, reactive black, and acid red at an approximate range of 70 % is observed by viable *Klebsiella oxytoca* with Pd (Wang et al. 2018). This might be due to its enhanced catalytic property and increased transfer of electrons outside the cell membrane region. The utility of complex Pd/Fe₃O₄-PEI-RG nanohybrids for wastewater treatment by mineralization of several contaminants stimulated through catalysts has enticed the interest of recent researchers. These nanohybrids showed excellent breakdown of methylene blue with a significantly enhanced removal of over 99% through sodium borohydride within the reaction mixture. Simultaneously, the nanoparticle could be detached from the

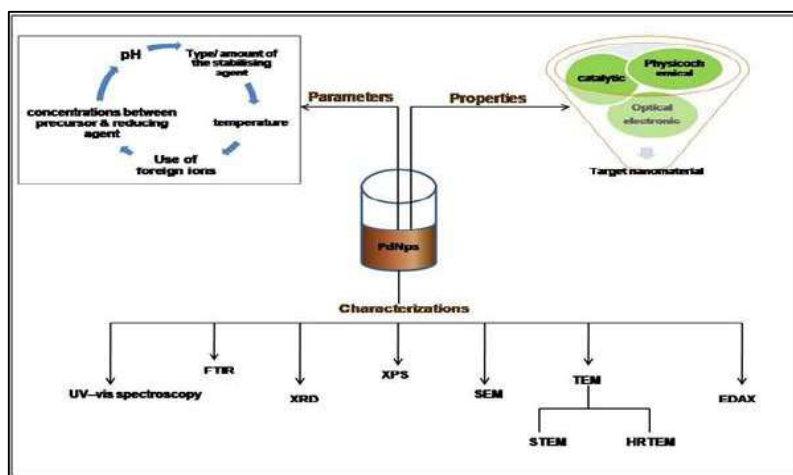


Fig. 4: Properties and instrumental analysis of nano-palladium particles.

synthetic solution in the presence of the magnetic field applied externally. These nanomaterials can be reused after being cycled nine times without any potential effect on their catalytic activity. Therefore, these qualities enabled this catalyst to be an encouraging contestant in the arena of energy and environment (Li et al. 2015).

Removal of nitrophenol: Nitrophenols are one among various contaminants that lead to cancer and must be reduced to aminophenols. Experiments have been conducted by using *Phoenix dactylifera* synthesized PdNPs for the degradation of 4-nitrophenol to 4-aminophenol in the presence of a catalyst within 2 min, thereby forming a 4-nitrophenolate ion intermediate (Tahir et al. 2016). Palladium-loaded hydrogel conjugates (PEI/Pd) reduce 4-nitrophenol to 4-aminophenol in wastewater (Feng et al. 2020). In 2021, Advanced nanoparticle formation core-shell nanomaterial Fe₃O₄@CS-Starch/Pd for mineralization of 4-nitrophenol in the presence of ultrasound waves. After the recovery process, the catalyst was utilized for consecutive repeated cycles (Veisi et al. 2021). Certain nanomaterials are used for dual catalytic application in removing nitrophenol as well as dyes simultaneously. Anand et al. (2016) showed the catalyst-oriented degradation of organic pollutants like para-nitrophenol (PNP) and methylene blue present in industrial effluents by biosynthesized PdNPs from *Moringa oleifera* flower extract. The green technology used for the formulation of PdNPs utilizing *Anogeissus latifolia* (gum ghatti) showed better antioxidant activity even at a minimum quantity. The particle also possessed efficient catalytic properties while reducing several dyes like methylene blue, coomassie brilliant blue G-250, methyl orange, and 4-nitrophenol through sodium borohydride. A single-pot, cost-effective technology was established for palladium nanoparticle materialization using a biocompatible polymer named gum olibanum obtained from *B. serrata*. This bifunctional agent reduces and stabilizes the nanomaterial simultaneously. Spherical-sized nanoparticles ranging from 2.5–8.8 nm were resistant to bacteria with different cell walls, even at high concentrations. They also imparted antioxidant activity. The nanoparticles showed homogeneous catalytic activity by reducing the aforementioned dyes. Therefore, these dyes revealed degradation activity for brilliant dye and pigment (Kora & Rastogi 2016).

Removal of pesticides and heavy metals: Pesticides are one such pollutant that, upon being discharged into the effluent or solid waste, cause harm to human health. Biosynthesized Palladium-sized particles effectively degrade dehalogenated PCBs (Windt et al. 2005), perchlorate and nitrate (Windt et al. 2006), PBDEs (Harrad et al. 2007, Deplanche et al. 2009), trichloroethylene (TCE) (Hennebel et al. 2009), lindane

and chlorobenzenes (Mertens et al. 2007), and diatrizoate (Hennebel et al. 2011), and chlorpyrifos and tebuconazole (Hamid et al. 2024). Chromium Hexavalent ion is a serious contaminant that must be reduced to trivalent chromium so that it can be used for various metabolic activities. Productive formation of ferric oxide conjugated palladium nanobioparticles could eliminate hexavalent chromium to trivalent chromium in the synthetic liquid environment in the presence of formic acid (Kalantari et al. 2021).

Removal of antibiotics: Essential pharmaceutical components like antibiotics are being utilized by patients at around 30 %, and the rest remain deposited in wastewater or sludge due to human excretory activities. Thus, it is important to take the necessary steps to reduce the dispersion and aggregation of relentless antibiotics into the ecosystem (Gavrilescu et al. 2015). Ciprofloxacin is one such antibiotic that induces mutation, as well as cancer, and has been linked to the incidence of hydrogen. The ciprofloxacin and hydrogenated palladium ion conjugates are removed at an approximate range of 88.0%. The presence of hydrogen boosts the sequestration of the pollutant by the conjugate (He et al. 2020). However, a different method was used for its removal in the presence of microbes. *Desulfovibrio vulgaris* synthesized PdNPs because of sulfamethoxazole (Martins et al. 2017).

Challenges

BioPdNPs dominate other processes in terms of cost and scalability, but there may be certain challenges that can be addressed. They are not monodispersed, and the rate of production is not speedy (Prasad et al. 2020). They may show irregular, uniform surfaces (Matsena & Chirwa 2021). Crucial parameters, viz., localization and morphology of nanoparticles, depend on microbial species. Biomolecules are involved in the process; hence, nanomaterials may be of different sizes (Filice et al. 2021). It may harm seed germination and accumulate in living organisms of the environment (Xiaodi et al. 2024). With the increase in the use of PdNPs for controlling automobile exhaust pollutants, an imbalance in biogeochemical cycles may occur. So, risk analysis of palladium is crucial to regulate its utility as a catalytic converter to obtain a sustainable Earth (Aarzo & Nidhi 2022).

Recovery of Palladium Towards the Achievement of Circular Economy

Economic upliftment requires commercialization that consequents in pollution of the ecological system because of the disposal of toxic metals. Hence, technologies must be there to recover, recycle, and reuse them, especially certain

heavy metals, viz. Au, Ag, Pd, etc., are precious in terms of cost and various applications such as catalyst hydrogenation, oxidation, dichlorination, gas sensing, and hydrogen storage (Klinkova et al. 2017). Moreover, eco-friendly processes must be applied to recover a high rate (~99.0 %) and purity of palladium through the union of copper capture and electrodeposition technique in the presence of 0.5 molar nitric acid, which had no adverse effect on the environment (Liu et al. 2020). Speedy, cheap, and eco-sustainable methods are to be involved in the recycling and recovery of the PdNPs so that they can be reused. For example, the pulsed laser ablation process is a powerful and energetic process that can isolate nanoparticles within 30 min of its irradiation (Lee et al. 2021). Recently, microbes have been used in the formulation of nanoparticles. Although heavy metal hazards affect microbial life, they can still be combated by diffusing the toxicity of metals through their actions at the gene and protein levels (Veisi et al. 2016, Patel et al. 2021, Law et al. 2022). Economically profitable and sustainable technologies involving microbes are required for their zero negative impact on the environment. One such microbe applied was *Desulfobibrio desulfuricans* for palladium recovery from aqueous solution and electronic wastes (Creamer et al. 2006). *B. thuringiensis* Y9 could reduce Pd^{2+} at 93 mg/g and 60 mg/g efficiency in the absence and presence of O_2 to PdNPs at around 99.18 % recovery (Chen et al. 2022). *E. coli* could modify waste Pd-based catalysts to recover approximately 99% of PdNPs. They may carry out Cr(VI) removal at around five recycles (Yu et al. 2023). However, work on recycling and recovery of PdNPs is still at its naïve; hence, research must be conducted on it. Regulatory and ethical issues during the recovery of nanoparticles and their application in biomedicine are mainly concerned with the health risk to workers handling them. To date, the main scientific explanation regarding environmental application remains unknown.

CONCLUSION AND FUTURE TARGETS

In recent years, nanotechnology has developed immensely because of its wide implications in various fields of science and technology. Alternative biological methods, compared to the physico-chemical approach, have several advantages, like being eco-friendly, simple, and lucrative. Thus, the sustainable production of these nanoscale components utilizing biotic assets has immense prospects, like stable, controlled size and shape nanomaterials. In the present review, we have delivered an overview of the biological production of PdNPs by employing numerous microbes as well as chemical extracts derived from unicellular and multicellular plants. The intrusion of biogenic formation of PdNPs over physicochemical methods

is preferable as it's ecologically benign, less charged, along uncomplicated character. In addition, various biological products like exopolysaccharides may be useful together as reducers and stabilizers for palladium nanoparticle formulation in the single-pot method.

The biological approach for nanopalladium material formation encompasses a widespread choice of sources like plants, bacteria, and actinomycetes. Reports are still rare on the implications of yeast and fungi regarding the biological production of PdNPs. Nowadays, the exploration of the formation of bioPdNPs is still in the innovative phase. Thus, as a prospect, (i) researchers should try to find out the answer to some questions that are still unanswered. (ii) Investigations are further required in the future to find out the optimized factors to apprehend the influence of varied process parameters during the development of PdNPs. (iii) Continuous attempts should be made to emphasize the mode of PdNPs synthesis and the effect of its structural properties for its diverse applications. Moreover, an inspection of former reports suggests the synthesis of palladium nanoparticles on a small scale. To date, no single report has focused on the large-scale production of PdNPs exhausting biological sources. Therefore, (iv) developing palladium nanoparticles by exploring biological sources at an industrial scale can be of great interest to researchers. Thus, it can be considered the best platform for researchers to propose a safer scheme for developing nanoparticles for better perception and knowledge towards green and sustainable approaches.

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Heat Index Effect on the Fecundity and Growth of *Decapterus macrosoma* in the Selected Fishing Waters of Caraga Region, Philippines

Julie S. Berame^{1†}, Minie L. Bulay¹, Leah R. Cruzado², Glenn Hayahay³ and Romar Pait⁴

¹Caraga State University, Butuan City, Philippines

²Agusan National High School, Butuan City, Philippines

³Sangay School of Living Tradition, Surigao del Sur, Philippines

⁴Agusan del Sur College, Agusan del Sur, Philippines

†Corresponding Author: Julie S. Berame; jberame386@gmail.com

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ABSTRACT

The fecundity of fish can be utilized to predict the fish catch in the future becoming to be the most vulnerable to the negative impact of global warming. This study aimed to determine the effect of heat index on the growth and fecundity of *D. macrosoma* "Budloy" from the selected locations of marine waters in the Caraga region. The study was conducted at Buenavista, Agusan del Norte, Placer Surigao del Norte, and Tandag Surigao del Sur, where Shortfin scad is common. This study used Stratification random sampling to collect the samples. To calculate the present heat index of the sampling areas, present temperature, and humidity during the capture of fish samples were determined. To determine the fecundity, six parameters of *D. macrosoma* were calculated: length, body weight, total weight of ovary, weight of ovary samples, number of eggs, and maturity stage of *D. macrosoma* ovaries. In terms of the correlational analysis between heat index and length of *D. macrosoma*, results showed that there is a moderate positive correlation, with $r = .55$, $p = .000$, for heat index and weight, results were found to have a strong positive correlation, with $r = .71$, $p = .000$. Based on the findings on the correlation between heat index and fecundity, with $r = .007$, $p = .959$, results indicate that although there is a positive correlation between heat index and growth, there is no significant relationship between heat index and fecundity. Based on the computed values of the heat index, growth, and fecundity of *D. macrosoma* samples in all sites, it is concluded that Buenavista waters have the highest numerical value of the reproductive potential of *D. macrosoma* as the basis for predicting the fish catch in the future.

INTRODUCTION

The fecundity of fish can be utilized to predict the fish catch in the future. As global warming is likely to be the greatest threat in the 21st century (Solar Impulse Foundation 2022), the fecundity of fish is also becoming the most vulnerable to the negative impact of global warming. Earth has entered a new era of rapid and potentially irreversible climate warming triggered by greenhouse gas emissions caused by human activities (Albouy et al. 2020). They also stated that oceans have taken up 93% of the extra energy (IPCC 2019) That has been accumulated in the Earth system in recent decades, and its temperature has increased much faster since 1991 than has been recorded previously (Cheng et al. 2019, Albouy et al. 2020).

Climate change brought changes in the oceanic environment and is predicted to have a large socioeconomic impact through its effects on commercial fisheries (Barange et al. 2014, King et al. 2015, Ijima et al. 2019). These temperature changes were found to have negative impacts on marine biota, including negative impacts on the fecundity of fish. Little is known about the effects of heat index on the growth and fecundity of *Decapterus macrosoma* (Albouy et al. 2020).

The world's marine ecosystems are continuously experiencing aquatic biological change at an unprecedented rate. Climate change, pollution, overfishing, invasive species, habitat loss and degradation, and over-exploitation are all contributing to the prevailing threats in aquatic ecosystems (Arthington et al. 2016). The intensifying resource of marine fish faces a multitude of threats that are ultimately driven by the increasing human population (Harding et al. 2018), projected to reach 9.7 billion human population in the year 2050 (UN-DESA 2015). The increase in the human population resulted in overfishing. In the study of Asni & Tauhid (2019), overfishing was observed wherein the maximum sustainable yield (MSY) of shortfin scads fish in Bulukumba waters in 2016-2017 exceeded the maximum sustainable production (MSY) of 10,739 tons. year⁻¹. According to Ye and Gutierrez (2017), one-third of the world's fish stocks are currently classified as overfished. Anthropogenic climate change has the potential to alter human and natural systems conditions such that the onset of unprecedented climatic extremes will outpace evolutionary and adaptive capabilities (Tan et al. 2018). Fish is more valuable than a diamond (Sumaila 2021). This directly supported the idea that fish and fisheries directly impact millions of people around the globe by feeding (Hicks et al. 2019) and providing livelihoods (Sumaila 2021).

According to Gola et al. (2021), recent studies showed that the human population increase resulted in serious issues of marine litter. Human-generated wastes like plastics are broken down into smaller pieces and are continuously accumulated in the marine environment in the form of microplastics. Microplastics are generally composed of polyvinyl chloride (PVC), polyethylene terephthalate (PET), polystyrene (PS) and nylon, etc. Microplastics (MPs) smaller than 5 mm have become an emerging global concern (Phuong 2022). In the study by Wootton et al. (2021), it was found that 49% of all fish sampled globally for microplastic ingestion had plastic (an average of 3.5 pieces per fish), with fish from North America ingesting more plastic than fish from other regions. The concentration of these microplastics is increasing at an unprecedented, alarming rate, which not only affects the marine environment but directly affects marine life Gola et al. (2021). The need for consistent guidelines in methods used to evaluate microplastic in fish to ensure data are unambiguous, comparable, and can be widely used to support mitigation and management strategies in marine life conservation Wootton et al. (2021). According to Zhang & Feng (2019), microplastics potentially contribute to climate warming. This indicates that the presence of microplastics in marine environments will contribute to

In tropical regions, the reproduction-related physiological activities of fish are greatly affected by the environmental

temperature. As such, tropical fish are particularly sensitive to climate change since they develop in a relatively stable thermal marine environment (Brule et al. 2022). Climate change-amplified temperature anomalies resulted in alarming threats to coral reef ecosystems. According to Magel et al. (2020), "while much focus has been placed on the effects of heat stress on scleractinian corals-including bleaching, mortality, and loss of reef structural complexity-and many studies have documented changes to reef fish communities arising indirectly from shifts in benthic composition, the direct impacts of heat stress on reef fish are much less well understood".

According to PSA & Lamarca (2017), the Philippines is an archipelago that is made up of 7,641 islands which made the country blessed with rich marine fishing grounds. The fishing industry is of great contribution to the country as it provides employment to over 1.6 million Filipinos. In 2017, the Philippines had a population of about 103 million, and the mean per capita consumption of fish and fishery products of 40 kg per year or 109 grams per day, with a total intake of fish and fishery products at 12.8%. They also highlighted that the increase in the Philippine population and the occurrence of improved fishing technology brought stress to the country's marine and coastal ecosystem, thereby greatly affecting fishery resources. The data of (the PSA) Philippine Statistics Authority showed that there is a decrease in the volume of fisheries production by sector in the Philippines, which was 941,870.86 metric tons in 2018, 968,758.60 metric tons in 2019, and 952,188.62 metric tons in 2020 (PSA 2021). In the study of Joson et al. (2019) on the *Sardinella lemuru* (Bleeker 1853) species from Balayan Bay, Batangas, they stated that as the human population steadily increased, the demand for the fish also increased, such that the fish population may be threatened with overexploitation, and fecundity of fish is also threatened. Another pressing threat to the fishery sector is climate change. Boyce et al. (2022) stated that climate change is impacting virtually all marine life.

Uncontrolled fishing efforts, along with economic growth and the threat of climate change (Boyce et al. 2022), will result in the decline of capture fisheries and productivity (Asni & Tauhid, 2019). However, there is no control instrument for the exploitation of shortfin scads (*Decapterus macrosoma*) fish until now. Hence, there is a concern that the sustainability of shortfin scad fish resources will be threatened (Asni & Tauhid 2019).

The *Decapterus macrosoma* (Shortfin scad) Budloy is a schooling species that typically occurs in open waters (Magallanes et al. (2022). This fishery serves as a source of income for the coastal community in some parts of the world and is also found in the Philippines (Jiménez Prado

& Béarez 2020). It is commonly distributed in tropical waters such as the Indo-Pacific, East Africa, Red Sea, Eastern Pacific, Galapagos Islands, and Peru (Smith-Vaniz & Williams 2016). In the Philippines, *D. macrosoma* is one of the popular commercial species caught in many parts of the country (Magallanes et al. 2022, Sangalang & Quinay, 2015). According to Jiménez Prado and Béarez (2020), *Decapterus macrosoma* was a cheap source of protein for Filipinos, and its low price made this known as a poor man's fish. However, overexploitation is threatening the natural stock of *Decapterus macrosoma* due to increased fishing pressure brought about by increasing demand resulting from the continuous increase of the human population (Jiménez Prado & Béarez 2020). According to Rinaldi et al. (2017), to predict the fish catch in the future, a fecundity study should be conducted to know the reproductive potential of fish. Rinaldi et al. (2017) studied the fecundity of *Sardinella fimbriata* in Alas Strait, and Kudale & Rathod (2016) studied the fecundity of *Sardinella fimbriata* from Karwar waters. The result showed that the value of $R^2=0.984$ is closer to one; thus, there is a highly positive relationship between fecundity and the standard length of *S. fimbriata* (Kudale & Rathod 2016). The study of Gonzales et al. (2021) investigates the spawning characteristics vis-a-vis fisheries management of *D. macrosoma* in the waters of Romblon Province; however, no studies have been conducted on the growth parameters and fecundity of *D. macrosoma*. To address the issue, the study was conducted to help predict the Shortfin scad (Budloy) future fish catch in the three water areas of Buenavista, Agusan del Norte, Placer, Surigao del Norte, and Tandag, Surigao del Sur.

Therefore, there is a need to generate in-depth knowledge on the determination of the effect of heat index on the growth and fecundity of *D. macrosoma* (Shortfin scad) "Budloy" from the marine waters in Buenavista, Agusan del Norte, Placer, Surigao del Norte, and Tandag, Surigao del Sur, Caraga. Also, the findings will help to predict the fish catch from the selected sampling stations.

MATERIALS AND METHODS

Research Design

This study used a quantitative experimental research design with a test configuration performed in a laboratory. Before, during, and after the test experiments, comparative assessments, study guides, and related articles were considered to ensure that it is clear and cut evidence to provide adequate results that could be based on by future researchers who would follow suit in the same or related research. The collected data were analyzed using correlation

measures to determine the heat index, growth, and fecundity of *D. macrosoma* relationship in various sampling areas.

Research Locale

The study sites were conducted in three different coastal areas in the Caraga region, particularly on the selected waters of Agusan del Norte, Surigao del Norte, and Surigao del Sur. *D. macrosoma* fish species were commonly found and selected on purpose by the researchers in each area respectively at Buenavista, Agusan del Norte, Placer, Surigao del Norte, and Tandag, Surigao del Sur. These sampling areas were considered market fishing grounds areas due to the large volume of fish caught in Caraga Region, Philippines where three sampling areas were located.

Sampling Technique

During the wet season (November-December), when the (Shortfin scad) *D. macrosoma* are abundant (Magallanes et al. 2022), specifically around 5:00 dawn on November 26, 2022, fresh *D. macrosoma* fish samples were collected. Newly caught *D. macrosoma* fish samples were bought from local fishermen from the three sampling sites along the seashores of Buenavista, Agusan del Norte, Placer, Surigao del Norte, and Tandag, Surigao del Sur, where local fishermen landed their newly caught fish. The ten kilos samples of fish in every station were placed in separate containers. The air temperature and relative humidity of the area during the collection of samples from local fishermen were determined and recorded using the alcohol laboratory thermometer. Examination, weighing, and measuring of the growth and maturity stages of the ovary, and counting of eggs were conducted at the Biology Laboratory of Agusan National High School-Senior High School Building, Butuan City. The estimation of fecundity and mature and ripe ovaries of *D. macrosoma* were examined using a light microscope.

Data Gathering Procedure

The researchers requested permission to collect *D. macrosoma* fish species and conduct fecundity tests through sending a request letter to the City Agriculture, and Bureau of Fisheries and Aquatic Resources office. The air temperature and relative humidity of the area were determined and recorded using the alcohol laboratory thermometer. The present heat index was calculated using the meteorological heat index calculator of the National Weather Services of the National Oceanic and Atmospheric Administration. Dissection of fish samples for ovary extraction was carried out in the laboratory of Agusan National High School-Senior High School. Examination, weighing, and measuring of the growth and maturity stages of the ovary and counting of eggs were conducted at the Biology

Laboratory, Agusan National High School, Senior High School Building, Butuan City. For the estimation of fecundity, mature and ripe ovaries of *D. macrosoma* were examined using a compound microscope. The numerical data for heat index, length, and weight of the fish samples were then used to obtain the fecundity in each sampling area.

Growth of *D. macrosoma*

In the laboratory, the individual lengths of fish samples in three sampling areas were independently taken to the nearest centimeter in a straight line via a ruler following the TL method - from the anterior tip of the longest jaw to the most posterior part of the caudal fin to the nearest centimeter scale respectively (Laevastu et al. 1965). The length is measured from the most forward point of the head, with the mouth closed to the farthest tip of the tail compressed while the fish is lying on its side. The weight of the specimens was taken by an electric balance and the weights were recorded to the nearest gram.

Fecundity of *D. macrosoma*

Fecundity has been considered as the number of ripening eggs in the female fish before spawning (Bagenal & Braum 1978). The ovary of the individual fish was taken out carefully and placed in an individual container. The gravimetric method or weight method (Lagler & Vallentyne 1956) was used for the estimation of the fecundity of *D. macrosoma*. The gravimetric method offers the best possibility of minimizing error due to its simple and easy sampling techniques. The Gravimetric method has been used for its greater efficacy than the other methods. The Gravimetric or weight method has been successfully used by Doha & Hye (1970), Boonkusol et al. (2020), and Limbu et al. (2021). The gravimetric method was done in the following way: before estimation of the fecundity of the fish species under study, ovaries were cut into sections from where pieces were removed and weighed in the nearest gram. This value was proportional to the total ovary weight, and the number of eggs (F1), and the mean (Fe) of sub-sample fecundities (F1, F2, F3, ...), the individual fecundity for each female fish were calculated by the following equations:

$$\text{Fecundity (Fe)} = \frac{\text{F1} + \text{F2} + \text{F3} + \dots}{\text{Total Number of Samples}}$$

$$\text{Fecundity (F1)} = \frac{\text{No. of eggs in sub sample}}{\text{No. of eggs in sub sample}} \times \text{Gonad weight}$$

Present Heat Index

The present heat index of the selected locations was calculated using the meteorological heat index calculator

of the National Weather Services of the National Oceanic and Atmospheric Administration. Descriptive analysis was utilized to compare the present heat index of the three selected locations in Caraga.

Heat Index and Growth

Analysis of correlation was used to identify if there is a significant relationship between the heat index and growth of *Decapterus macrosoma* within the waters of Buenavista, Placer, and Tandag.

Heat Index and Fecundity

Analysis of correlation was utilized to identify if there is a significant relationship between the present heat index and fecundity of *Decapterus macrosoma* within the waters of Buenavista, Placer, and Tandag.

Reproductive Potential of *D. macrosoma*

Correlational and descriptive analyses were utilized to determine the reproductive potential of *Decapterus macrosoma* as the basis for predicting the fish catch in the future along the waters of Buenavista, Placer, and Tandag, Caraga Region, Philippines.

RESULTS AND DISCUSSION

Length and Weight of Fish

The individual length and weight measurement of fish is extremely important in aquaculture, both production systems and breeding programs (Fernandes et al. 2020). Most of the current methods are based on manual measurements.

Table 1 shows the average length and weight in each sampling area, which includes the length of fish in millimeters and the weight of fish in grams. According to Dash et al. (2019), length-weight relationships are used to derive the 'condition factor', and an index is often utilized to describe the 'well-being' of fish if the heavier fishes belonging to a particular length class are in better condition than the lighter counterparts.

Further, Sani et al. (2010) stated that the length-weight relationship (LWR) is essential for fisheries management and conservation in gathering crucial information on biomass

Table 1: Average Length (mm) and Weight (g) of (Shortfin scad) *Decapterus macrosoma* in Selected Waters of Caraga.

Location	Length	Weight (g)
Buenavista	289.300	262.019
Placer	187.200	187.856
Tandag	280.700	399.938

for any stock by directly collecting data on fish weight is quite complicated, time-consuming and expensive, but such information can be easily derived without any hassle by just converting the ubiquitously available length-based data.

In the study of Famoofo & Abdul (2020) on the biometry and length-weight relationships of sixteen fish species in Lekki Lagoon, the researchers explained that length-weight data are often used to study the indication of fatness or general well-being of fish. However, the small sample size may lead to an overestimation of the length-weight relationship of fish.

In the study of Afdhila et al. (2019) on the length-weight relationship and condition factors of *D. macrosoma*, their results showed *D. macrosoma* had a negative allometric growth pattern with a value of $b = 2.61$. The value of 103,46 from the relative weight conditions factor indicates a stable water condition, and the value of 3,00 from the factor of Fulton's conditions indicates it allows the availability of abundant food that supports the life of *Decapterus macrosoma* fish well. Further, based on related studies that also conducted weight and length of fish, their findings showed that weight and length relationships were significantly interrelated ($R^2 > 0.942$) (Kara & Acarli 2020).

In this study, the results of the length-weight data were obtained from the research conducted on seventy-nine fish samples from the waters of Buenavista, Agusan del Norte, Placer, Surigao del Norte, and Tandag, Surigao del Sur. Table 1 shows that the fish sample in Buenavista has the highest average length of fish samples, which is 289.300 mm, while Tandag has the highest average weight of 399.938 grams. Thus, based on the results of the descriptive analyses of data, it shows that the increase in length of *D. macrosoma* does not follow an increase in weight. This is supported by the study of Famoofo & Abdul (2020), wherein they explained that the length-weight relationship is greatly affected by many factors related to pollution, population variability, sampling season, availability of food, feeding intensity, fish size, age, sex, stage of maturation, and estimation methods. Hence, the growth of *D. macrosoma* in terms of length and weight may vary and does not follow a length-weight pattern.

Fecundity is defined as the total number of eggs present in the ovary of a fish. This also refers to the ability of an individual or group of fish to generate a certain number of offspring for a certain amount of time.

Number of Eggs

Table 2 shows the average number of eggs in the ovary sample of *Decapterus macrosoma* (Budloy). From the three selected waters in Caraga, the ovaries of forty-five females of *Decapterus macrosoma* were used. Matured eggs were

Table 2: Average number of eggs of ovary sample of (Shortfin scad) *Decapterus macrosoma* in Selected Waters of Caraga.

Location	Average No. of Eggs
Buenavista	1098
Placer	625
Tandag	739

counted manually which was taken from an ovary sample of individual fish. In this study, the Batch Fecundity technique by De Vlaming (1983) was utilized to determine the fecundity of fish samples. Batch Fecundity refers to the number of eggs spawned per batch, and the sum of batch fecundities represents the realized annual fecundity and estimated fecundity from field measurements (i.e., total length (TL), relative weight [Wr], or ovary weight) were determined to estimate reproductive potential (Lenaerts et al. 2021).

Based on the result of the descriptive analysis of the average number of eggs of the ovary samples of *Decapterus macrosoma* (Budloy) in selected waters of Caraga, among the three selected water sampling areas, the most abundant average fish eggs area was Buenavista, Agusan del Norte with one thousand nine hundred-eight (1098) average count, while Placer, Surigao del Norte has the least number of eggs with six hundred twenty-five (625) average count, and Tandag, Surigao del Sur has seven hundred thirty-nine (739) average count of eggs. The result showed a wide range of differences in terms of values of the number of eggs in the three selected water areas. This result can be explained using the study of Servali et al. (2020), variations in temperature and photoperiod are known to strongly affect the timing and the spawning period in several fish species. According to Servali et al. (2020), the temperature, changes in salinity, and water acidification are also directly associated with the reduction of sperm quality and reproductive output. Thus, the average number of eggs of ovary samples of *Decapterus macrosoma* (Budloy) in the selected waters of Caraga were varied due to the differences in some environmental factors, such as temperature, and salinity of the sampling areas.

Maturity Stage of Eggs

From the waters of Buenavista, Placer, and Tandag, matured ovaries of forty-five female *Decapterus macrosoma* were used. Table 3 shows the average maturity stage of eggs of *Decapterus macrosoma* in the three selected sampling areas. Fifteen (15) female fish ovaries were observed to be in the maturity stage III-IV in the waters of Buenavista, Agusan del Norte, and thirty (30) female fish ovaries are in the maturity stage I-II in Placer, Surigao del Norte and Tandag, Surigao del Sur waters.

Based on the observation of individual ovary samples of morphology and histology of *D. macrosoma* in the selected

Table 3: Average Maturity Stage of Eggs of (Shortfin scad) *Decapterus macrosoma* in Selected Waters of Caraga.

Location	Maturity Stage of Eggs	Number of Female Samples
Buenavista	III-IV	15
Placer	I-II	20
Tandag	I-II	10

Caraga waters, the proportion of the maturity stage of ovaries dominated by maturity stage I-II that was observed in Placer, Surigao del Norte and Tandag, Surigao del Sur *D. macrosoma* samples, while the maturity stage of ovaries III-IV was less found which was only in the waters of Buenavista.

Fig. 1: (a-d) Ovaries of *D. macrosoma* at different stages of maturation (a) Stage I: Immature (Placer, Surigao del Norte), (b) Stage II: Early Maturation (Tandag, Surigao del Sur), (c) Stage III: Later Maturation (Buenavista, Agusan del Norte), (d) Ripe and running (Buenavista, Agusan del Norte), (O) Oogonia, (PO) Primary oocyte, (PVO) Primary vitellogenic oocyte, (SVO) Secondary vitellogenic oocyte Based on the external characteristics of the ovary and on the internal organization of oocytes, it was possible to classify the ovary of bigeye tuna into four states of maturity, which are described below.

Shown in Fig. 1 are the different stages of ovary maturation of *Decapterus macrosoma*. Stage I-Immature: The ovaries filled up with oogonia and oocytes in the peri nucleolar stage. This kind of ovary was found in females with the fork length ranging from 62.0 to 150.0 cm (average of 112.6 cm). Stage II-Early Maturation: The ovaries were bigger than in the former stage, and showed oocytes at

the early lipidic and vitellogenesis stage. Stage III-Later Maturation: Pre-hydrate and hydrated oocytes were present, and some oogonia and peri nucleolar oocyte stages were still present in the ovary. Stage IV-Ripe and Running: The ovary showed oocytes at different stages, with most of them being at oogonia and peri nucleolar stages.

In the study of Rinaldi et al. (2017), among the 750 *S. fimbriata* catch, the total of gonad stage IV was found in 28 fishes, and the total stage V was found in 41 fishes. Based on the results, there is a high difference in the average number of eggs observed.

In this study, the descriptive analysis result on the average maturity stage of eggs showed that many of the samples were in maturity stages I-II. According to Rilani et al. (2017), the maturity of ovaries indicates the spawning potential of fish. Also, the average maturity stage of eggs can be supported by the conclusion of Sulistiono et al. (2011) that variations in the maturity of ovaries are caused by the changes in the annual rainy season, the condition of fish, and geographical location.

Ratio of Male and Female Fish

Table 4 shows the ratio of male and female fish samples from the three selected waters in Caraga. The *Decapterus macrosoma* samples consist of eighteen (18) from the waters of Buenavista, Agusan del Norte, thirty-one (31) from Placer, Surigao del Norte, and thirty (30) from Tandag, Agusan del Sur. From the waters of Buenavista, the male and female ratio is 3:15, Placer fish sample ratio is 11:20, and Tandag is 20:10. Data shows that Buenavista and Placer fish sample ratio showed a higher rate in female fish and a lower female

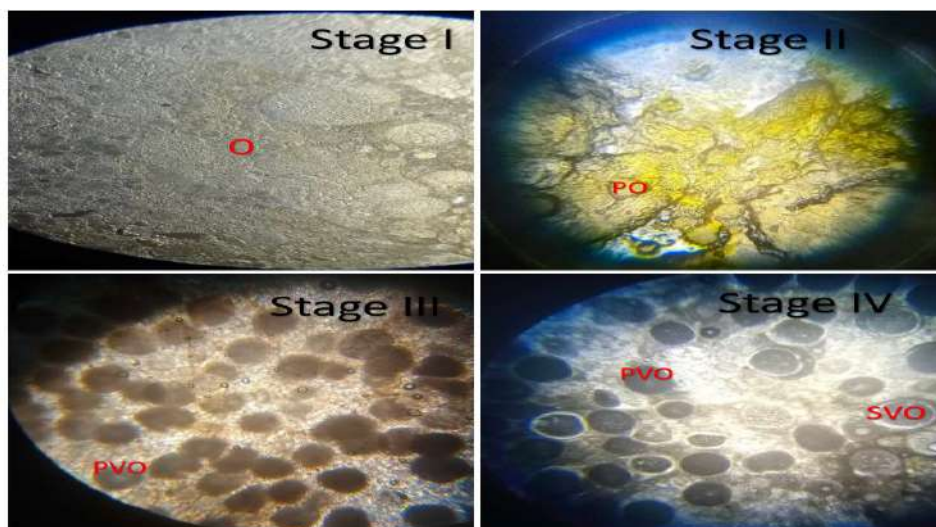


Fig. 1: Ovary Histology of (Shortfin scad) *Decapterus macrosoma*.

Table 4: Average Maturity Stage of Eggs and Ratio of (Shortfin scad) *Decapterus macrosoma* in Selected Waters of Caraga.

Location	Ratio	
	Male	Female
Buenavista	3	15
Placer	11	20
Tandag	20	10

rate in Tandag waters. The highest number of *D. macrosoma* male samples is twenty (20) from Tandag, Surigao del Sur, eleven (11) males from Placer, Surigao del Norte, and three (3) male fish samples from Buenavista, Agusan del Norte.

In the study of Geffroy & Wedeking (2020), a total of 155 fish (96 females and 59 males) were collected during the wet and dry seasons. The female-to-male ratios of *S. pleugostigma* were significantly different from the ratio of 1:1 within the dry (21 females and 12 males) and the wet seasons (75 females and 45 males).

In this study, the observed male and female *D. macrosoma* ratio can be supported by the study of Geffroy & Wedeking (2020). In fishes, sex is determined by genetics, the environment, or an interaction of both. Geffroy & Wedeking (2020) stated that “temperature is among the most important environmental factors that can affect sex determination”. Consequently, changes in temperature at critical developmental stages of fish can induce biases in primary sex ratios. “However, early sex ratios can also be biased by sex-specific tolerances to environmental stresses that may, in some cases, be amplified by changes in water temperature” (Geffroy & Wedeking 2020).

Further, this is also supported in the study of Edmands (2021), which stated that the rising global temperatures pose a threat to population sex ratios, which, particularly when ratios are male-biased, can lead to mate shortages, lower population growth, and adaptive potential, and raise extinction risk. Moreover, according to Sass et al. (2022), “sex differentiation may often be dictated by genetics, environmental conditions, and population density during critical embryonic development periods”.

The heat index is an index that combines air temperature and relative humidity during the actual collection of fish samples from the local fishermen at 5:00 at dawn in the three sampling areas. Heat index changes at a constant temperature with increasing air moisture. Table 5 shows the heat index in the selected waters in Caraga. Tandag has the highest heat index of 39°C, and the lowest heat index value is 27°C in Placer. According to Opitz & Rhoten (2016), the heat index is increasing worldwide due to land-use development and climate change. This explains why urban temperatures may be up to 10°C warmer than the surrounding suburban areas.

Table 5: Calculated Heat Index in the Selected Sampling Areas in Caraga.

Location	Heat Index (°C)
Buenavista	29
Placer	27
Tandag	39

Correlational analysis was utilized to determine the significant relationship between the heat index and the growth of *D. macrosoma* in terms of length and weight. The correlational analysis is a statistical tool to test if two variables have any relationship, whereas the p-value tells us if the result of an experiment is statistically significant. A significant relationship is determined through correlational analysis. It is a statistical method used to measure the strength of the linear relationship and association of variables. In this study, the present heat index of the three selected water areas was calculated using the meteorological heat index calculator of the National Weather Services of the National Oceanic and Atmospheric Administration, and the growth of *D. macrosoma* was determined through the length and weight of fish samples.

Temperature changes, specifically climate change, affect marine ecosystems worldwide, and there have been measurable and increasing consequences to fish populations. As such, affecting the individual physiology of fish, including growth and population dynamics (Cheung et al. 2013).

Table 6 shows the result of the correlational analysis between the heat index and growth in terms of length and weight of *Decapterus macrosoma*. The Pearson Correlation Coefficient was used to answer the hypothesis that there is a significant relationship between the present heat index and the growth of *Decapterus macrosoma* within the waters of Buenavista, Placer, and Tandag, which is tested on a 0.05 significant difference. Heat index and length of *D. macrosoma* samples in the three selected waters of Caraga were found to have a moderate positive correlation, $r = .55$, $p = .000$. The result indicates that there is a significant relationship between the present heat index and growth in length of *Decapterus macrosoma* within the waters of Buenavista, Placer, and Tandag. Heat index and weight of *D. macrosoma* samples in the three selected waters of Caraga were found to have a strong positive correlation, $r = .71$, $p = .000$. The result indicates that there is a significant

Table 6: Correlational analysis between the heat index and the growth of (Shortfin scad) *Decapterus Macrosoma* (Tested at 0.05 Level of Significance).

Factor	r-value	p-value	Remark
Length (mm)	0.546	0.00	Reject Ho
Weight (g)	0.708	0.00	Reject Ho

relationship between the present heat index and growth in weight of *Decapterus macrosoma* within the waters of Buenavista, Placer, and Tandag. The results indicate that there is a tendency for high heat index values to go with high length values and vice versa, and there is a tendency for high heat index values to go with high weight values and vice versa.

In this study, the significant relationship between the present heat index and the growth of *Decapterus macrosoma* is supported by the study of Laven et al. (2022), that temperature showed a significant relationship with the body size of fish. The larger-bodied species of fish may experience the strongest temperature-size responses. They also found out that temperature was more important than dissolved oxygen concentration in determining the maximum body length of fish.

According to Laven et al. (2022), the temperature-dependent characteristic is driving a reduction in the maximum body length of fish in warmer temperatures. In the study of Servali et al. (2020), the highest mean body depth (BD) was observed in *Dagetichthys lakdoensis* (0.14 ± 6.0 cm) and automatically had the highest mean body girth (BG) value (0.55 ± 13.0 cm) of the fishes. The least mean body depth value was observed in *Pellonula afzeliusi* (0.20 ± 2.5 cm) with (0.51 ± 5.7 cm) mean body girth value as the fish species with the least body girth value.

According to Servali et al. (2020), the varying temperature greatly influences the fish size, age, and degree of muscular development. Further, the availability of food, the life history of fish, and environmental conditions also influence the development of fish. However, in the study of Denderen et al. (2020), they concluded that higher temperatures increase the metabolic rate of ectothermic organisms up to a certain level and make them grow faster. Hence, the growth of fish is sensitive to temperature, which is frequently used to predict the long-term effects of climate warming on ectotherms. They further concluded that growth also depends on ecological factors and evolutionary adaptation.

The result of this study is also supported by the Temperature-Size Rule (TSR), which describes how ectothermic species grow faster and mature at a smaller size when reared at warmer temperatures (Atkinson 1994). The result can also be explained based on the findings of Ljungström et al. (2020), that “spatial and temporal variation in temperature is the generally invoked driver but food abundance and quality are also emphasized”. This can also be explained in the study of Ikpewe et al. (2022), which stated that “the increasing sea temperatures are predicted to decrease body size of marine ectotherms based on the temperature size rule”. This will impact

fisheries yields, but empirical evidence of the process is still limited.

Table 7 shows the result of the correlational analysis between the heat index and fecundity of *Decapterus macrosoma*. The Pearson Correlation is used to answer the hypothesis if there is a significant relationship between the present heat index and fecundity of *Decapterus macrosoma* within the waters of Buenavista, Placer, and Tandag, which is tested on 0.05 significant difference. Heat index and fecundity of *D. macrosoma* samples in the three selected waters of Caraga were found to not correlate, $r = .007$, $p = .96$. The result indicates that there is no significant relationship between the present heat index and fecundity of *Decapterus macrosoma* within the waters of Buenavista, Placer, and Tandag. The results indicate that although technically there is a positive correlation, the relationship between heat index and fecundity is weak. The nearer the value is to zero, the weaker the relationship.

The result is supported by the study of Žák & Reichard (2020), that female fish exposed to fluctuating temperatures effectively compensated egg production for their smaller size. They concluded that there was no difference in absolute fecundity between thermal regimes and body size corrected fecundity was higher in females in fluctuating temperatures. This indicates that the heat index has no direct effect on the fecundity of fish. Also, the findings of Žák & Reichard (2020) suggest that the expression of life history traits such as fecundity and their associations under stable temperatures are a poor representation of the relationships obtained from ecologically relevant thermal fluctuations. This can be further explained in the study of Dillon et al. (2016) that “temperature, light intensity, and food availability fluctuate in predictable daily and seasonal cycles and organisms adapt their endogenous biorhythms to optimize performance”. This indicates that the numerical value of the fecundity of fish is unstable due to daily and seasonal fluctuations of the temperature as well as the heat index. The result of the study can be further explained by Laven et al. (2020), that the fecundity or reproductive output of fish depends on the interactions of population abundance, size structure, and reproductive condition and varies geographically and from year to year.

Table 8 shows the result of the descriptive analysis of the fecundity of *Decapterus macrosoma* in Buenavista, Placer, and Tandag. The data shows that Placer has the highest

Table 7: Correlational Analysis between the Heat Index and the Fecundity of (Shortfin scad) *Decapterus Macrosoma* (Tested at 0.05 Level of Significance).

Factor	r-value	p-value	Remark
Fecundity	0.0077	0.959	Accept Ho

percentage of fecundity in the cumulative percent, which is 44.4%. This indicates that among the three selected locations of the study, Placer has the highest reproductive potential of *Decapterus macrosoma* as the basis for predicting fish catch in the future. Tandag has the lowest percentage of fecundity in the cumulative percent, which is 22.2%, while Buenavista fish samples have 33.3%. This indicates that among the three selected locations of the study, Tandag has the highest reproductive potential of *Decapterus macrosoma*.

In the study of Ali et al. (2020) on the reproductive potential of fish, the potential fecundity of fish was determined through the fecundity-weight correlation of fish. The results of their study showed that the absolute fecundity of the fish varied from 3559 to 15712 ova, while relative fecundity (per gram of body weight) ranged from 30.52 to 63.45 ova. The average absolute fecundity recorded was 7698.4 ova, whereas the average relative fecundity was 43.85 ova per gram body weight. Fecundity was found to have a strong correlation with total weight ($R^2 = 0.6375$), total length ($R^2 = 0.5379$), and ovary weight ($R^2 = 0.5804$), while a low correlation was observed with ovary length ($R^2 = 0.2880$).

The result can be explained in the study of Petit et al. (2020), wherein they stated that the “reproductive success of aquatic animals depends on a complex web of relationships between the environment, the attributes of the reproductive individuals, and human-induced selection”. They stated that these factors can directly or indirectly affect the fecundity of fish and can also be compensated for certain external impacts. Parental effects include the influence that the phenotype and environmental conditions that individuals develop exert on the phenotype of their offspring, and they can even have a transgenerational impact.

Based on the study of Thorne et al. (2020), the impact of lifelong exposure to two concentrations (0.7 and 5.3 $\mu\text{g}\cdot\text{L}^{-1}$) of the antidepressant fluoxetine - often used to treat depression and sometimes obsessive-compulsive disorder, fish highly affects its fecundity. When exposed to the highest concentration of fluoxetine (5.3 $\mu\text{g}\cdot\text{L}^{-1}$), fish were smaller at maturation, but they more frequently engaged in mating. They also concluded that, in both fluoxetine treatments, females roughly doubled their overall fecundity, while egg fertilization rates were the same for exposed and

Table 9: Descriptive Analysis between the Average Fecundity and Average Weight of the Ovary of *Decapterus Macrosoma*.

Location	Average Fecundity	Average Weight of Ovary (g)
Buenavista	1 098	3.30
Tandag	739	2.15
Placer	625	1.44
Total	2 462	

unexposed fish. The result of their study indicates that the fecundity of fish is highly affected by different environmental factors.

However, according to Lenaerts et al. (2021), ovary weight was the strongest predictor of fecundity. Their findings study supported the result of our study that fecundity is strongly predicted by the weight of fish. Based on the results shown in Table 9, *D. macrosoma* samples in Buenavista waters have the highest average ovary weight of 3.30 g and the highest average fecundity of 1 098. This indicates that Buenavista waters have the highest numerical value of reproductive potential of *Decapterus macrosoma* as the basis for predicting fish catch in the future. *D. macrosoma* samples in Placer waters have the lowest average ovary weight of 1.44 g and the lowest average fecundity of 625. This indicates that Placer waters have the lowest numerical value of reproductive potential of *Decapterus macrosoma* as the basis for predicting the fish catch of the selected waters in Caraga in the future.

In the study of Osho & Usman (2019), they concluded that “fecundity was weakly correlated with total length and standard length. However, there were negative correlations between fecundity and condition factor, egg size and condition factor, total length, and condition factor with the values of -0.113, -0.030, and -0.270, respectively. Among all correlated variables, fecundity and gonad weight were the highest”. According to Oshom & Usman (2019), the results may be attributed to different factors such as sex, age, state of maturity, size, state of stomach fullness, and environmental factors affecting fish in water bodies. In their study, the sampled fish exhibited wide variations in the number of eggs, with larger samples producing more eggs than the smaller ones. However, they concluded that the highest number of eggs was not found in the largest fish, and the lowest number of eggs was not found in the smallest fish. This was also like the study of Murua et al. (2013), wherein different fish species present a lot of differences in their reproductive potential. Hence, the fecundity of fishes varies, depending on the reproductive characteristics of a species and changes in environmental conditions such as temperature, food availability, habitat, and predation intensity. Similarly,

Table 8: Descriptive analysis of the fecundity of (Shortfin scad) *Decapterus macrosoma* in the Buenavista, Placer, and Tandag.

Location	Frequency	Percent	Cumulative Percent
Placer	20	44.4	44.4
Buenavista	15	33.3	77.8
Tandag	10	22.2	100.0
Total	45	100.0	

in the study of Mekki & Hassan (2011), they stated that variability in fecundity may be attributed to age, sex, size weight, gonad weight, and locality. In the study of Olurin & Savage (2011), the result showed a range of 1711 to 4000 for *P. obscura* weighing between 161.94 and 380.78 g from the Oshun River, Southwest Nigeria. There was a high correlation coefficient between the Fecundity (F) and Gonad Weight (GW), which is $r = 0.992$. Hence, fecundity and growth may vary, which are attributed to different species characteristics, food availability, and environmental factors.

CONCLUSIONS

Based on the fecundity values of the (Shortfin scad) *D. macrosoma* samples in all sites, it is concluded that Buenavista waters have the highest numerical value of the reproductive potential of *D. macrosoma* as the basis for predicting the fish catch in the future. The large disparity in fecundity values obtained across all three sites is attributable to the differentiated values of the ovary weight of the fish samples. Even though the study indicates that there is a significant relationship between fecundity and growth in terms of length and weight of *D. macrosoma*, there are still varied environmental factors that need to be considered in determining fecundity. As such, results showed that fecundity values of *D. macrosoma* have no significant relationship with the present heat index within the three selected waters in the Caraga region.

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Evaluation of Black Oil Biodegradation by a Consortium of Indigenous *Bacillus cereus* and *Pseudomonas aeruginosa*

Zeena Ghazi Faisal†, Huda Mohammed Mahmoud, Mohannad Mohammed Jameel and Othman Abbas Abdullah

Department of Biology, College of Education, Al-Iraqia University, Baghdad, Iraq

†Corresponding author: Zeena Ghazi Faisal; zeenaalbayati76@gmail.com

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ABSTRACT

Black oil is a refined oil product that poses a significant environmental risk. It contains complex multi-hydrocarbons that decompose slowly. Black oil remains in the environment for a long time, which causes various toxic effects. This study was focused on three aspects. First of all, the local bacteria were isolated. Then, the potential of these bacteria for degrading black oil was determined. Finally, the efficiency of the bacterial consortium in degrading black oil was evaluated. Three black oil-degrading bacteria were isolated from crude oil-contaminated soil and molecularly identified by 16s rDNA sequencing as *Bacillus cereus* strain ZG.S6, *Bacillus cereus* strain ZG.S12, and *Pseudomonas aeruginosa* strain ZG.S11. Based on the measurement of optical density and chromatogram analysis, *B. cereus* strain ZG.S6, *B. cereus* strain ZG.S12, and *P. aeruginosa* strain ZG.S11 degrade black oil efficiently by reducing the number of their compounds to 10, 16, and 14, respectively. The results were compared to the complex combination of black oil (control group), which consists of 25 compounds of aliphatic and aromatic substances. The bacterial consortium demonstrated compatibility with each other. This helped them to degrade black oil more efficiently than individual strains, reducing its compounds to seven. Consequently, the consortium is a promising candidate for black oil bioremediation.

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INTRODUCTION

Urbanization and industrialization have led to the generation of hazardous, non-biodegradable pollutants that cause many toxic effects on human health and the environment (Bekele et al. 2023). Petroleum and its derivatives are serious compounds that significantly impact the soil, surface, and groundwater, as well as the stability and diversity of the microbial population (Ja'afaru et al. 2017). Therefore, there is a demand for rapid and effective methods to remove these contaminants.

Among petroleum derivatives, black oil is one of the most hazardous heavy fuels for ecosystems and is a common pollutant in Iraq (Owaied et al. 2018). It comprises heavy hydrocarbons such as resins, asphaltene, solid paraffin, long-chain alkanes, cyclic alkanes, polycyclic aromatics, unsaturated compounds, sulfuric compounds, and heavy metals (Khorasani et al. 2013). Black oil is slowly degraded, and it can remain in the environment for a long time. Black oil causes various toxic effects through the possibility of some components accumulating within susceptible organisms and being transferred to other levels in the food chain (Al-Wasify & Hamed 2014).

In general, conventional remediation processes for petroleum hydrocarbons are inappropriate due to their high application costs and the hazardous intermediates they produce. Bioremediation strategies, using biological agents such as plants and microbes, are a better option for restoring contaminated sites. It ensures a simple, cheap, and eco-friendly cleanup method (Chandran et al. 2020, Alsayegh et al. 2021). Microorganisms are utilized primarily in bioremediation because of



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their rapid growth, nutritional flexibility, and ability to adapt to extreme environments, thus enhancing their function as bioremediation agents (Ayilara & Babalola 2023).

A group of microorganisms, including many species of bacteria, yeast, and fungi, that inhabit polluted sites and have high metabolic potential are important for microbial bioremediation (Rahayu et al. 2019, Chandran et al. 2020). Numerous studies have demonstrated that many bacterial species are common hydrocarbon degraders (Rahayu et al. 2019, Tuhuloula et al. 2019, Alsayegh et al. 2021). *Pseudomonas* and *Bacillus* species are recognized as proficient degraders of hydrocarbons by utilizing them as sole sources of carbon and energy (Ja'afaru et al. 2017, Rahayu et al. 2019, Abdal-Satter, 2020, Abena et al. 2020, Faisal et al. 2023). These bacteria have improved their enzymatic systems and produced surface-active compounds, enabling them to utilize such pollutants and modify them into nontoxic and eco-friendly products (Owaied et al. 2018, Tuhuloula et al. 2019, Faisal et al. 2023).

The present study aimed to estimate the possibility of a local bacterial consortium, *Bacillus cereus* strain ZG.S6, *Bacillus cereus* strain ZG.S12, and *Pseudomonas aeruginosa* strain ZG.S11, to degrade black oil.

MATERIALS AND METHODS

Black Oil Sample

A sample of black oil was collected from the Al-Doura refinery located southeast of Baghdad, Iraq. It was stored in a dark, tightly sealed bottle, then transported to the laboratory and kept in a cool, dark place until use.

Isolation and Identification of Black Oil-Degrading Bacteria

A selective enrichment method, using mineral salt medium (MSM) pH 7, enriched with 2% (v/v) black oil, was used to isolate black oil-degrading bacteria from a sample of soil contaminated with crude oil. The soil sample was obtained from a depth of 10-20 cm from an electrical generator in Baghdad, Iraq. MSM contained (g.L⁻¹): KH₂PO₄ (1.0), K₂HPO₄ (1.0), NaCl (1.0), CaCl₂ (0.05), (NH₄)₂SO₄ (1.0), MgSO₄·7H₂O (0.5), FeCl₃ (0.002), and Yeast extract (0.1) (Faisal et al. 2023). Bacterial isolates were molecularly identified by 16S rRNA sequencing, using universal primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-TACGTTACCTTGTTACGACTT-3' and compared with those of 16S rRNA genes from NCBI Gene Bank. PCR products were sent for Sanger sequencing using ABI3730XL, an automated DNA sequencer, by Macrogen Corporation-Korea. The results were received by e-mail and then analyzed

using Genius software.

Preparation of the Bacterial Cultures and Bacterial Consortium and Estimating Their Ability to Degrade Black Oil

This experiment was performed in triplicate. One milliliter of fresh culture (OD₆₀₀ = 1.0) was inoculated individually into a 500 mL Erlenmeyer flask containing 100 mL MSM with 2% black oil and incubated in a 150 rpm shaker incubator at 30 °C for 14 days. To prepare bacterial consortia, a 500-mL Erlenmeyer flask containing the same medium composition was inoculated with 1 mL of each fresh bacterial culture (OD₆₀₀ = 1.0) and kept under the same conditions. A flask with the same culture media composition but no inoculum was used as a control. After 14 days of incubation, bacterial growth was assessed using a UV/Vis spectrophotometer (OD₆₀₀) and compared to MSM as a blank (Lima et al. 2020).

Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

After 14 days of incubation, the residual black oil was collected separately from each bacterial culture and the bacterial consortium by chloroform (3 sample:1 chloroform v/v) with continuous shaking. By using a separation funnel, the organic phase containing the residual black oil was separated from the aqueous phase, collected in a glass Petri dish, and then dried at 40-45°C. The precipitate was analyzed by a GC-MS instrument that contains a ZB-5MS capillary column (30 mm × 0.25 mm, I.D. 0.25 µm), using helium (He) as carrier gas with a flow rate of 2 mL.min⁻¹. The temperatures of the injector and detector were 230°C and 280°C, respectively. The column was initially heated to 80°C for 3 minutes and then raised to 280°C for 10 minutes at a rate of 8°C.min⁻¹. First, 0.1 g of samples were dissolved in chloroform at a concentration of 100 µg.mL⁻¹, and then 1 µL of the sample was used with a split ratio of 10:1.

Data Analysis

The computerized database structure was used to perform statistical analyses and report on the obtained data. One-way analysis of variance (ANOVA) was used, with a statistical significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Isolation and Identification of Bacteria

Three effective black oil-degrading bacteria were isolated using a selective enrichment approach. This shows that bacteria can survive and utilize black oil in soil contaminated with petroleum derivatives. Previous studies indicate

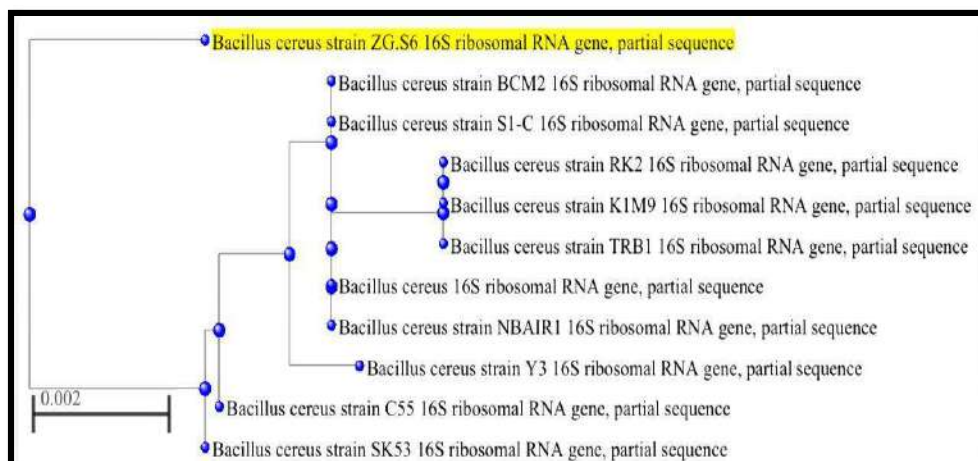


Fig. 1: *Bacillus cereus* strain ZG.S6 phylogenetic tree from the NCBI database.

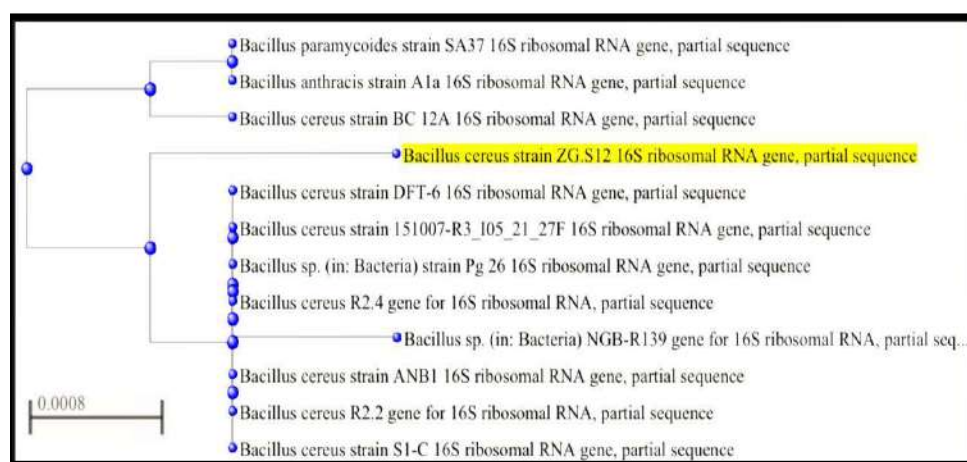


Fig. 2: *Bacillus cereus* strain ZG.S12 phylogenetic tree from the NCBI database.

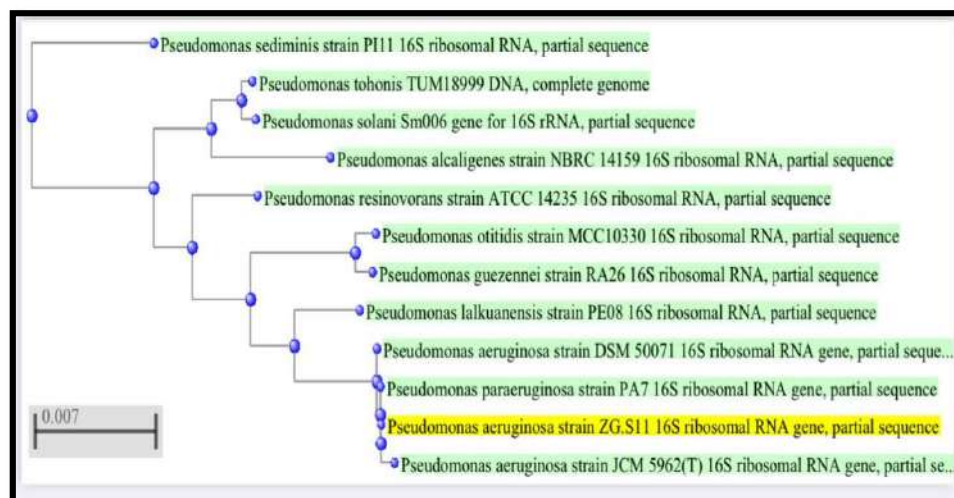


Fig. 3: *Pseudomonas aeruginosa* strain ZG.S11 phylogenetic tree from the NCBI database.

the possibility of isolating many types of bacteria from petroleum-contaminated soil that can utilize crude oil and hydrocarbon residues (Rahayu et al. 2019, Abdal-Satter, 2020, Abena et al. 2020, Faisal et al. 2023).

The sequence analysis indicated that two isolates were classified as *Bacillus cereus*, while one was identified as *Pseudomonas aeruginosa* (Figs. 1, 2, and 3). Strains were documented in the NCBI GenBank nucleotide sequence database as *Bacillus cereus* strain ZG.S6, *Bacillus cereus* strain ZG.S12, and *Pseudomonas aeruginosa* strain ZG.S11 with accession numbers PP408317, PP408320, and PP408321, respectively.

Bekele et al. (2022) explained that *Bacillus cereus* and *Pseudomonas aeruginosa* play significant roles in the environment, particularly in the decomposition of hydrocarbons and their derivatives. This is attributed to the diverse metabolic capacity of bacteria to decompose hydrocarbons and recycle metals in nature (Kebede et al. 2021). Additionally, many species of bacteria can generate surface-active compounds with different chemical structures, which can minimize interfacial and surface interactions, thereby facilitating the emulsification process of hydrocarbon contaminants and improving their bioavailability (Faisal et al. 2023).

Estimating the Ability of Bacterial Consortium to Degrade Black Oil

By measuring the optical density of bacteria grown on MSM containing 2% black oil as a carbon and energy source, the ability of bacteria to decompose black oil was assessed. Bacterial proliferation is an indicator of their ability to degrade black oil. The variation in OD was related to the intensity of bacterial growth and the breakdown of the black oil molecular structure as a result of its utilization by bacteria as a carbon and energy source.

Through the first three days of incubation (lag phase), there was no discernible change in turbidity. This period is responsible for the bacteria adapting to their new environment in preparation for the next stage (experiential phase). After 14 days of incubation, bacterial growth reached a maximum of 1.214 ± 0.01 , 1.186 ± 0.03 , and 1.124 ± 0.01

for *B. cereus* strain ZG.S6, *B. cereus* strain ZG.S12, and *P. aeruginosa* strain ZG.S11, respectively. The OD of the bacterial consortium recorded a significant growth intensity of 1.237 ± 0.03 , indicating an increase in the rate of black oil degradation. Table 1 shows the optical density (OD₆₀₀) of individual bacterial strains and their consortium after 14 days of incubation in MSM containing 2% black oil.

Table 1 shows variation in OD, and *B. cereus* strain ZG.S6 was the most efficient in utilizing black oil. This variation is due to the different metabolic capacities of bacteria to decompose hydrocarbons (Kebede et al. 2021). Furthermore, lipopeptides and glycolipid biosurfactants generated by *Bacillus* and *Pseudomonas* species can aid in the emulsification process and increase hydrocarbon bioavailability (Viesser et al. 2020, Kebede et al. 2021, Bekele et al. 2023, Faisal et al. 2023). *Bacillus* species can form spores that allow them to thrive in various environments, including hydrocarbon-polluted soils (Viesser et al. 2020). Also, *Pseudomonas* species are successful in the biodegradation of petroleum because they are less vulnerable to the toxic effects of hydrocarbons (Bilen Ozyurek & Seyis Bilkay, 2020).

GC-MS Analysis

GC-MS was used to determine the residual black oil of individual bacterial cultures (*B. cereus* strain ZG.S6, *B. cereus* strain ZG.S12, and *P. aeruginosa* strain ZG.S11) and their consortium, contrasting them with the control. Table 2 shows the complicated mixture of black oil (control group), which contains 25 compounds of aliphatic and aromatic substances. Fig. 4 shows several peaks that illustrate variations between black oil compounds caused by differences in the number of carbon atoms.

Chromatogram analysis of individual bacterial strain cultures (*B. cereus* strain ZG.S6, *B. cereus* strain ZG.S12, and *P. aeruginosa* strain ZG.S11) reveals a reduction in black oil compounds compared to the control. *B. cereus* strain ZG.S6 and *B. cereus* strain ZG.S12 were able to reduce the compounds of black oil to 10 and 16, respectively (Tables 3 and 4), while *P. aeruginosa* strain ZG.S11 could reduce black oil compounds to 14 (Table 5). Figs. 5, 6, and 7 illustrate

Table 1: The optical density (OD₆₀₀) of individual bacterial strains and their consortium after 14 days of incubation in MSM containing 2% black oil.

No.	Symbol	Bacterial Strain	The optical density (OD ₆₀₀) after 14 days
1	S6	<i>Bacillus cereus</i> strain ZG.S6	1.214 ± 0.01
2	S11	<i>Pseudomonas aeruginosa</i> strain ZG.S11	1.124 ± 0.01
3	S12	<i>Bacillus cereus</i> strain ZG.S12	1.186 ± 0.03
4	Consortium (S6, S11, and S12)		1.237 ± 0.03

Data: Mean $\pm$ SEM. The experiments were performed in three replicates.

Table 2: The chemical composition of black oil (control group).

No.	RT (min)	Area %	Name	Quality	CAS Number
1	11.341	0.38	Cyclopentasiloxane, decamethyl-	80	000541-02-6
2	15.362	13.88	Dodecamethylcyclohexasiloxane	93	000540-97-6
3	16.545	0.37	Tetradecane	93	000629-59-4
4	17.894	0.19	n-Eicosane	60	000112-95-8
5	18.61	0.55	Pentadecane	96	000629-62-9
6	19.02	13.08	Dodecamethylpentasiloxane	52	000141-63-9
7	20.561	0.84	Cetane	96	000544-76-3
8	21.536	0.29	Tridecane, n-	90	000629-50-5
9	22.294	6.88	: Cyclooctasiloxane, hexadecamethyl-	59	000000-00-0
10	24.183	2.22	Octadecan	98	000593-45-3
11	25.132	3.17	Cyclononasiloxane, octadecamethyl-	90	109007-87-6
12	25.858	3.06	Nonadecane	99	000629-92-5
13	27.462	5.31	pentadecane	97	000629-62-9
14	27.68	1.85	Boldenone, di-trimethylsilyl	62	109007-87-6
15	28.998	6.73	n-Heneicosane	98	000629-94-7
16	30.461	8.18	Normal-docosane	99	000629-97-0
17	31.867	6.59	n-Tricosane	99	000638-67-5
18	33.216	6.82	Tetracosane	98	000646-31-1
19	34.518	4.92	n-Heneicosane	98	000629-94-7
20	35.769	4.03	n-Hexacosane	99	000630-01-3
21	36.972	3.28	n-Heptacosane	98	000593-49-7
22	38.13	1.98	Heptacosane, 1-chloro-	98	062016-79-9
23	39.255	1.98	n-Eicosane	95	000112-95-8
24	40.345	1.82	n-Eicosane	96	000112-95-8
25	40.791	1.58	Baccharene	53	036441-74-4

the disappearance of several peaks compared to a control sample as the number of chemical components decreased. It is noteworthy that any GC reduction between the control and inoculated samples is referred to be a biodegradation process since any loss induced by non-biological factors would affect both the control and inoculated samples. This indicated that *B. cereus* strain ZG.S6, *B. cereus* strain ZG.S12, and *P. aeruginosa* strain ZG.S11 are significant black oil decomposers.

Comparing the chromatogram analysis of the control group, individual bacterial strains, and the bacterial consortium, the consortium showed a decrease in black oil compounds, recording seven chemicals (Table 6). Fig. 8 shows the disappearance of several peaks, revealing the efficiency of the bacterial consortium in decomposing black oil, which led to a decrease in the chemical components of black oil. This indicates the compatibility of bacterial strains with each other, as they work by fragmenting aliphatic and aromatic components.

Results were in accordance with Bilen Ozyurek and Seyis Bilkay (2020), who revealed that the synergistic impact of a consortium containing gram-positive and gram-negative bacterial strains is more effective than a consortium containing only gram-positive or gram-negative bacteria. Tuhuloula et al. (2019) found that a consortium of *B. cereus* and *P. putida* at a concentration of 15% (v/v) and a bacteria ratio of 1:1 can successfully degrade oil sludge with a biodegradation percentage of 97.42%. Patowary et al. (2016) also found that a consortium of two *Bacillus* strains, *B. pumilus* KS2 and *B. cereus* R2, showed the best crude oil degradation, up to 84.15%. Allamin et al. (2020) revealed a favorable correlation between the microbial population and hydrocarbon degradation.

Bioremediation processes rely on the diverse catabolic activity of microorganisms to break down contaminants. While various microorganisms can detoxify organic compounds, bacteria are known to be the most effective in biodegradation. Bacteria offer several advantages, including

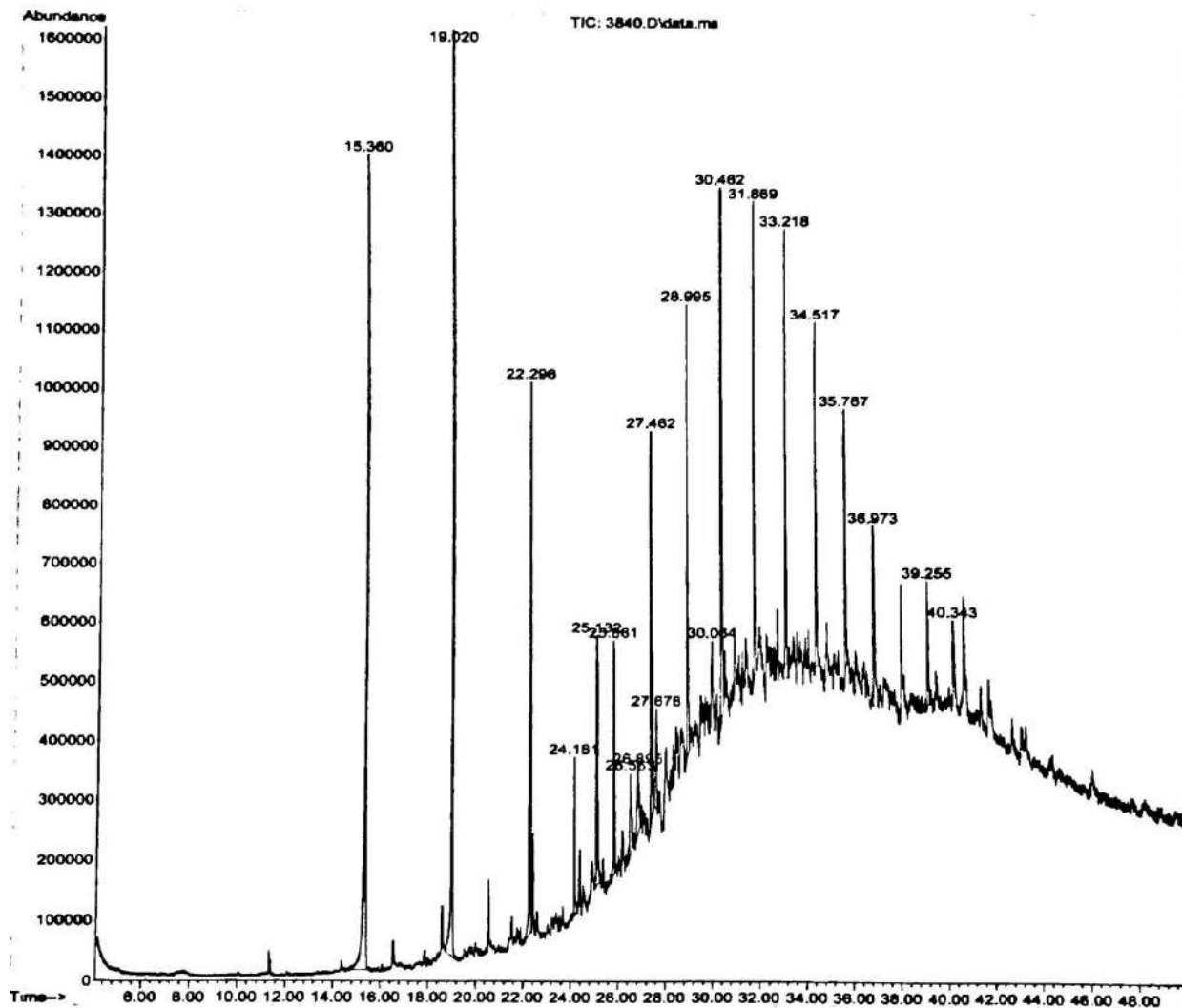


Fig. 4: Chromatogram analysis of black oil (the control group).

Table 3: The residual combination of black oil degraded by *B. cereus* strain ZG.S6.

No.	RT (min)	Area %	Name	Quality	CAS Number
1	11.336	0.58	Cyclopentasiloxane, decamethyl-	83	000541-02-6
2	15.367	37.94	Cyclohexasiloxane, dodecamethyl	94	000540-97-6
3	19.025	30.38	Cyclononasiloxane, octadecamethyl-	46	013450-70-9
4	22.299	16.72	Tetracosamethyl-cyclododecasiloxane	58	018919-94-3
5	25.132	10.29	Cyclononasiloxane, octadecamethyl-	74	109007-87-6
6	30.015	1.71	Tetracosamethyl-cyclododecasiloxane	53	018919-94-3
7	30.45	0.57	Heptadecane	93	000629-78-7
8	31.862	0.58	Nonadecane	96	000629-92-5
9	32.147	0.72	Cardigin	93	109007-87-6
10	33.211	0.51	n-Eicosane	96	000112-95-8

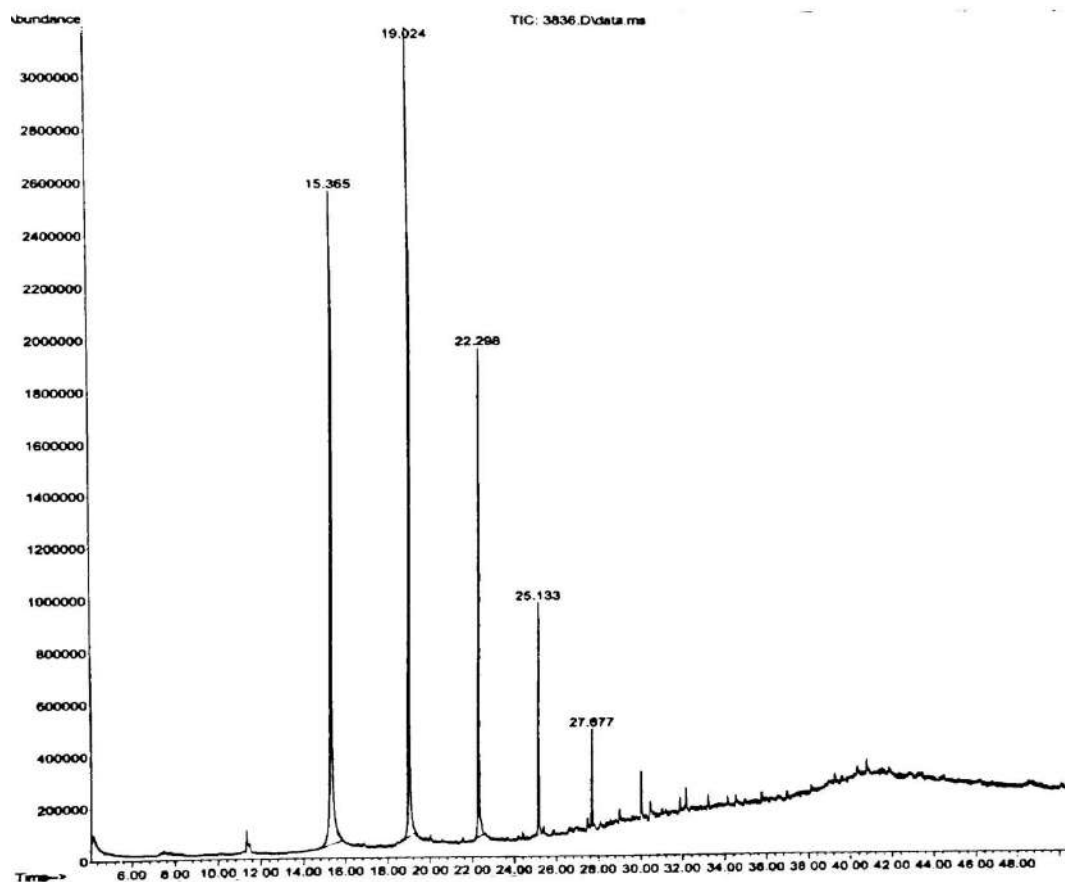


Fig. 5: Chromatogram analysis of residual black oil degraded by *B. cereus* strain ZG.S6.

Table 4: The residual combination of black oil degraded by *B. cereus* strain ZG.S12.

Number	RT (min)	Area%	Name	Quality	CAS Number
1	11.335	0.56	Cyclopentasiloxane, decamethyl-	87	000541-02-6
2	15.367	33.99	Dodecamethylcyclohexasiloxane	93	000540-97-6
3	19.025	31.17	Cyclodecasiloxane, eicosamethyl-	50	000995-82-4
4	22.299	14.18	Cyclooctasiloxane, hexadecamethyl-	64	000000-00-0
5	24.39	0.82	Isotetradecane	94	000629-59-4
6	25.132	6.83	Cyclononasiloxane, octadecamethyl-	87	000556-71-8
7	27.456	0.95	pentadecane	98	000629-62-9
8	27.674	2.78	Cyclononasiloxane, octadecamethyl	53	000000-00-0
9	28.992	1.06	Heptadecane	97	000629-78-7
10	30.02	2.24	Tetracosamethyl-cyclododecasiloxane	80	018919-94-3
11	30.455	2.27	Docosane	99	000629-97-0
12	33.211	0.72	n-Heneicosane	98	000629-94-7
13	34.513	0.60	Docosane	96	000629-97-0
14	35.763	0.65	n-Nonadecane	95	000629-92-5
15	39.245	0.56	1,3-Bis(trimethylsilyl)benzene	55	002060-89-1
16	40.786	0.63	Lupan-3-ol	46	000541-05-9

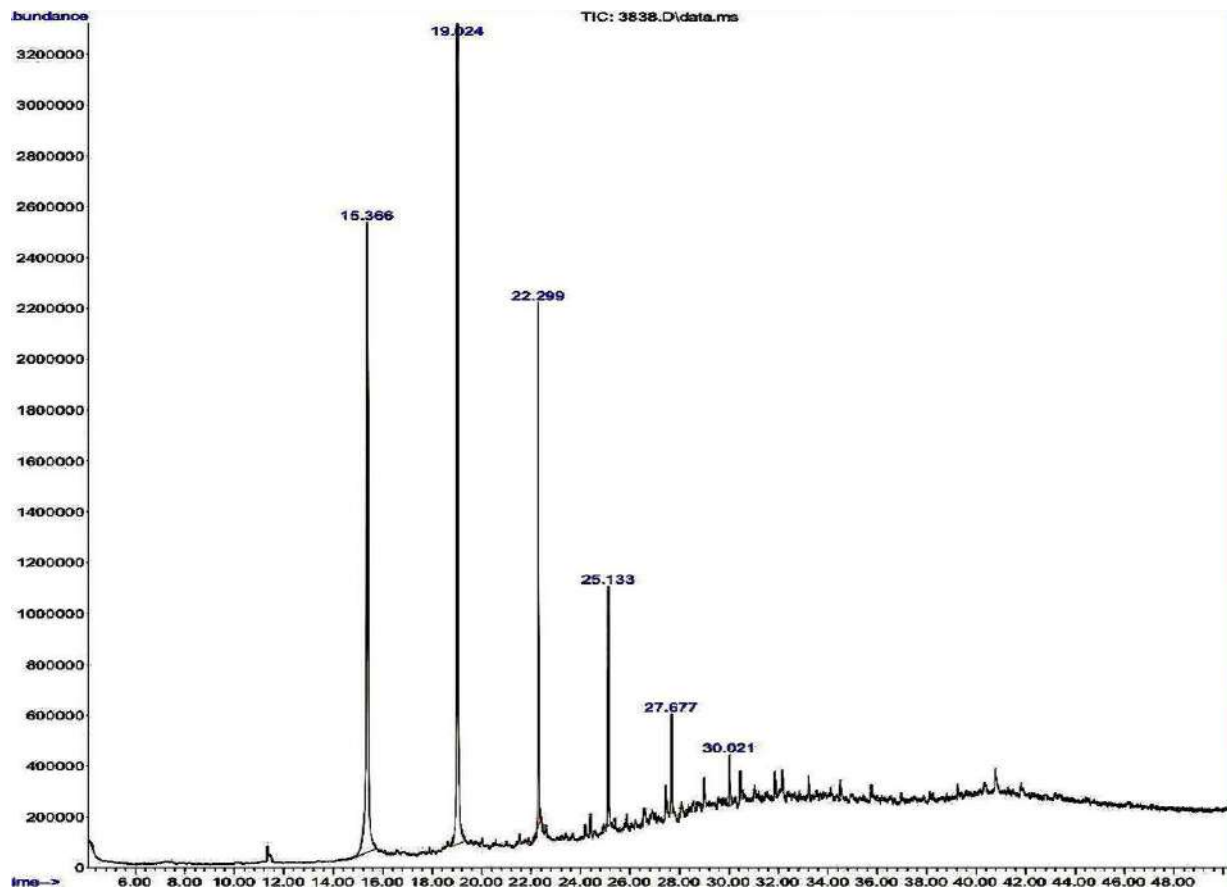


Fig. 6: Chromatogram analysis of residual black oil degraded by *B. cereus* strain ZG.S12.

Table 5: The residual combination of black oil degraded by *P. aeruginosa* strain ZG.S11.

No.	RT (min)	Area %	Name	Quality	CAS Number
1	11.34	0.85	Cyclopentasiloxane, decamethyl-	91	000541-02-6
2	15.367	37.80	Dodecamethylcyclohexasiloxane	94	000540-97-6
3	19.025	31.24	1,11-Dihydrogendodecamethylhexasiloxane	50	000995-82-4
4	22.299	14.17	6-Aza-5,7,12,14-tetrathiapentacene	64	000000-00-0
5	25.132	6.49	Cyclononasiloxane, octadecamethyl-	49	000556-71-8
6	27.456	0.75	Pentadecane	91	000629-62-9
7	27.674	2.80	Heptasiloxane, hexadecamethyl-	53	019095-24-0
8	28.987	0.77	Eicosane	92	000112-95-8
9	30.02	1.88	6-Aza-5,7,12,14-tetrathiapentacene	47	000000-00-0
10	30.455	0.96	Normal-heptadecane	98	000629-78-7
11	31.862	0.69	Octadecan	98	000593-45-3
12	32.147	0.53	Morphine, bis(trimethylsilyl) ether	74	109007-87-6
13	33.216	0.62	Heneicosane	96	000629-94-7
14	35.758	0.44	Docosane	95	000629-97-0

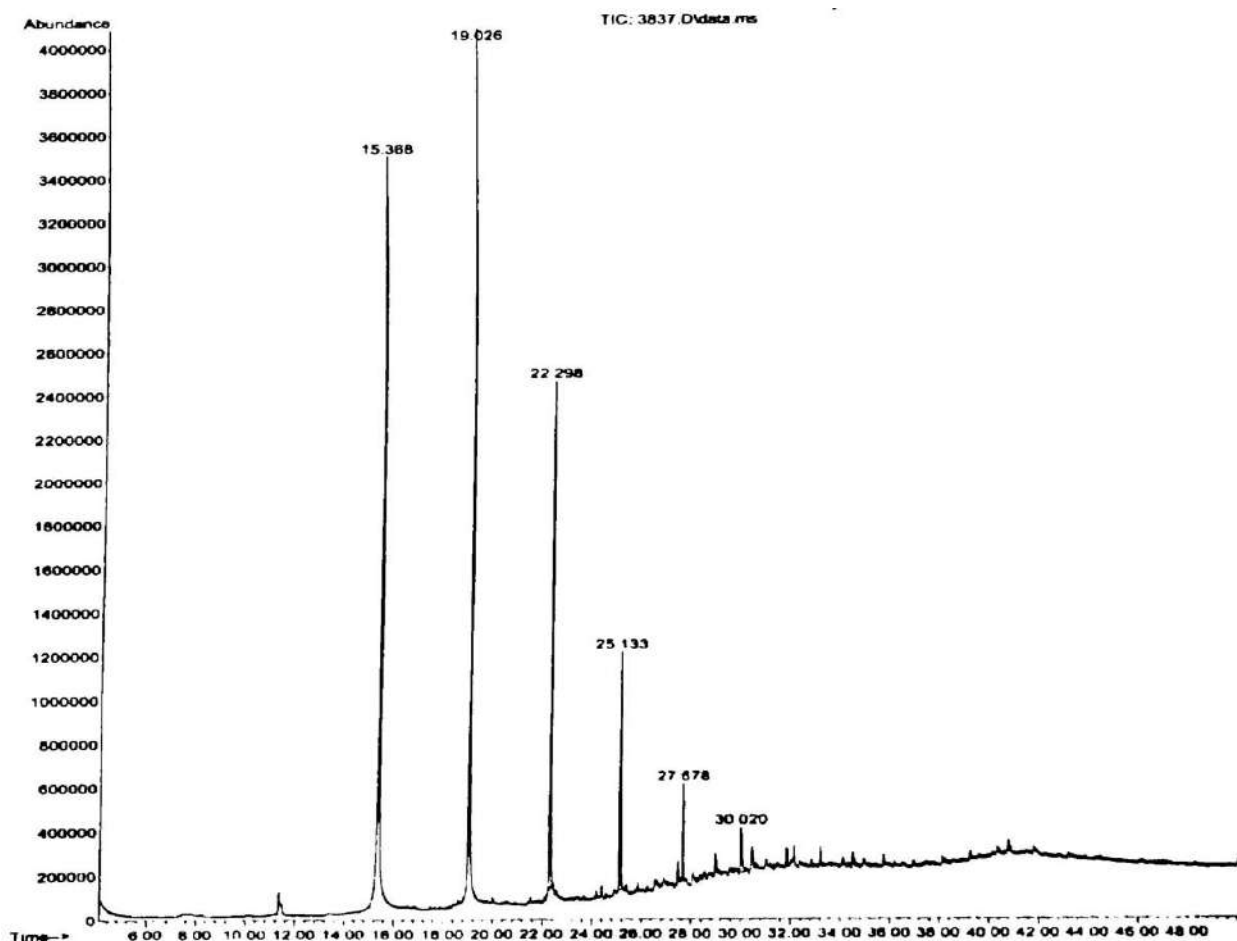


Fig. 7: Chromatogram analysis of residual black oil degraded by *P. aeruginosa* strain ZG.S11.

Table 6: The residual combination of black oil degraded by the bacterial consortium.

No.	RT (min)	Area %	Name	Quality	CAS Number
1	11.335	0.54	Cyclopentasiloxane, decamethyl-	83	000541-02-6
2	15.362	35.23	Dodecamethylcyclohexasiloxane	94	000540-97-6
3	19.025	33.37	Cycloheptasiloxane, tetradecamethyl-	50	000995-82-4
4	22.299	17.54	Cyclooctasiloxane, hexadecamethyl-	64	000000-00-0
5	25.132	10.67	Cyclononasiloxane, octadecamethyl-	43	000556-71-8
6	30.014	1.49	Cyclooctasiloxane, hexadecamethyl-	49	000000-00-0
7	32.147	1.12	Cyclononasiloxane, octadecamethyl-	53	109007-87-6

genetic diversity, rapid reproduction, and metabolic versatility. Over millions of years, bacteria have evolved catalytic enzymes to degrade hydrocarbons, enabling them to survive in polluted environments (Bilen Ozyurek & Seyis Bilkay, 2020, Kebede et al. 2021, Bekele et al. 2023). Furthermore, many species of bacteria are capable of producing biosurfactants that can emulsify hydrocarbons (Viesser et al. 2020, Bekele et al.

2023, Faisal et al. 2023). *Bacillus cereus* is known to produce surfactin (Wang et al. 2024), whereas *P. aeruginosa* produces rhamnolipids (Faisal et al. 2023). Rhamnolipid and surfactin increase the bioavailability of contaminants by enhancing their emulsification. The emulsification process is essential for microorganisms to take up and assimilate hydrocarbons (Viesser et al. 2020).

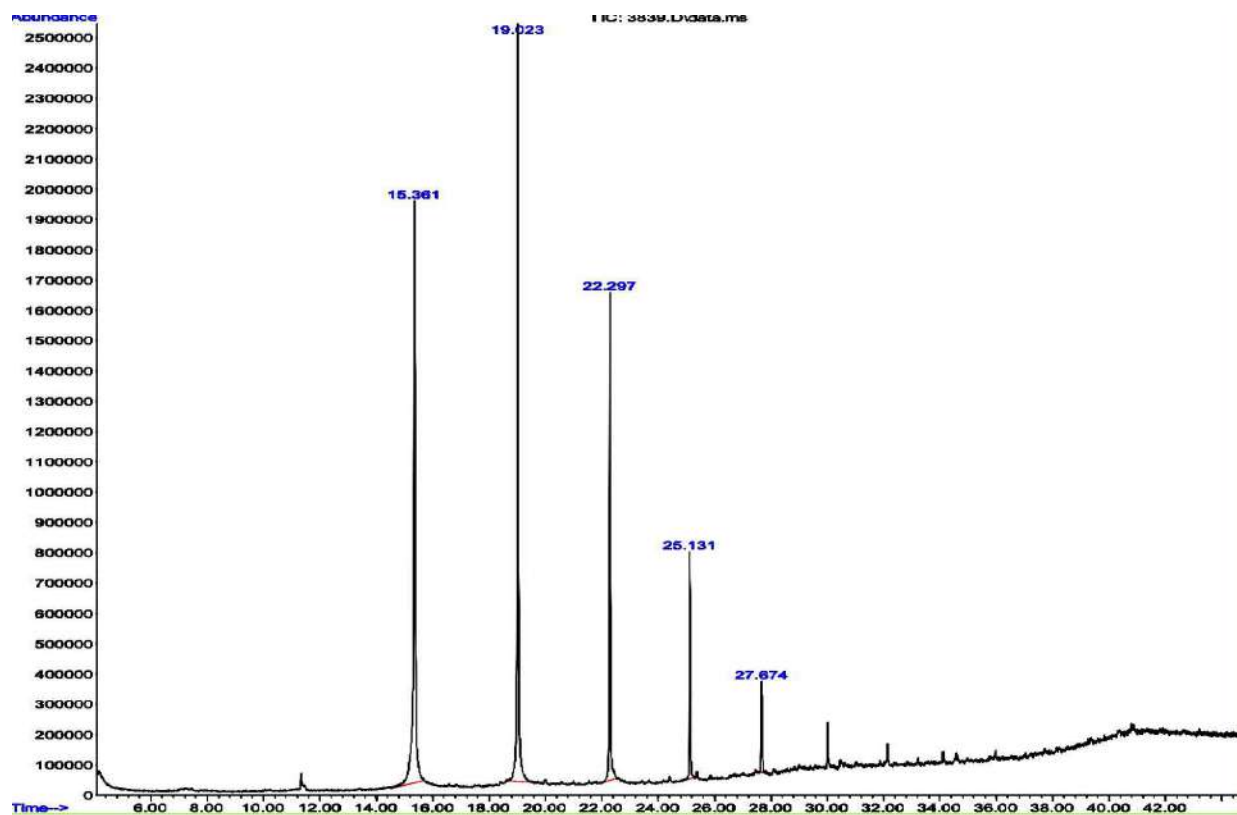


Fig. 8: Chromatogram analysis of residual black oil degraded by *the* bacterial consortium.

There are few studies on the biodegradation of black oil compared with other petroleum compounds. Petroleum containing aliphatic, aromatic, and saturated compounds at different ratios is biodegraded at different rates by the same microorganism (Bilen Ozyurek and Seyis Bilkay, 2020). Various bacterial species can degrade several hydrocarbons. Each bacterial species specializes in a few hydrocarbons as distinct carbon sources, and no single bacterium can produce all the enzymes essential to the degradation process (Ja'afaru et al. 2017). Because an individual bacterium can metabolize a limited range of hydrocarbons, the bioremediation of complicated hydrocarbons typically requires the collaboration of many species (Patowary et al. 2016). Therefore, natural microbial communities with broad enzymatic capacities can improve biodegradation processes through synergistic interactions between microbial strains, where any bacterial strain may remove the toxic metabolites produced by the metabolic activity of other microbes (Bilen Ozyurek & Seyis Bilkay 2020). Consequently, the biodegradation of black oil necessitates a combination of different microbial strains with wide enzymatic activity.

CONCLUSIONS

The present study demonstrates that *B. cereus* and *P. aeruginosa*, isolated from a soil sample contaminated with crude oil, perform well in black oil biodegradation. The efficacy of the black oil degradation process varies with strains, depending on catabolic activity. The bacterial consortium was typically more effective than individual strains, degrading a considerable amount of black oil. Consequently, this bacterial consortium can be employed in contaminated soil to biodegrade black oil.

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Innovative Azimuth Tracking for Multi-Directional Inclined Basin Solar Desalination Systems

Maheswari Kasi¹, Mayandi. K.^{1†}, Joe Patrick Gnanaraj. S.² and Vanthana Jeyasingh²

¹Department of Mechanical Engineering, Kalasalingam Academy of Research and Education, Krishnankovil, Tamil Nadu, India

²Department of Mechanical Engineering, St. Mother Theresa Engineering College, Vagaikulam, Thoothukudi, Tamil Nadu, India

†Corresponding author: K. Mayandi, k.mayandi@gmail.com

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ABSTRACT

The paper presents a multi-directional inclined compartmental basin solar desalination system with a unique design aimed at enhancing water purification through solar energy. The system consists of a central basin surrounded by four inclined compartmental basins, each equipped with a thick glass cover of 4 mm tilted at a 30° angle to facilitate condensation. Techniques such as one-step azimuth tracking are employed, where the entire setup is rotated 15° daily to optimize solar exposure, improving distillate productivity. The methodology includes the construction of basins with pyramid-like structures to concentrate solar energy, increase water temperature rapidly, and maintain it for prolonged periods. Experimental tests were conducted at different orientations (0°, 15°, 30°, 45°, 60°, 75°, and 90°), measuring yields across basins and analyzing the effects of solar radiation and temperature. This innovative system, leveraging azimuth tracking and optimized basin configurations, offers a supportable solution for potable water production in solar-rich areas. The study's results show that the multi-directional solar desalination system achieved its highest yield of 20.305 liters/day at a 0° orientation, with Basin 1 (south-facing) producing 5.780 liters/day. Rotating the setup to different angles (e.g., 15°, 30°) yielded minor increases (up to 0.90%) in overall productivity due to optimized solar exposure. The findings confirm that one-step azimuth tracking enhances daily distillate production in solar-rich environments.

INTRODUCTION

Globally, every nation experiences water stress, characterized by a fast decline in per capita water availability alongside an increase in water use. As a result, international apprehension is increasing, prompting the adoption of significant actions to address water scarcity. Scientists are allocating substantial resources to wastewater treatment, investigating the origins of wastewater generation, and its adverse impacts. 97% of the world's available water is contained in the ocean and is unsuitable for consumption due to its salinity. Consequently, although water supplies are plentiful, almost 99% of Earth's water is non-potable (Luo et al. 2022). Water scarcity and ineffective wastewater treatment result in insufficient sanitation, leading to waterborne infections and detrimental effects on aquatic ecosystems. Only sustainable water treatment can satisfy the current water demand. An eco-friendly method of obtaining potable water is solar desalination (Zhang et al. 2023).

Potable water is generated through a process that is exclusively powered by solar energy in solar desalination. This radiation possesses pasteurization and germicidal properties, efficiently eliminating pollutants from wastewater (Girimurugan et al. 2023, Muruganandhan et al. 2023). Water research has demonstrated that distilled water obtained through solar desalination is safe for human consumption.

The sun still (SS) is a significant technique in direct solar desalination, attracting interest due to its optimal components, straightforward design, dependence on renewable energy, environmentally benign operation, and ease of building with local resources. Salty water is transformed into potable water using a solar still, which is powered by sunlight through the processes of evaporation and condensation (Chauhan et al. 2021). It is particularly advantageous in coastal regions and can be installed in any location that absorbs solar radiation.

An SS is a solar-powered device engineered to generate potable water from polluted sources. It has multiple advantages, rendering it an exceptional remedy for groups or individuals lacking access to potable water. SS has two advantages. Simple designs are among the primary benefits of SSs, as is their portability (Balan et al. 2024). The fundamental design of an SS is a shallow trench or receptacle that is entirely encased in a transparent medium, such as plastic or glass. Water that is contaminated is introduced into the basin, where it is evaporated by solar radiation. Vaporized water condenses and deposits into a collection receptacle on the interior surface of the transparent cover. It is capable of being constructed spontaneously from components that are easily accessible and transported to remote or disaster-affected regions that require potable water (Mohanasundaram et al. 2024). Currently, researchers in the desalination field have observed that passive solar distillation is an inefficient technique for cleaning and desalinating brackish water. Given a daily production of around 1 kg.m^{-2} , passive solar stills may lack economic feasibility. The efficacy of solar stills has been improved through the implementation of numerous improvements. The productivity of the basin is increased during the day and decreased at night as the water profundity decreases (Khalifa & Hamood 2009, Murugavel et al. 2008).

Researchers have developed diverse designs of inclined solar stills that utilize wicks and steps to sustain minimal water depth and improve production. Our objective is to assess the current state of the various designs that are being employed to improve the efficacy of slanted solar stills. It fosters the development of SS systems that are more efficient and effective, which has the potential to provide real-world benefits in large-scale operations (Balan et al. 2024).

Enhanced total output was demonstrated in Mar, April, Aug, Nov, and Dec by the development of a one-basin DSSS, which yielded approximately 4.1 L.day^{-1} (Kulandaivel & Karuppian 2014). Experiments were conducted by Gnanaraj and Velmurugan (2019) to improve the output of a DSSS. They evaluated SS by employing a finned corrugated receptacle, black granite, a flame, and an external reflector. Contrasting the results of these four modified solar stills with

those of a conventional, unmodified solar still. The traditional solar still yielded 1880 ml/m^2 per day, whereas the modified stills generated 2995, 3210, 2690, and 3655 mL.m^{-2} per day, correspondingly, all exceeding the output of the traditional still (Gnanaraj & Velmurugan 2019). In an evaluation of the double slope solar still (DSSS), the maximum production was achieved with light black cotton cloth (Murugavel & Srithar 2011). Other wick materials tested included coir matting, discarded cotton, sponges, and light cotton fabric. Based on testing results, a DSSS can generate $7.80 \text{ l/m}^2/\text{day}$ in passive mode and $10 \text{ l/m}^2/\text{day}$ in active mode (Morad et al. 2015). The efficiency of solar stills with two basins, one with a DSSS and one with a solo basin single slope, with the latter producing 85 percent more energy than the former (Rajaseenivasan & Murugavel 2013). Energy storage media, like gravel, black rubber, quartzite rock, iron remnants, red brick fragments, asphalt, glass, washed stones, and cement concrete fragments, increase the production of the still and the heat storage capacity of the basin by 50% (Abdel-Rehim & Lasheen 2005, Murugavel et al. 2010, Badran 2007, Nafey et al. 2001).

The experimental comparisons were conducted to assess the efficacy of single slope, single basin, and DSSS (Al-Karaghoul & Alnaser 2004a, 2004b). The twin basin solar still exhibited superior output. Furthermore, it was noted that insulation considerably affects production, particularly for the twin basin variant. Cappelletti conceptualized and assessed the experimental effectiveness of a double basin DSSS (Cappelletti 2002). Gnanaraj et al. (2018) conducted a study on a double slope SS featuring both internal and external enhancements. The basin area was filled with pebbles, and an exterior mirror was employed to concentrate sunlight onto the still. The upgraded double slope solar still obtained a 40.86% better efficiency compared to the original version (Gnanaraj et al. 2018). Authors (Gnanaraj & Ramachandran 2017) conducted desalination experiments and constructed a solar still and a solar pond that were combined. The researchers determined that the daily output of a conventional still is 95.5% lower than that of a single slope solar still related to a solar pond.

Nagarajan et al. (2017) demonstrated that the production of freshwater and the evaporation of water within the solar still are fully governed by the duration water remains exposed to sun radiation. Researchers (Kumar et al. 2017) illustrate that an inclined SS coupled with a triangular pyramid SS generates greater water temperatures with minimal water depth. Author (Anburaj et al. 2013) designed and experimentally assessed a solar still with a slanted absorber plate incorporating rectangular grooves and ridges, evaluating its performance at different angles and

employing various wick materials like clay pots, discarded cotton fragments, jute fabric, black cotton cloth, and mild steel components, under real solar conditions. The optimal orientation determined was a 30° inclination toward south. Gupta et al. (2024) examined the energy demands of wastewater treatment plants (WWTPs) and evaluated the integration of microalgae cultivation, biogas production, and solar power as sustainable energy sources. They found that traditional WWTPs are energy-intensive and proposed that incorporating these renewable energy technologies can enhance energy efficiency and reduce environmental impact. The findings suggest that utilizing microalgae for nutrient removal and biomass production, harnessing biogas from anaerobic digestion of organic waste, and implementing solar power can collectively contribute to the sustainable operation of WWTPs. Ansari et al. (2024) assessed the economic viability of installing on-grid photovoltaic (PV) solar power systems at private universities in Indonesia. The study analyzed factors such as initial investment costs, energy savings, payback periods, and potential environmental benefits and despite the substantial upfront costs, the long-term financial savings and positive environmental impact make on-grid PV systems a feasible and sustainable energy solution for private universities in Indonesia and it contributed valuable insights into the adoption of renewable energy technologies in different sectors, emphasizing the importance of sustainable development and energy efficiency. Abdullah et al. (2024) investigated the performance of a hemispherical solar distillation system enhanced with rock salt balls, optimizing its efficiency and comparing its effectiveness with a system without storage. Tareemi et al. (2024) conducted a thermoenviroeconomic analysis of an upgraded solar desalination system incorporating a heat pump and various active and passive enhancements. Elashmawy and Ahmed enhanced the productivity of tubular solar stills by utilizing composite aluminum/copper/sand sensible energy storage tubes. Panchal et al. (2023) evaluated the performance of solar stills incorporating evacuated tube collectors, perforated

fins, and pebbles, along with a CO_2 mitigation analysis. Elsheikh et al. (2024) improved solar still performance using heat pipe/pulsating heat pipe evacuated tube collectors and phase change materials, with experimental and environmental analysis. Abdullah et al. (2024) explored the use of rock salt balls in hemispherical solar stills, achieving enhanced performance through cost-effective sensible energy storage. Tareemi et al. (2024) evaluated solar desalination systems integrated with heat pumps, highlighting improvements in energy efficiency and environmental impact. Liu et al. (2023) explored the integration of evacuated tubes and nanofluids, achieving enhanced thermal efficiency and improved desalination rates. Jathar and Ganesan (2021) highlighted the role of nanoparticles and cooling arrangements in boosting the productivity of stepped solar stills. Tareemi et al. (2023) identified optimal design configurations for solar desalination systems integrated with heat pumps, maximizing their efficiency. Elashmawy et al. (2023) demonstrated tubular solar stills utilizing composite energy storage materials to ensure consistent thermal performance. Panchal et al. (2023) showcased the integration of evacuated tube solar collectors, fins, and pebbles for improved productivity and CO_2 mitigation. Elsheikh et al. (2024) enhanced solar still efficiency by employing heat pipe solar collectors and phase change materials for effective energy storage.

The main aim of this plan is to develop a multi-directional inclined compartmental basin solar desalination system. While many configurations of water desalination systems exist, this design is a new initiative with higher efficiency compared to other systems.

MATERIALS AND METHODS

Experimental Arrangement

Fig. 1 illustrates a newly designed solar still, which comprises a square central basin with four sloping compartmental basins affixed to each of its sides, constructed from 2 mm-thick



Fig. 1: Newly designed solar still.

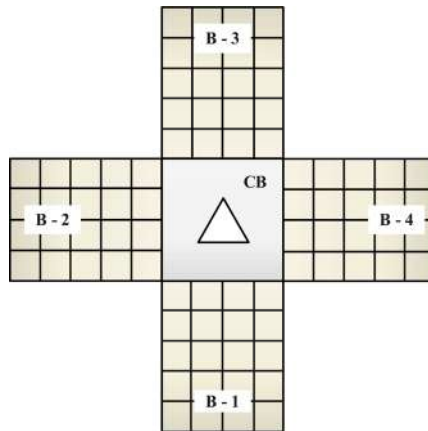


Fig. 2: An illustration of the Cross-sectional perspective of the trays.

iron sheet. The central basin was constructed with inner dimensions of $0.5\text{m} \times 0.5\text{m} \times 0.25\text{m}$. The central basin is converted into a compartmental basin, which is positioned on a metal platform and is tilted, and it was kept the plate is kept at the bottom. This plate exhibits a cross-step configuration. The four compartmental basins attached to each side of the center basin measure $1\text{m} \times 0.5\text{m} \times 0.25\text{m}$.

The compartmental plate features four pyramid patterns that span from one side of the still to the other. The 0.12-meter-wide compartment between each pair of pyramids is where the water to be distilled is stored.

The pyramid-shaped structures absorb solar energy and transfer it to the water in the basin, enabling the water to achieve a higher temperature more rapidly and maintain it for a longer period. Drains are attached to two sides of the basin to collect the distilled water. Inclined compartmental basins are connected to each of the four edges of the central basin. A glass cover that is 4 mm thick and inclined at a 30° angle is affixed to each compartmental basin. This inclined shape facilitates smooth condensation. Include clear and well-labeled diagrams to illustrate the following:

- The multi-directional inclined basin with pyramid-shaped compartments.
- The arrangement of basins (central and compartmental basins) and their dimensions.
- The 4 mm-thick inclined glass covers and their positioning for condensation.
- The one-step azimuth tracking mechanism and its 15° daily rotation.
- Locations of thermocouples, water drains, and sawdust insulation within the experimental setup.

The materials used in the experimental setup were carefully chosen to optimize performance and sustainability.

The 4 mm-thick glass covers, with a solar transmittance of over 90%, ensured maximum solar radiation penetration while minimizing heat loss during condensation. The 2 mm iron sheets used for the basins offered excellent thermal conductivity and durability, ensuring consistent heat transfer and prolonged system lifespan. To enhance insulation, sawdust was employed in the wooden enclosures, leveraging its high thermal resistance to prevent heat dissipation and maintain internal temperatures. The pyramid-shaped compartments utilized a cross-step configuration to accelerate heat absorption and retention. Various wick materials, such as black cotton and discarded fabrics, were evaluated for their superior water absorption properties and thermal stability. This meticulous selection of materials ensures cost-effective scalability and high operational efficiency for real-world applications.

Fig. 2 illustrates a cross-sectional representation of the boxes installed within the system. Each tray measures $0.2\text{m} \times 0.125\text{m} \times 0.25\text{m}$. They are arranged in a staircase pattern. Thus, it seems that the setup includes five interconnected basins. Because these basins are connected, the vapor is able to move freely between them. Additionally, the colder basin absorbs excess heat. The basin is entirely enclosed in a wooden box, and the space between the basin and the box is filled with sawdust to reduce heat loss to the ambient air given in Table 1.

Experimental Procedure

In this desalination setup, rectangular inclined basins are connected to all four sides of a central square basin. As illustrated in Fig. 3, the basin on the south side is designated as Basin 1, while the basins on the west, north, and east sides are labeled as Basin 2, Basin 3, and Basin 4, respectively. The central basin is referred to as Basin 5. The apparatus is intended to be spun to adjust the still's orientation for azimuth tracking.

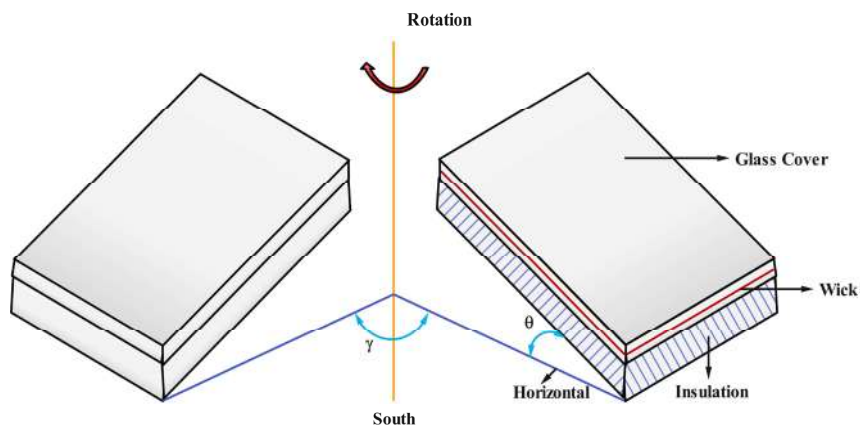


Fig. 3: Schematic representation of tilted azimuth angle tracking.

Researchers found that rotating the still daily to align with the sun's southern position might markedly enhance its distillate output. The still should be oriented according to the time and location of its installation. By rotating the inclined stepped basin solar still once a day, or using one-step azimuth tracking, distillate productivity can be significantly improved. This one-step azimuth tracking can be performed manually and does not require advanced technical skills, making the construction and maintenance of the still relatively easy.

The evaporating wick materials and glass covers are 4 mm thick and inclined at 30° over each compartmental basin. This inclined design facilitates smooth condensation. The orientation of the still, γ , tilted angle of the basins, θ , are manually adjusted for solar tracking to simplify construction and maintenance. The tilt angle of all basins remains fixed, and the still's orientation is adjusted just once daily to align with the sun's southern position. All the basins are rotated 15° southward in a clockwise direction each day. Consequently, Basins 1, 2, 3, and 4 are rotated daily towards the southwest, northwest, northeast, and southeast, respectively, and experiments are conducted accordingly. The experiments took place at Francis Xavier Engineering College in Tirunelveli (8.7321° N, 77.7241° E), Tamil Nadu, during March and April 2024. Measurements were recorded from 6 AM to 6 PM at one-hour intervals. The total yield of the still was analyzed and optimized across six different orientations.

Energy Balance Equation

Heat transport occurs through numerous means, both inside and outside, within the still. Heat exchange occurs between the water and glass cover through radiation, convection, and evaporation, as well as between the glass cover and the atmosphere via convection and radiation. Conductive heat transmission has been overlooked by researchers in

prior numerical studies, and our work adheres to the same omission. This experimental setup has four inclined basins and one horizontal central basin, necessitating consideration in the formulation of the energy balance equation. Heat transfer transpires both within and outside the stills.

Combined with the heat emitted by the vapor inside the still, the thermal energy received by the glass of the still is equivalent to the thermal dissipation through convection and radiation from the outside surface of the glass.

The energy balance equation is articulated as per the reference (El-Sebaai et al. 2004).

$$[A \sum_{n=1}^4 I_n \alpha_{gn} + A_{g5} I_5 \alpha_{g5}] + A \sum_{n=1}^4 [(h_{en,w-g} + h_{cn,w-g} + h_{rn,w-g})(T_{wn} - T_{gn})] + A_5 [(h_{e5,w-g} + h_{c5,w-g} + h_{r5,w-g})(T_{w5} - T_{g5})] = A \sum_{n=1}^4 [(h_{cn,g-a} + h_{rn,g-a})(T_{gn} - T_a)] + A_5 [(h_{c5,g-a} + h_{r5,g-a})(T_{g5} - T_a)] \quad \dots(1)$$

Here is the expression for the $Q_{c,w-g}$ (Q_c between water and glass):

$$\sum_{n=1}^4 Q_{cn,w-g} + Q_{c5,w-g} = A \sum_{n=1}^4 h_{cn,w-g} (T_{wn} - T_{gn}) + h_{c5,w-g} A_5 (T_{w5} - T_{g5}) \quad \dots(2)$$

Nagarajan et al. (2017) describe a method for determining the heat transfer coefficient (HTC) of evaporative ($h_{e,w-g}$) through the application of mass and heat transfer analogies, incorporating heat flux as detailed below, Sayigh & El-Salam (1977)

$$Q_{e,w-g} = 0.016 h_{c,w-g} A_b (P_w - P_g) \quad \dots(4)$$

and

$$Q_{e,w-g} = h_{e,w-g} A_b (T_w - T_g)$$

Applying Qe and w-g into the aforementioned equation yields Tiwari and Lawrence (1989).

$$h_{e,w-g} = 0.016 h_{c,w-g} A_b [(P_w - P_g) / (T_w - T_g)] \quad \dots(5)$$

Applying the previous formulas to our system, we get the subsequent equation.

$$\begin{aligned} \sum_{n=1}^4 h_{en,w-g} + h_{e5,w-g} &= 0.016 \sum_{n=4}^4 h_{cn,w-g} \\ &[(P_{wn} - P_{gn}) / (T_{wn} - T_{gn})] + \\ &0.016 h_{c5,w-g} [(P_{w5} - P_{g5}) / (T_{w5} - T_{g5})] \quad \dots(6) \end{aligned}$$

The volume of water collected on the glass's inside surface is referred to as (Zurigat & Abu-Arabi 2004)

$$\sum_{n=1}^4 m_{en,w-g} + m_{e5,w-g} = [(\sum_{n=1}^4 Q_{en,w-g} \times 3600) / h_{fgn}] + [(Q_{e5,w-g} \times 3600) / h_{fg5}] \quad \dots(7)$$

The HTC of radiative (hr, W/g) per unit area between water and cover glass is expressed by Nagarajan et al. (2017) in accordance with Charters (1977).

$$\sum_{n=1}^4 h_{rn,w-g} + h_{r5,w-g} = [\sigma \epsilon \sum_{n=1}^4 (T_{wn}^2 + T_{gn}^2) (T_{wn} + T_{gn})] + [\sigma_5 \epsilon_5 (T_{w5}^2 + T_{g5}^2) (T_{5n} + T_{g5})] \quad \dots(8)$$

Heat Exchange Between the Atmosphere and Glass Surface

Typically, convective and radiative heat transfers happen on the side wall, glass surface, and bottom of the still; however, due to the insulating layers on the bottom and side wall, heat transfer transpires just from the glass surface. Nagarajan et al. (2017) assert that the convective transfer of heat from the still is influenced by the surrounding wind flow and the HTC of convective, represented as:

$$\begin{aligned} \text{Wind speed} &\leq 5 \text{ m.s}^{-1} \text{ Madhlopa \& Johnstone (2009)} \\ h_{c,g-a} &= 2.8 + 3u \quad \dots(9) \end{aligned}$$

$$\begin{aligned} \text{wind speed} &> 5 \text{ m s}^{-1} \text{ (Tanaka 2010)} \\ h_{c,g-a} &= 5.7 + 2.8u \quad \dots(10) \end{aligned}$$

Qc is defined as follows:

$$\begin{aligned} \sum_{n=1}^4 Q_{cn,g-a} + Q_{c5,g-a} &= \sum_{n=1}^4 h_{cn,g-a} (T_{gn} - T_{an}) \\ &+ h_{c5,g-a} (T_{g5} - T_{a5}) \quad \dots(11) \end{aligned}$$

The radiative transfer of heat (hr) from the solar still is dependent on the Tg and Tsky. The Tsky is defined as a function of Ta, as articulated by Duffie & William (1977).

$$T_{sky} = 0.05525 T_a^{1.5} \quad \dots(12)$$

The Qr is provided by Shukla & Sorayan (2005).

$$Q_{r,g-a} = \sigma \epsilon_g (T_g^4 - T_{sky}^4) \quad \dots(13)$$

and

$$Q_{r,g-a} = h_{r,g-a} (T_g - T_a) \quad \dots(14)$$

Utilizing the aforementioned formulas in our system yields the subsequent equation.

$$\sum_{n=1}^4 h_{rn,g-a} + h_{r5,g-a} = \sigma \epsilon_g \sum_{n=1}^4 [(T_{gn}^4 - T_{sky}^4) / (T_{gn} - T_{an})] + \sigma \epsilon_g [(T_{g5}^4 - T_{sky}^4) / (T_{g5} - T_{a5})]$$

RESULTS AND DISCUSSION

The Impact of Temperature and Solar Intensity on Productivity

Fig. 4 depicts the variations in air temperature and global radiation during the experimental duration. During the experimental duration, the atmosphere was consistently clear and devoid of frost. The experimental data from days exhibiting standard atmospheric conditions, as seen in Fig. 4, were analyzed. Maximum temperatures occurred during midday when solar radiation peaked, thereafter decreasing as solar energy diminished.

The yield of the sloped compartmental SS is typically augmented by the progressive rise in ambient temperature. The trials recorded the Tg and Tw in the basins, with glass temperatures designated as Tg1, Tg2, Tg3, Tg4, and Tgcb, and water temperatures as Tw1, Tw2, Tw3, Tw4, and Twcb, respectively. The output rate of the sloped stepped solar system was assessed despite an increase in the temperature differential between the inside and outside glasses. Fig. 5 and Fig. 6 depict the fluctuations in water and glass temperatures within the system.

Effect of One-Step Azimuth Tracking on the Productivity

Anburaj et al. (2013) showed that it functions well at a 30° angle when directed southward. In their experiments, just the orientation of the still was modified while maintaining a constant inclination angle. The configuration facilitates rotation, permitting azimuth tracking through the alteration of the still's orientation. Our findings demonstrate that moving the still once daily towards the sun's southern position markedly influences performance. The outcomes of experiments performed with the still in different orientations are outlined below.

One-step Azimuth tracking at $\gamma = 0^\circ$: Basin 1 (B1) was situated on the southern side and served as the reference point, while Basin 2 (B2), Basin 3 (B3), and Basin 4 (B4) were located on the western, northern, and eastern sides, respectively. Basin 5 was identified as the central basin (CB). As a result, the orientation of the still was established at $\gamma = 0$. Fig. 7 illustrates the hourly yield for each basin

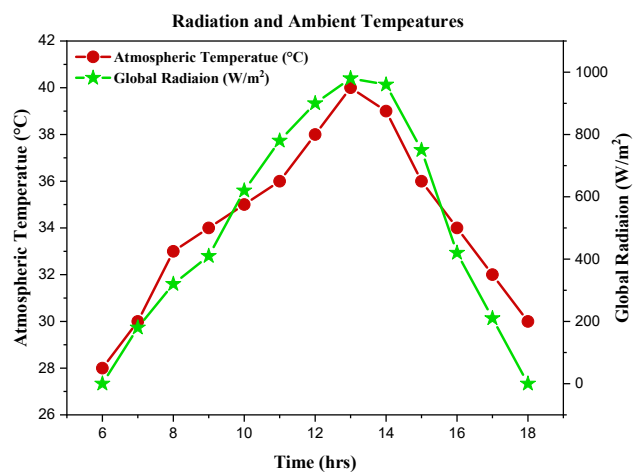


Fig. 4: Comparison of ambient temperature (T_a) and global radiation over time.

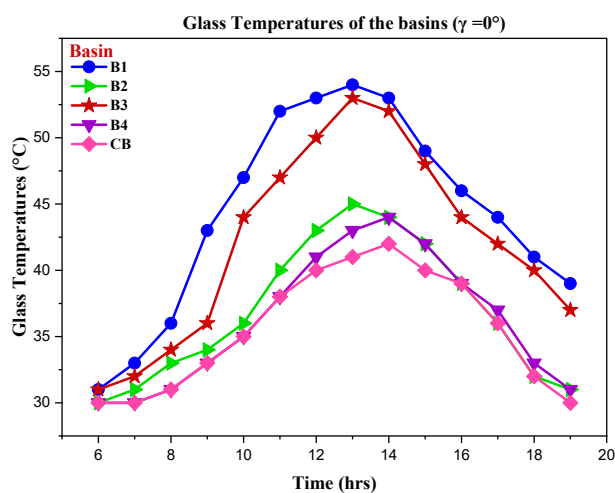


Fig. 5: Evaluation of glass temperature of various basins with respect to time of $\gamma = 0^\circ$.

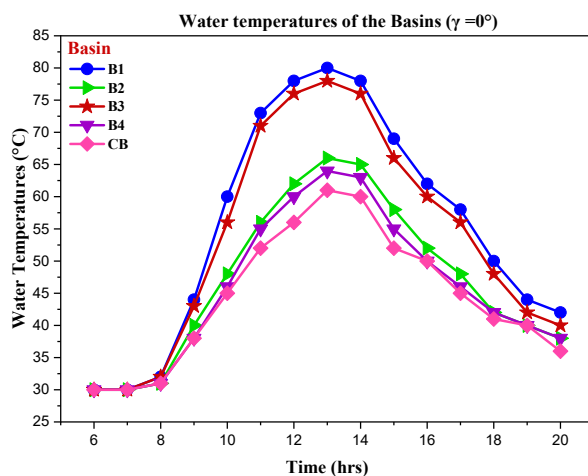


Fig. 6: Evaluation of water temperature of various basins with respect to time at $\gamma = 0^\circ$.

in that configuration. On the inaugural day of the trial, Basin 1, oriented to the south, yielded the highest output of 5.780 L.day^{-1} . After that, Basin 3, which was facing north, created 5.560 L.day^{-1} . Basin 2, facing west, produced approximately 3.740 L.day^{-1} , while basin 4, facing east, yielded about 3.890 L.day^{-1} . In contrast, basin 5, located in the middle, generated 1.33 L.day^{-1} . The total daily yield was 20.305 liters. Owing to India's location, basins 1 and 3, oriented southward and northward, respectively, receive increased sun radiation, resulting in superior yields. Basin 2, oriented westward, receives solar radiation exclusively post-noon, whereas basin 4, oriented eastward, is illuminated solely in the morning. Notwithstanding the central basin's 0° inclination angle, it features a pyramid-shaped structure

intended to harness solar energy and absorb surplus heat from the other basins, enabling it to generate a certain yield. Table 2 shows the temperature variation for water for various basins on Day 1, Solar Still, with its productivity.

One-step Azimuth tracking at $\gamma = 15^\circ$: The following day, the entire system was turned 15° clockwise for one-step azimuth tracking, correcting the still's orientation to $\gamma = 15^\circ$. Subsequently, the readings were documented. Fig. 8 illustrates the hourly yield for each basin in that orientation. Basin 1, formerly located on the southern side, shifted southwest, resulting in a reduction in morning production. Similarly, basin 2, originally on the west side, switched to the northwest direction, Basin 3, from the north side, went to the northeast direction, and basin 4, on the east side,

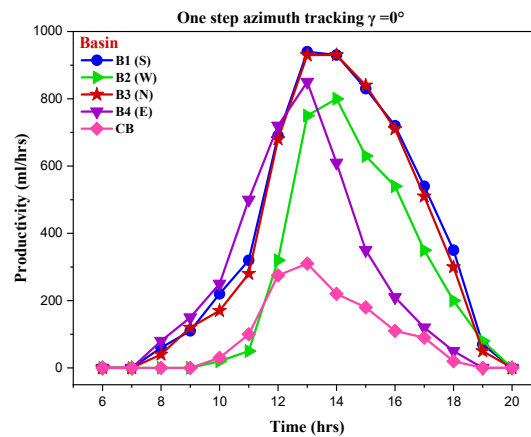


Fig. 7: Evaluation of the productivity of one-step Azimuth tracking at $\gamma = 0^\circ$ with respect to time.

Table 2: Temperature variation for water for various basins on Day 1, Solar Still with its productivity.

Time	Global Radiation	Basin 1	Basin 2	Basin 3	Basin 4	Central Basin	Productivity
hour	W/m^2	$^\circ\text{C}$	$^\circ\text{C}$	$^\circ\text{C}$	$^\circ\text{C}$	$^\circ\text{C}$	mL/hrs
6:00	0	30	30	30	30	30	0
7:00	180	30	30	30	30	30	0
8:00	320	32	31	32	31	31	180
9:00	410	44	40	43	38	38	380
10:00	620	60	48	56	46	45	690
11:00	780	73	56	71	55	52	1250
12:00	900	78	62	76	60	56	2685
13:00	980	80	66	78	64	61	3780
14:00	960	78	65	76	63	60	3490
15:00	750	69	58	66	55	52	2830
16:00	420	62	52	60	50	50	2290
17:00	210	58	48	56	46	45	1610
18:00	0	50	42	48	42	41	920
19:00	-	44	40	42	40	40	200
20:00	-	42	38	40	38	36	0

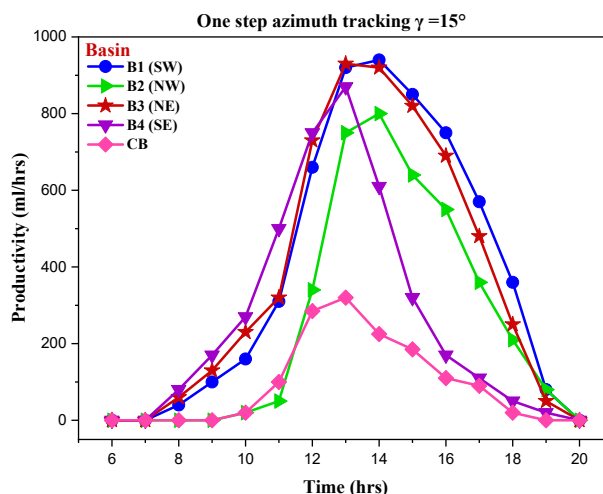


Fig. 8: Evaluation of the productivity of one-step Azimuth tracking at $\gamma = 15^\circ$ with respect to time.

transitioned to the southeast direction. In this arrangement, the yield of Basin 1 is inferior to that seen in the initial experiment, although the yields in the other three basins are marginally elevated. The total yield rises by 0.59% relative to the initial experiment.

One-step Azimuth tracking at $\gamma = 30^\circ$: On the third day, a 30° clockwise rotation of the complete setup resulted in a modification of basin-wise yield. Fig. 9 depicts the hourly yield of each basin in that configuration. Basin 1 is advancing southwestward, leading to a diminished production. Basin 2, heading towards the northwest, has a little higher yield. Basin 3, oriented northeast, exhibits a little enhancement in morning output. Likewise, Basin 4, located in the southeast, exhibits an elevated morning output. These modifications affected the yield as a result of fluctuations in solar radiation.

Although particular basins exhibited a significant variation in production, the overall output in this experiment increased by 0.76%.

$\gamma = 45^\circ$ of Azimuth tracking: On the fourth day of the experiment, the rig was turned 45° clockwise, and the experiment was repeated. As a result, the yields in Basin 1 and Basin 3 declined dramatically. This was mostly attributable to a drop in morning yield in Basin 1, which was now oriented in the southwest direction.

Similarly, the evening yield in Basin 3, now oriented in the northeast direction, has reduced. Nonetheless, the yields in Basin 2 and Basin 4 experienced an increase. The rise in Basin 2, located in the northwest, resulted from elevated afternoon temperatures, which contributed to an increased yield. In Basin 4, now oriented southeast, the morning output

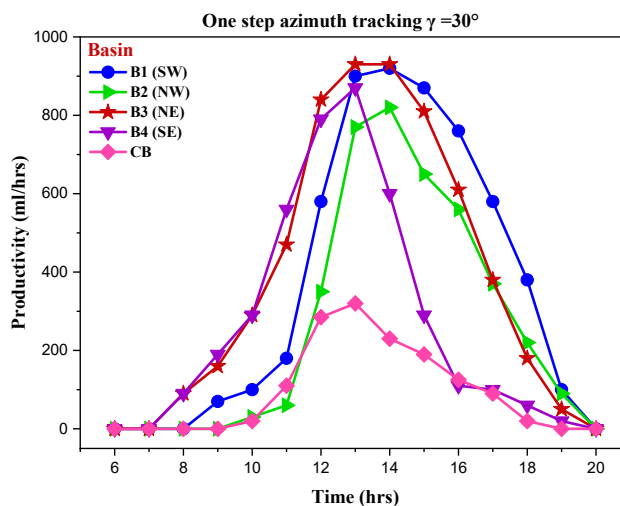


Fig. 9: Evaluation of the productivity of one-step Azimuth tracking at $\gamma = 30^\circ$ with respect to time.

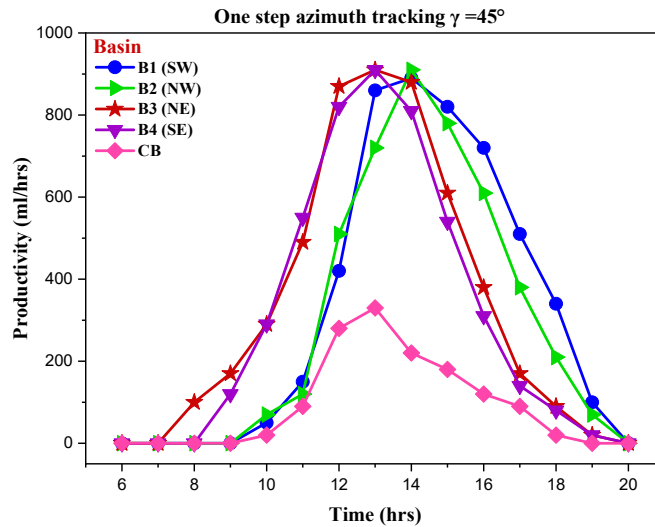


Fig. 10: Evaluation of the productivity of one-step Azimuth tracking at $\gamma = 45^\circ$ with respect to time.

varied due to elevated temperatures. The yield decline in Basins 2 and 4 surpasses the yield increases in Basins 1 and 3, resulting in a net reduction of 0.71%. The variations are illustrated in Fig. 10.

$\gamma = 60^\circ$ of Azimuth tracking: On the fifth day of the experiment, the apparatus was rotated by 60° . Consequently, yields in Basin 1 and Basin 3 diminished for the aforementioned reasons, whereas yields in Basin 2 and Basin 4 augmented. In Basins 2 and 4, situated in the northwest and southeast, the yield rose in both the morning and evening, culminating in a total yield increase of 0.90%. Fig. 11 depicts the hourly basin-specific yields in such orientations.

$\gamma = 75^\circ$ of Azimuth tracking: On the sixth day, the

experimental equipment was rotated 75° , and the experiment was conducted. Fig.12 depicts the hourly-based basin-wise yields in that orientation. As a result, yields declined in Basin 1 and Basin 3. Basin 1, currently oriented nearly westward, encountered a substantial decline in morning yield, whereas Basin 3, virtually oriented eastward, experienced a dramatic loss in yield post-noon. In contrast, yields in Basin 2 and Basin 4 rose. Basin 2, nearly facing north, and Basin 4, practically facing south, both saw higher temperatures due to sun radiation throughout the day, resulting in an overall yield increase of 0.81%.

$\gamma = 90^\circ$ of Azimuth tracking: Finally, when the experimental setup was rotated 90° , the directions of the four basins

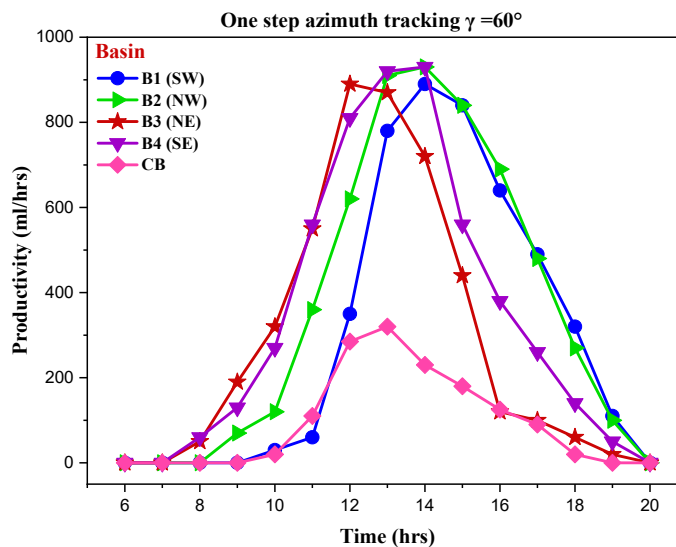


Fig. 11: Evaluation of the productivity of one-step Azimuth tracking at $\gamma = 60^\circ$ with respect to time.

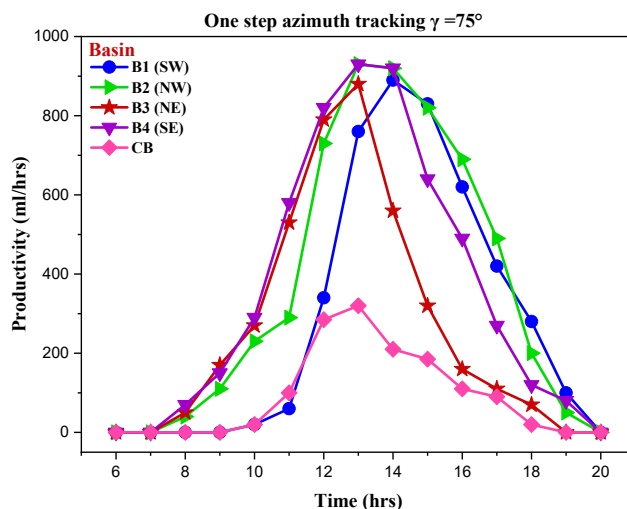


Fig. 12: Evaluation of the productivity of one-step Azimuth tracking at $\gamma = 75^\circ$ with respect to time.

changed: Basin 1 traveled from south to west, Basin 2 from west to north, Basin 3 from north to east, and Basin 4 from east to south. The basin-wise yield in this experiment was nearly identical to that on the first day. The total yield was $20.295 \text{ L.day}^{-1}$, as illustrated in Fig. 13.

Overall Productivity

A multi-directional inclined compartmental basin solar still was designed and evaluated utilizing diverse wick materials within the basins to optimize heat absorption and augment yield. The still has multiple basins, with testing revealing that Basin 1, facing south, had the maximum yield at 5.780 L.day^{-1} , followed by Basin 3, facing north, with

5.560 L.day^{-1} . The aggregate production from all basins was $20.305 \text{ L.day}^{-1}$. The apparatus facilitates rotation, permitting studies with constant tilt angles (θ) and diverse orientations ($\gamma = 0^\circ, 15^\circ, 30^\circ, 45^\circ, 60^\circ, 75^\circ$, and 90°) in various azimuth tracking configurations. Daily, the basins are rotated 15° clockwise, with Basin 1, 2, 3, and 4 positioned southwest, northwest, northeast, and southeast, respectively, for experimental purposes.

The experimental apparatus was rotated to a distinct orientation angle daily, and measurements were recorded. The differences depicted in Fig. 14 demonstrate the efficacy of the experimental setup irrespective of direction. The overall productivity for all azimuth angles for 7 days is illustrated in Fig. 15.

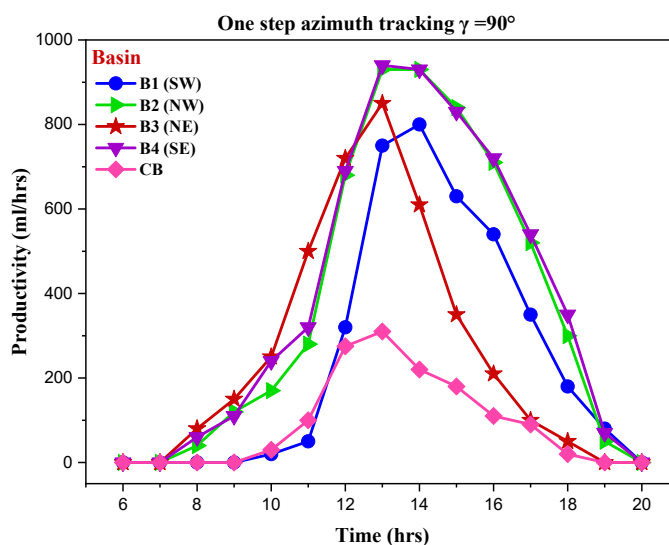


Fig. 13: Evaluation of the productivity of one-step Azimuth tracking at $\gamma = 90^\circ$ with respect to time.

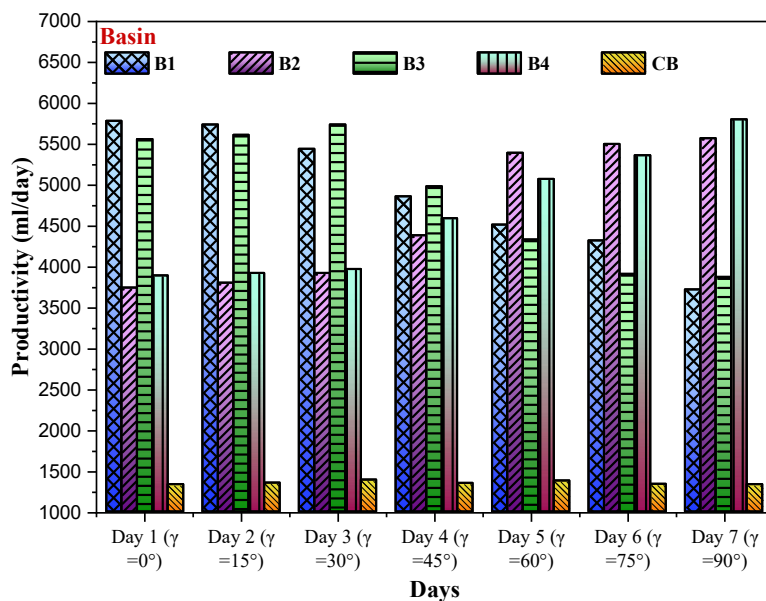


Fig. 14: Evaluation of productivity for all azimuth angles with respect to the individual basin for 7 days.

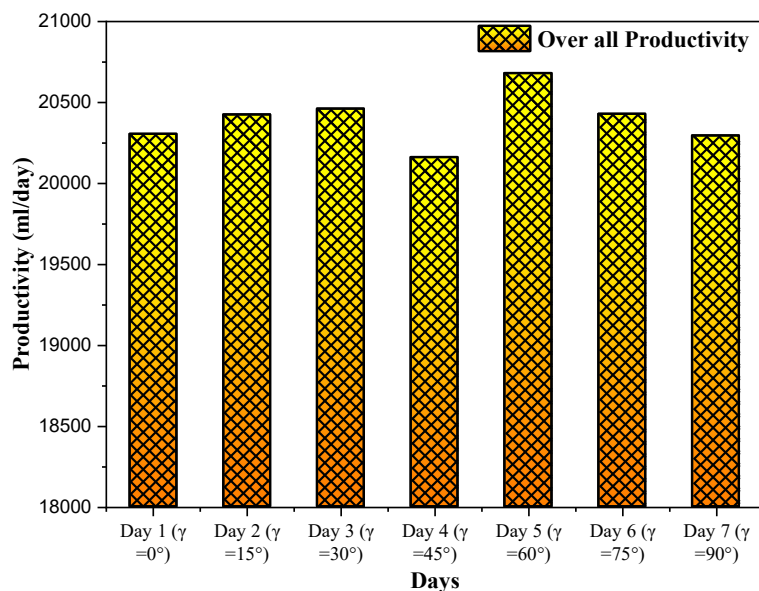


Fig. 15: Evaluation of overall productivity for all azimuth angles for 7 days.

Cost-Benefit Analysis

A cost-benefit analysis of implementing the system in real-world scenarios was shown in Tables 3,4,5.

Productivity and Cost Per Liter

Daily Productivity: 20.305 L.day⁻¹

Annual Productivity:

$$20.305 \text{ liters/day} \times 365 \text{ days} = 7,411.33 \text{ liters/year}$$

Cost Per Liter (First Year):

$$\frac{\text{₹}25,000}{7,411.33 \text{ liters}} = \text{₹}3.37 \text{ per liter}$$

Payback Period

Water Procurement Cost Savings:

Initial Cost Breakdown

Table 3: Initial cost breakdown for solar still setup and estimated productivity.

Component	Quantity	Unit Cost [₹]	Total Cost [₹]
Iron Sheets (2mm)	5 sheets	1,500/sheet	₹7,500
4mm Glass Covers	4 covers	1,000/cover	₹4,000
Wooden Frame (Insulated)	1 frame	5,000/unit	₹5,000
Sawdust (for Insulation)	10 kg	50/kg	₹500
Drain Pipes and Accessories	4 units	250/unit	₹1,000
Labor Costs	1 setup	5,000	₹5,000
Miscellaneous (Paint, Sealants)	-	2,000	₹2,000
Total Initial Cost	-	-	₹25,000

Assuming ₹10/liter (average cost of bottled water in rural areas):

$$7,411.33 \text{ L.day}^{-1} \times 74,113.30 \text{ ₹} = 10 \text{ ₹ savings/year}$$

Payback Period:

$$\frac{₹25,000(\text{initial cost})}{₹74,113.30(\text{annual savings})} = 0.34 \text{ years (approx. 4 months)}$$

- **Initial Investment:** ₹25,000
- **Operational Cost:** ₹2,333/year
- **Payback Period:** 4 months
- **Lifetime Cost Per Liter (5 Years):** ₹0.99
- **Benefit:** Low-cost potable water production, scalable

design, and rapid ROI make this system highly feasible for real-world deployment.

Environmental Benefits and Scalability Analysis

The proposed solar desalination system significantly reduces the carbon footprint by relying solely on renewable solar energy, eliminating the need for fossil fuels. Its low-cost materials and minimal operational requirements make it scalable across diverse geographic regions, especially in solar-rich areas like arid and coastal regions. Additionally, the system's eco-friendly design minimizes waste, contributing to sustainable development goals. By offering a decentralized solution, it enhances water security in remote and underdeveloped regions, promoting environmental and social sustainability shown in Table 6.

Operational Costs

Table 4: Annual operational costs for solar still maintenance and component replacement.

Component	Frequency	Annual Cost [₹]
Cleaning and Maintenance	Quarterly (₹500/visit)	₹2,000
Glass Replacement	Every 3 years	₹1,000 (₹333/year prorated)
Total Annual Operational Cost	-	₹2,333
Component	Frequency	Annual Cost [₹]
Cleaning and Maintenance	Quarterly (₹500/visit)	₹2,000

Lifetime Cost Analysis (Over 5 Years)

Table 5: Five-year cumulative cost-benefit analysis for solar still system.

Year	Initial Cost (₹)	Operational Cost [₹]	Cumulative Cost [₹]	Cumulative Productivity (Liters)	Cost Per Liter (₹)
1	₹25,000	₹2,333	₹27,333	7,411.33	₹3.69
2	₹0	₹2,333	₹29,666	14,822.66	₹2.00
3	₹0	₹2,333	₹31,999	22,233.99	₹1.44
4	₹0	₹2,333	₹34,332	29,645.32	₹1.16
5	₹0	₹2,333	₹36,665	37,056.65	₹0.99

Table 6: Environmental Implications Table.

Aspect	Details	Environmental Benefit
Energy Source	100% solar-powered, eliminating fossil fuel dependency	Significant carbon footprint reduction
Material Usage	Low-cost, eco-friendly materials like iron sheets and sawdust insulation	Reduces waste and promotes resource sustainability
Geographic Scalability	Adaptable for arid, coastal, and solar-rich regions	Provides clean water in areas with water scarcity
Waste Reduction	Minimal operational waste and no harmful by-products	Low environmental impact and better ecosystem preservation
Decentralized Implementation	Suitable for remote and underdeveloped areas, requiring no grid electricity	Promotes environmental sustainability in off-grid locations

Comparative Analysis

The proposed multi-directional solar desalination system demonstrates a significant productivity improvement of up to 20.305 L.day⁻¹, surpassing conventional systems producing 4–10 L.day⁻¹. Its one-step azimuth tracking mechanism enhances solar exposure by up to 0.90%, compared to limited or costly tracking in existing designs shown in Table 7. The pyramid-shaped compartments improve thermal efficiency without requiring nanotechnology or hybrid setups. Low-cost materials like iron sheets and sawdust insulation make the system scalable and eco-friendly. Comprehensive testing across seven orientations ensures broader applicability in diverse solar-rich regions. This design effectively combines

sustainability, affordability, and high productivity to address global water scarcity depicted in Table 8.

CONCLUSIONS

The study reveals that the multi-directional inclined compartmental basin solar desalination system obtained its best yield of 20.305 L.day⁻¹ at the 0° orientation, with individual basins producing as follows: Basin 1 (south-facing) produced 5.780 L.day⁻¹, Basin 3 (north-facing) 5.560 liters/day, Basin 2 (west-facing) 3.740 L.day⁻¹, Basin 4 (east-facing) 3.890 L.day⁻¹, while the middle Basin 5 produced 1.33 L.day⁻¹. Upon rotating the setup to 15°, the overall productivity experienced a marginal improvement

Table 7: A comparison of results with existing systems.

Feature/Parameter	Existing Systems (Literature Review)	Proposed System (Your Study)	Improvement (%)
Daily Productivity (L/day)	4–10 L.day ⁻¹ (Jathar & Ganesan, 2021, Morad et al. 2015, Panchal et al. 2023)	20.305 L.day ⁻¹ at 0° orientation	103–408% higher productivity than conventional systems
Basin-wise Yield Distribution	Uneven distribution, with the highest yields typically in southern-facing basins (Liu et al. 2023, Tareemi et al. 2023)	South: 5.780 L.day ⁻¹ , North: 5.560 L.day ⁻¹ , West: 3.740 L.day ⁻¹ , East: 3.890 L.day ⁻¹	Optimized and balanced yields across basins, utilizing multi-directional design
Impact of Tracking Mechanism	Limited tracking or expensive automated systems (Tareemi et al. 2024, Panchal et al. 2023)	One-step azimuth tracking improves yield by up to 0.90% with a daily 15° rotation.	Low-cost, effective tracking system ensuring enhanced solar exposure.
Thermal Efficiency Enhancements	Nanofluids, hybrid collectors, or reflectors moderately improve performance (Elsheikh et al. 2024, Liu et al. 2023)	Pyramid compartments concentrate heat and maintain elevated temperatures	Achieves sustained thermal efficiency without reliance on costly external modifications
Cost and Material Feasibility	Advanced systems rely on nanotechnology or expensive automated components (Tareemi et al. 2023, Abdullah et al. 2024)	Low-cost materials (iron sheets, sawdust insulation, manual tracking)	Scalable and highly affordable for diverse environments
Orientation Testing	Limited to static or semi-dynamic setups with restricted azimuth angles (Abdullah et al. 2024, Panchal et al. 2023)	Comprehensive testing across 7 orientations (0°, 15°, 30°, 45°, 60°, 75°, 90°)	Detailed productivity analysis across diverse angles ensures broader applicability.
Environmental Impact	Passive systems with low carbon footprint but limited scalability (Panchal et al. 2023, Elsheikh et al. 2024)	Sustainable design with significantly enhanced productivity	Combining sustainability with practicality, addressing water scarcity in solar-rich regions

Table 8: A deeper analysis of why certain orientations yield higher productivity.

Orientation	Productivity Characteristics	Reasons for Productivity Variation	Key Advantage in Proposed System
South-facing (0°)	Highest productivity (e.g., 5.780 L.day ⁻¹ for Basin 1)	Receives maximum direct solar radiation throughout the day in the Northern Hemisphere.	Optimized design maximizes solar exposure in the most productive direction.
North-facing (180°)	High productivity (e.g., 5.560 L.day ⁻¹ for Basin 3)	Benefits from indirect radiation and scattered sunlight, particularly in regions with reflective surfaces or diffuse radiation.	Pyramid compartments enhance heat retention, compensating for reduced direct exposure.
East-facing (90°)	Moderate productivity during morning hours (e.g., 3.890 L.day ⁻¹ for Basin 4).	Captures early-morning solar radiation, which is lower in intensity but critical for kickstarting the evaporation process.	Efficient thermal conductivity and design prevent energy loss during low-intensity radiation periods.
West-facing (270°)	Moderate productivity during afternoon hours (e.g., 3.740 L.day ⁻¹ for Basin 2).	Absorbs solar radiation in the afternoon when sunlight intensity increases, but the duration is shorter compared to the south-facing basin.	Heat concentration and insulation maintain effective evaporation in afternoon periods.
Inclined at 15°–60°	Slight productivity increase (up to 0.90%) compared to 0° orientation.	Adjustments in azimuth angle ensure improved solar alignment, reducing shadow effects and enhancing energy absorption throughout the day.	Manual azimuth tracking optimizes exposure with minimal complexity or cost.
Inclined at 75°–90°	Slight decline in productivity (e.g., ~0.71%) as basins shift away from optimal alignment.	Reduced alignment with direct solar radiation leads to lower energy absorption, especially in the morning or late afternoon.	Maintains reasonable productivity through thermal retention and efficient heat distribution in pyramid-shaped compartments.

to 20.425 L.day⁻¹, reflecting a 0.59% enhancement from the 0° orientation. At a 30° tilt, the yield increased to 20.460 L.day⁻¹, reflecting a 0.76% rise relative to the baseline. Following a 45° rotation, productivity fell somewhat to 20.161 L.day⁻¹, attributable to diminished sun exposure for certain basins, indicating a 0.71% decline. At a 60° orientation, the apparatus attained a yield of 20.487 L.day⁻¹, representing the most significant increase of 0.90%. For the 75° orientation, productivity was 20.471 L.day⁻¹, a 0.81% increase from the baseline. Finally, at 90° orientations, the yield was restored close to the initial production level at 20.295 L.day⁻¹, practically identical to the 0° configuration. The findings validate that the one-step azimuth tracking technique improves distillate production, exhibiting minor fluctuations depending on orientation, and indicate ideal angles for various climatic circumstances to optimize water purification by solar energy.

ABBREVIATIONS

A: Surface area of the basin or cover (m²)

A5: Surface area of the secondary region (m²)

Tw: Temperature of Water (°C)

Tg: Glass temperature (°C)

Ta: Ambient temperature (°C)

hr: Heat transferring rate for Radiative (W/m²·K)

hc: Heat transfer coefficient for Convection (W/m²·K)

Qc: Heat transferring rate for Convective (W)

he: Heat transfer coefficient for evaporation (W/m²·K)

Qr: Heat transferring rate for Radiative (W)

Qe: heat transferring rate for Evaporation (W)

Pw: Water vapor pressure at the water surface (Pa)

mf: Mass flow rate of water vapor (kg/s)

hfg: Latent heat of vaporization (J/kg)

Pg: Water vapor pressure at the glass surface (Pa)

ε: Surface Emissivity (dimensionless)

σ: Stefan-Boltzmann constant

Cp: Water Specific heat capacity (J/kg·K)

u: Wind velocity (m/s) Tsky: Sky temperature (°C)

ΔT\Delta TΔT: Temperature difference between surfaces (°C)

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Exploring the Role of Environmental Education in Promoting Sustainability Mindset: An Empirical Analysis Using Forward and Hierarchical Regression Methods

Archana Singh^{1†}, Roshan Kazi², Swapnil Gorde^{1,3}, Rishi Prakash Shukla⁴, Amol Gawande¹ and Atul Kumar¹

¹Dr. D. Y. Patil B-School, Bengaluru-Mumbai Express Bypass, Tathawade, Pune-411033, Maharashtra, India

²MCE Society, Dr. P. A. Inamdar University, Allana Institute of Management Sciences, Azam Campus, K. B. Hidayatullah Road, New Modikhana, Pune-411001, Maharashtra, India

³Symbiosis International (Deemed) University, Lavale, Pune, India

⁴Jaipuria Institute of Management, Jaipur, India

†Corresponding author: Archana Singh; drarchana.singh@dpu.edu.in

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ABSTRACT

This study employed Forward Regression Analysis and Hierarchical Regression Analysis to explore the connection between ecological literacy, ecological regulations, ecological awareness, ecological education, and the Sustainability Mindset. The study participants were higher education students from Pune who had received environmental education and could provide information on their ecological education, literacy, awareness, regulation, and sustainability mindset. The results indicate that elements such as knowledge levels among individuals and communities, alongside policies implemented for environmental protection measures and public awareness campaigns, coupled with educational endeavours, collectively account for a significant portion of the variations observed in fostering sustainable attitudes and behaviours. Ecological knowledge has become increasingly crucial in promoting development beyond the environment. Experts have emphasized the importance of understanding how these factors are interconnected. This study's findings have several implications. It could help shape tailored educational initiatives and policies that support sustainable growth. Moreover, the valuable insights from this research could greatly benefit decision makers and academics dedicated to enhancing sustainability. The results revealed that the strongest predictor of a Sustainability Mindset is Ecological literacy, followed by ecological regulations, ecological awareness, and ecological education. All four independent variables were significant predictors of Sustainability Mindset. This study remains a rich area for future exploration in education and sustainable development, with important implications for policymakers and sustainability professionals.

INTRODUCTION

In light of the current climate crisis, global initiatives towards sustainable development are crucial for a more sustainable future. This study uses forward and hierarchical regression analyses to investigate the connection between environmental sustainability and the promotion of the United Nations Sustainable Development Goals (SDGs). This study aims to better understand how ecological literacy, regulations, awareness, and education contribute to sustainable development by fostering a sustainable mindset by integrating sustainability principles into higher education institutions' curricula, research, and community engagement.

Universities emphasize sustainability by engaging in research and educational programs to support Sustainable Development Goals (SDGs). Education is crucial for promoting nations' shared goals. The Education for Sustainable Development (ESD) initiative by UNESCO is an approach to fostering a community and

making progress towards achieving the SDGs by 2030. This study bridges the knowledge gap by demonstrating how these educational initiatives effectively contribute to achieving development outcomes and fostering enduring consciousness among students and alumni from areas such as the Global South.

This study employs forward and hierarchical regression analyses to examine how various elements of education collaborate to enhance results in Higher Education Institutions by recognizing and elucidating crucial sustainability dimensions and obstacles. Addressing this gap in understanding and offering insights derived from the study's findings could potentially assist policymakers, educational institutions, and organizations in devising more effective sustainability education strategies and policies tailored to motivate students to embrace behaviors aligned with the sustainability development goals (SDGs).

Background

This study highlights the importance of education in driving development and achieving the United Nations Sustainable Development Goals (SDGs). Many colleges and universities have incorporated sustainability values into their curricula and study initiatives as community engagement activities to recognize their contribution to promoting sustainable development (Asdlori & Yahya 2023, Atthakorn et al. 2024).

The global significance of sustainability has led universities to play a role in contributing towards Development Goals (SDGs) through their focus areas of research efforts, educational initiatives, and community involvement (Saleh & Adly 2024). UNESCO has strongly advocated Education for Sustainable Development (ESD) as a tool for establishing a society & accomplishing the SDGs by 2023 (Handayani 2019). Higher education institutions are highly valued, as they shape students into individuals who understand the importance of sustainability in balancing the environment, economy, and society (Handayani 2019).

Incorporating sustainability into leadership and management is essential for instilling a culture of practice and achieving long-term objectives (Atthakorn et al. 2024). This goes beyond curriculum development to include implementing policies and encouraging leadership while actively involving stakeholders in supporting sustainable growth and addressing economic, environmental, and social issues. There is an emphasis on encouraging the industry to embrace responsibility and promote social entrepreneurship as part of this approach (Diez-Busto et al. 2022).

Adopting an educational approach that supports progress is crucial (Annan-Diab & Molinari 2017). Universities must align their policies, funding, and course offerings with Sustainable Development Goals (Saleh & Adly 2024). The

advancement of development and management promotion depends heavily on educational institutions cultivating human resources by incorporating ESD principles into their curricula (Handayani 2019). This study is significant in advancing environmental development objectives. With the increasing focus on practices and the importance of responsible development and environmental care becoming a top priority for the public, this research is now more crucial than ever.

Aim/Objective

The main goal of this research was to analyze how “ecological literacy, regulations, awareness, and education” impact the sustainability mindset through forward and hierarchical regression analysis. The primary focus was to offer perspectives on the efficiency of environmental education initiatives and their influence on behavior by studying these associations. This analysis could assist decision-makers in refining their approaches to foster a culture of sustainability among individuals, while also aiding educators and environmental groups in enhancing their strategies for sustainability awareness.

Novelty

We used the forward and hierarchical regression methods to analyze the environmental education variables in promoting the sustainability mindset, a unique and novel approach in sustainable development research.

Rationale

This study aims to provide an understanding of how environmental education supports the achievement of development goals by investigating challenges that impede the implementation of environmental education programs and proposing solutions to overcome them while also exploring the enduring impact of “ecological literacy,” “ecological regulations,” “ecological awareness, and “ecological education” impacts sustainability mindset. Exploring the influence of awareness on attitudes and behaviors can offer perspectives for fostering a culture of sustainability across diverse populations. Understanding the impact of education could inspire strategies and programs to promote lasting behaviors and practices in communities.

This method supports the environmental education objectives of reshaping individuals' views and actions towards sustainability.

LITERATURE REVIEW

Previous studies have shown that environmental education plays a crucial role in promoting sustainability; however, it does not explain the specific mechanism through which we

can achieve sustainable development and the attainment of SDGs.

Various factors, such as demographics and shape attitudes towards sustainable development. Educational policies must understand the diverse needs and characteristics of different social groups and be sufficiently flexible. (Concina & Frate 2023, Fanea-Ivanovici & Baber 2022). A comprehensive higher education approach is needed to address environmental issues proactively. However, this process requires not only understanding the concept of sustainability but also implementing the process of sustainable development by educators and students (Gupta et al. 2023).

Higher education institutions currently focus on ecological education research. Highlighting the significance of cultivating a mindset in students' learning journeys, according to Barth et al. (2007), underscores the importance of environmental competence in achieving development goals. Existing education systems have not sufficiently incorporated education into their curricula, leading to a lack of awareness about development among future generations, as mentioned by Sterling & Orr (2001).

Ecological Education and Sustainable Development

Ecological education encompasses concepts aimed at enhancing individuals' comprehension of ecosystems and functions, emphasizing the significance of acquiring knowledge to foster a mindset centered on sustainability and conscientious environmental actions (Ha et al. 2021). When people participate in environmental education programs, they learn about the importance of conserving resources for growth and development (Zevallos 2024). Integrating education into the Sustainable Development Goals framework aligns with promoting sustainability and increasing awareness of conservation (Boca & Saraçlı 2019). Environmental education also involves sharing knowledge on practices and encouraging behaviors such as conservation and sustainable economic development (Xu 2023).

Ecological Literacy and Awareness

Understanding literacy entails understanding the relationship between nature and human conduct rather than just acquiring information; it involves nurturing principles and sentiments connected to the environment (Khanal et al. 2020). Ecological literacy contributes to improving our understanding of how human actions impact the planet's processes and outcomes, which is an element in promoting development (Prastiwi et al. 2019). Awareness of ecology is closely tied to knowledge, understanding the connection between humans and the natural world. Focusing on fostering connections with nature and encouraging activism is key here, as awareness is crucial

in implementing actions that bolster conservation efforts and sustainability practices.

Ecological Regulation and Sustainability Mindset

Ecological regulations play a role in driving advancements by establishing standards and approaches that promote environmental preservation (Xus 2023). Practices such as incentives help foster nature conservation by inspiring individuals and organizations to engage in conservation efforts (Xus 2023). Encouraging a mindset centered on sustainability involves embracing values and skills aimed at protecting the environment and working towards achieving Development Goals (Mulyadi 2020). Committing to sustainability inspires individuals to take steps towards meeting development goals by reducing waste production and conserving resources while also supporting preservation efforts (Busi 2023).

Research Design

The study structure involved an analysis utilizing the forward and hierarchical regression methods to investigate the relationships between the different facets of environmental education and their combined impact on achieving a sustainability mindset. Through the application of forward and hierarchical regression analyses in this research endeavor, we revealed the channels by which ecological education levels and literacy awareness, alongside regulations and sustainability attitudes, play a role in promoting development. The results of this research have the potential to guide decision-making on policies and educational approaches directed towards building a sustainable tomorrow.

MATERIALS AND METHODS

This research employs forward and hierarchical Regression Analysis as a method to explore the impact of Ecological literacy, ecological regulations, ecological awareness, and ecological education on Sustainability Mindset. Through an examination of data encompassing education and sustainable development indicators across regions and timeframes, this study sought to pinpoint factors and their contributions to attaining sustainable development outcomes using the forward and hierarchical Regression Methodology. In addition, the study analyses work dives into elements that might influence or interact with aspects of education to achieve a deeper understanding of how they all contribute to sustainability goals.

Application of Forward and Hierarchical Regression Analysis in Environmental Education Research

Forward and hierarchical Regression Analysis is a technique used in environmental education research to explore how

different independent variables are simultaneously affected by other variables. This method allows researchers to investigate the relationships between education levels, literacy rates, awareness levels, practices, and sustainability mindsets to comprehend their effects on development outcomes. By studying the interactions among these factors, a hierarchical Regression Analysis highlights the significance of education in promoting a Sustainability Mindset.

Data Collection

Data were collected through a survey questionnaire that assessed participants' ecological education, literacy, awareness, regulation, and perception of the sustainability mindset. Four hundred higher education students majoring in environmental science in Pune city were approached for data collection.

Study Participants

The study participants were higher education students from Pune City who had received environmental education and were able to provide information on their ecological education, literacy, awareness, regulation, and sustainability.

Inclusion Criteria

Participants were included in the study if they had received environmental education and were able to provide information on the five key variables of ecological education: literacy, awareness, regulation, and sustainability mindset.

Exclusion Criteria

Participants were excluded from the study if they were unable

to provide information on the five key variables or had not received any environmental education.

Statistical Analysis

Statistical analysis involves the application of forward and hierarchical regression analyses to identify the relationships between dependent and independent variables.

Application/ Implications

The findings of this study provide insights into how environmental education programs can contribute to achieving sustainable development objectives by exploring the mechanisms involved in promoting the acceptance and implementation of these goals. Furthermore, it delves into the factors that could enhance or hinder the efficacy of efforts in advancing Sustainable Development Goals (SDGs) through a sustainability mindset and offers valuable insights for designing impactful educational initiatives within the realm of sustainability.

RESULTS AND DISCUSSION

Reliability and Validity

Reliability is the ability of a scale to produce consistent results and is the extent to which a scale is trustworthy. The five variables under study, ecological literacy, ecological awareness, ecological regulations, ecological education, and sustainability mindset, are single-item scale variables. The reliability of the measuring instrument was examined using the test-retest method. The results of the test-retest method showed high consistency among the data collected

Table 1: Model summary and coefficients.

Model	Parameters	B	Std. Error	Beta	T	Sig.	R Square	R Sq Changed	F	Sig.
1	(Constant)	2.287	0.105		21.718	p<0.001	0.364	0.364	119.8	p<0.001
	Ecological Literacy	0.318	0.029	0.604	10.947	p<0.001				
2	(Constant)	2.03	0.106		19.098	p<0.001	0.459	0.095	88.35	p<0.001
	Ecological Literacy	0.23	0.031	0.436	7.52	p<0.001				
	Ecological Regulations	0.17	0.028	0.351	6.042	p<0.001				
3	(Constant)	1.912	0.108		17.624	p<0.001	0.491	0.032	66.5	p<0.001
	Ecological Literacy	0.193	0.031	0.367	6.149	p<0.001				
	Ecological Regulations	0.138	0.029	0.285	4.816	p<0.001				
	Ecological Awareness	0.101	0.028	0.212	3.594	p<0.001				
4	(Constant)	1.796	0.113		15.856	p<0.001	0.512	0.021	54.04	p<0.001
	Ecological Literacy	0.16	0.033	0.304	4.89	p<0.001				
	Ecological Regulations	0.128	0.028	0.264	4.505	p<0.001				
	Ecological Awareness	0.085	0.028	0.179	3.034	0.003				
	Ecological Education	0.089	0.03	0.174	2.977	0.003				

a. Dependent Variable: Sustainability Mindset

on two occasions. Validity refers to the ability of a scale to produce accurate results. This is the extent to which a scale measures what it is supposed to do. Validity is examined by computing the content validity index and content validity ratio, as suggested by Lawshe (1975). The content validity ratio and content validity index for all five variables were above the threshold of 0.741 for the seven experts.

It is hypothesized that “ecological literacy,” “ecological regulations,” “ecological awareness,” and “ecological education” are significant predictors of a “sustainability mindset.” Forward regression analysis was used to validate this hypothesis. Forward regression analysis is an entry-based method for developing a predictive model. We had a continuous dependent variable and several continuous independent variables. A forward regression approach begins with a null model (an empty model with only a constant in the model) and then sequentially adds independent variables to the model to improve it until the stopping criterion is met. Unlike the backward method, which employed a significance level of 0.1, the level of significance used in the forward method was 0.05. The analysis produced four models of the current data.

Model 1 ($F = 119.8^{***}$) includes the most meritorious predictor, “ecological literacy,” with an R square of 0.36, indicating that ecological literacy can explain 36% of the variance of the Sustainability Mindset. Model 2 ($F = 88.3^{***}$) includes two predictors, “ecological literacy” and “ecological regulation,” with an R square of 0.45, indicating that ecological literacy and ecological regulation can explain 45% of the variance in the Sustainability Mindset. Model 3 ($F = 66.5^{***}$) includes three predictors, “ecological literacy,” “ecological regulations,” and “ecological awareness,” with an R square of 0.49 indicating that ecological literacy, ecological regulations, and ecological awareness can explain 49% of the variance of Sustainability Mindset.

Finally, model 4 ($F = 54.04^{***}$) includes four predictors: “ecological literacy,” “ecological regulations,” “ecological awareness,” and “ecological education,” with an R square of 0.51 indicating the ecological literacy, ecological regulations, ecological awareness, and ecological education can explain 51% of the variance of Sustainability Mindset. “***” significant at 0.1% level of significance.

Predictor Importance

The strongest predictor of a Sustainability Mindset is Ecological literacy ($\beta = 0.304$, $t = 4.89^{***}$), followed by ecological regulations ($\beta = 0.264$, $t = 4.5^{***}$), ecological awareness ($\beta = 0.179$, $t = 3.03^{***}$), and environmental education ($\beta = 0.174$, $t = 2.97^{***}$). The inclusion of ecological regulation has improved the model by 9.5%, the inclusion of environmental awareness has improved the model by 3.2%, whereas the inclusion of ecological education has improved the model by 2.1%, “***” significant at 0.1% level of significance.

The accuracy of the results of multiple regression analysis is based on four assumptions: linearity, residual normality, homoscedasticity, and no outliers. If these assumptions are not supported, multiple regression analysis (MRA) results can be misleading, and the MRA model can be misspecified. Figure 1 shows that the red line is close to the horizontal axis, supporting the Assumption of linearity and ruling out the possibility of a nonlinear relationship between the “sustainability mindset” and our predictors, “ecological literacy,” “ecological regulations,” “ecological awareness,” and “ecological education.”

A standard QQ residual plot is a graphical technique used to assess whether residuals are approximately normally distributed. The residuals are plotted against a theoretical normal distribution such that the points form an

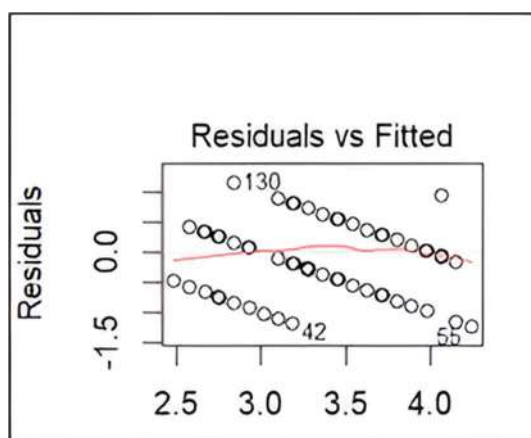


Fig. 1: Assumption of linearity.

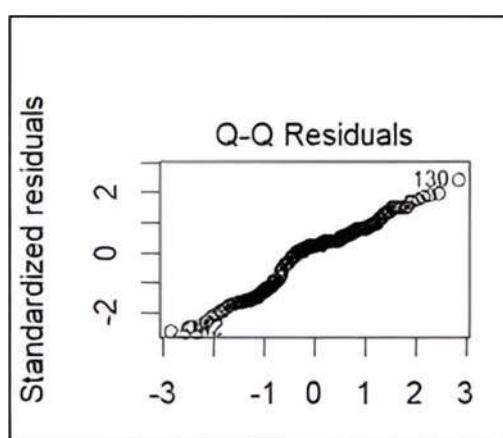


Fig. 2: Assumption of residual normality.

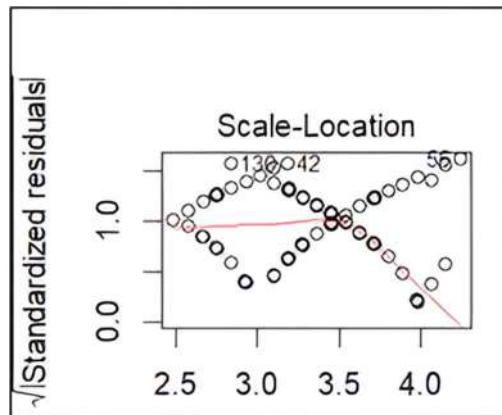


Fig. 3: Assumption of homoscedasticity.

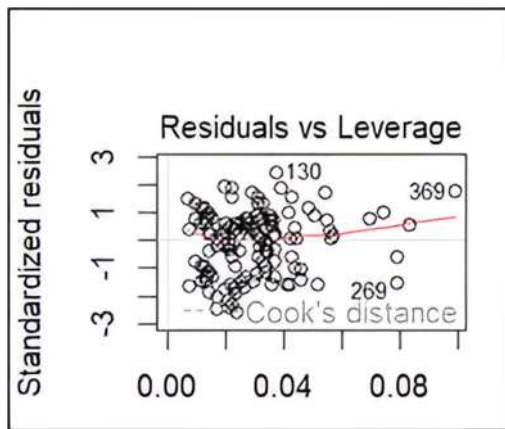


Fig. 4: Assumption of outliers.

approximately straight line. Departures from this straight line indicate a departure from normality. The error terms were close to the diagonal line of normality, supporting the assumption of residual normality, as shown in Figure 2.

The third important assumption was homoscedasticity. That is, the error terms are constant across the regression line; that is, the size of the error terms is independent of the four predictors. Figure 3 shows that the error terms were constant across the regression line. These results are also supported by an insignificant ($p > 0.05$) Breusch and Pagan test ($\chi^2 = 1.085$, $p = 0.298$).

The final assumption is that there are no outliers, that is, the data are free from outliers (abnormal values). Figure 4 shows that there are no potential outliers because no cases are beyond the Cook's distance threshold.

Hierarchical Regression Analysis

The above results were reinforced using a hierarchical regression analysis. Hierarchical regression analysis is a type

Breusch-Pagan Test for Heteroskedasticity ^{a,b,c}		
Chi-Square	df	Sig.
1.085	1	.298
a. Dependent variable: Sustainability mindset		
b. Tests the null hypothesis that the variance of the errors does not depend on the values of the independent variables.		
c. Predicted values from design: Intercept + D1 + D2 + D4 + D16		

Fig. 5: Breusch-Pagan Test.

of multiple regression analysis that allows us to examine the impact of an independent variable on a dependent variable after accounting for other variables. In this method, predictors are entered into a block. The evaluated variable is entered next (last) to check the predictive ability of the variable to be assessed over and above the covariates. The results of the hierarchical regression analysis with four block variables ("ecological literacy," "ecological regulations," "ecological awareness, and "ecological education") are presented below.

It is interesting to note that Ecological Education contributes 2.1% to the model over and above ecological literacy, ecological awareness, ecological regulations, Ecological Literacy contributes 5.7% to the model, over and above ecological education, ecological awareness, ecological regulations, ecological awareness contributes 2.1% to the model over and above ecological literacy, ecological education, ecological regulations, whereas ecological regulations contributed 4.8% to the model over and above ecological literacy, ecological awareness, ecological education.

Limitations and Future Research Directions

This study, while offering valuable insights, has certain limitations primarily related to data accessibility and the scope of investigation. The research focused on five core components of environmental education, ecological literacy, ecological regulations, ecological awareness, ecological education, and sustainability mindset within the context of higher education in Pune, India. Although this offers a meaningful exploration, it restricts the generalizability of the findings beyond this specific geographic and demographic context.

Table 2: Hierarchical regression analysis.

Variable to be evaluated	R square BEFORE	R square AFTER	R-squared changed
Ecological Education	0.491	0.512	0.021
Ecological Literacy	0.455	0.512	0.057
Ecological Awareness	0.490	0.512	0.021
Ecological Regulations	0.464	0.512	0.048

Despite these constraints, the study contributes significantly to the discourse on environmental education and sustainable development. It opens avenues for future research aimed at understanding the long-term influence of environmental education on development goals. Further investigations could adopt a cross-regional or cross-cultural approach to validate and extend the present findings, offering a broader perspective on the role of education in sustainability transformation.

The unique forward and hierarchical regression methods employed in this study may serve as a methodological foundation for other researchers interested in analyzing the relationship between educational constructs and sustainability outcomes. Future studies could incorporate additional variables or expand on interdisciplinary dimensions to explore how environmental education interacts with other educational domains to influence the achievement of Sustainable Development Goals (SDGs).

Moreover, longitudinal studies are recommended to assess the enduring effects of environmental education on individuals' attitudes, behaviors, and active participation in sustainability initiatives over time. The insights gained could inform administrative policies at the institutional, community, and national levels, guiding the design of impactful environmental education programs. Thus, this research not only contributes to academic understanding but also paves the way for a wide range of future explorations and practical applications in promoting sustainability through education.

CONCLUSIONS

This research underscores the critical role of environmental education in shaping a sustainability mindset among higher education students. By examining the predictive value of ecological literacy, regulations, awareness, and education through forward and hierarchical regression methods, the study offers empirical evidence of how these factors collectively contribute to sustainable attitudes and behaviors.

The findings highlight ecological literacy as the most significant predictor, followed by ecological regulations, awareness, and education, each playing a vital role in achieving sustainability objectives. The results affirm that well-structured environmental education programs can foster conscious and informed individuals who are equipped to support sustainable development.

Overall, the study bridges an important gap by empirically linking educational inputs with developmental outcomes. It sets a foundation for expanded and long-term research across diverse regions and academic disciplines. By aligning educational practices with sustainability goals, institutions can play a pivotal role in preparing future

generations for responsible global citizenship and ecological stewardship.

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Carbon Dioxide Gas Reduction Using the Absorption Method as an Effort to Support Carbon Capture and Storage (CCS) Technology

O. H. Cahyonugroho†, I. S. Putra, E. N. Hidayah and R. Novembrianto

Environmental Engineering Study Program, Faculty of Engineering and Sains, Universitas Pembangunan Nasional “Veteran”, Jawa Timur, Indonesia

†Corresponding Author: O. H. Cahyonugroho, okikhc@upnjatim.ac.id

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ABSTRACT

Significant changes in global temperature occurred when carbon dioxide gas produced increases from industrial combustion processes that still use fossil fuels. These fuels contribute to environmental damage that will result in greenhouse gas emissions. One form of effort made by using Carbon Capture and Storage (CCS) technology is effectively able to reduce global warming. The reduction process is carried out using NaOH and KOH solutions by comparing the effectiveness of the captured gas. This study was conducted to determine the effect of solution and gas flow rates between NaOH and KOH absorbents on the removal of dissolved CO₂ gas. The chosen methods were the sampling method and the alkalimetric method. The method was used to determine the variation of the absorbent flow rate of 2, 4, and 6 L.min⁻¹. While the gas flow rate used is 10, 20, and 30 L.min⁻¹. The results showed that the effective flow rate variation was obtained at a speed of 2 L.min⁻¹ with a gas flow rate of 30 L.min⁻¹. The concentration that is removed in the absorbent is 52% of CO₂ gas absorbed in NaOH solution with an electric voltage of 0.817 Volt, while KOH is obtained by 48% with an electric voltage of 0.798 Volt. The analysis results will be used as electrochemical-based electrical energy. It can be concluded that the effective absorbent solution is NaOH which can reduce CO₂ gas optimally, which has a correlation to the flow rate of the solution and gas and can produce electrochemical-based electrical energy.

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INTRODUCTION

Global warming occurs when emissions from industrial combustion processes continue to increase, resulting in extreme temperature increases that occur on Earth (Zubaydah et al. 2024). Based on the results of the Copernicus Climate Change Service (C3S) report on temperature changes in 2024, it is known that the global average temperature reached 1.52°C. The increase in global temperature exceeds the threshold limit from the previous year of 1.5°C (Okonkwo et al. 2023). This occurs because of greenhouse gas emissions. Greenhouse gases are gases found in the atmosphere or in the earth's layers that are naturally used to keep the earth's temperature stable (Nasir & Go 2024). Stable temperatures occur when sunlight enters the earth's surface and then they will be reflected into the sky. When sunlight is reflected to the sky, some of it is absorbed by some gases in the atmosphere in the form of short waves that are received by the earth's surface, and the rest will be forwarded back to space in the form of long-wave radiation or infrared radiation (Kurnia & Sudarti 2021). Long-wave radiation emitted by greenhouse gases in the lower layers of the atmosphere will cause hot temperatures called the greenhouse effect (Habibur 2022). An increase in global temperature that continues to increase will have a negative impact on climate so it will trigger ecological and social problems (Zaky & Sari 2024, Zahro et al. 2023).

Carbon dioxide or CO₂ emission is a type of greenhouse gas emission that is the main factor that can cause global warming. Carbon dioxide gas consists

of two oxygen atoms covalently bonded to a carbon atom (Dziejarski et al. 2023, Anggraini & Sutjahja 2023). Based on a report from the Global Carbon Project team of scientists in 2022, the amount of carbon emissions produced in Indonesia reached 700 million per year. This figure is an increase of 18.3% from the previous year. Various efforts have been made by the Indonesian government to reduce CO₂ gas emissions produced. In 2060 the government plans to reduce CO₂ gas emissions by implementing a strategy towards net zero emissions (Putri et al. 2024). The plan is to use renewable energy without fossil fuels by using CO₂ gas capture technology produced in the form of Carbon Capture and Storage (CCS) (Shu et al. 2023, Martin-Roberts et al. 2021). Technology is carried out to mitigate the release of greenhouse gas emissions from industries that use fossil fuels. This technology uses the principle of reducing the released carbon dioxide gas with the help of absorbents in liquid form (Roussanaly et al. 2021). The absorbents can be methyl diethanolamine (MDEA), monoethanolamine (MEA), potassium hydroxide (KOH), and sodium hydroxide (NaOH). These materials are commonly utilized as CO₂ capture systems (Arning et al. 2024, Yoshida 2022). Implementing technology will provide an opportunity to produce emissions but not allow the emissions produced to the earth's atmosphere by engineering technology so that the planned net zero emissions program in 2060 will be achieved (Shaw & Mukherjee 2022, Liang et al. 2024).

Gas absorption is the process of separating and purifying gas from other gas mixtures. By using the principle of gas diffusion in contact between gas and dissolved liquid, mass transfer will occur (Dziejarski et al. 2023, Hong 2022). These events will affect the flow rate between the solution and the injected gas during the absorption process. Absorption is used in the form of a packed column (Hanifa et al. 2023). The absorption process occurs when gas and air are mixed by being pushed at the bottom of the packed column while the absorbent solution is injected from above, which flows by gravity so that there will be a meeting between the gas and the solution (Zhou et al. 2024). This event will affect changes in temperature, pH, and EC in the solution so that the gas will be removed in the absorbent solution (Isya et al. 2021). The packed column has an elongated tube shape with packing inside. Packing is a material derived from gravel, glass, ceramic shards, copper iron, or materials that are not easily corrosive when reacting with solutions with strong alkaline conditions. Packing is arranged irregularly to facilitate condensation during reduction on the surface of the packed column. A packed column occurs in a closed column so that an empirical correlation value is obtained between the absorption rate and the mass rate of liquid and gas (Huda et al. 2023, Putro et al. 2023).

The implementation of Carbon Capture and Storage (CCS) technology results in the output of a carbonate solution that will be utilized as electrochemical-based electrical energy. Electrochemical processes that study electro-mass transfer occur in a medium that has electrodes as electrical conductors (Jiang & Ashworth 2021, Mao et al. 2023). The electrode has positive and negative caps so that when the electrode is dipped in a solution, it will produce an electrical energy source. The event will experience a redox reaction in the form of electron capture in the reduction reaction, while there is an electron release in the oxidation reaction (Chai et al. 2022). From the electrochemical process that utilizes acidic CO₂ gas emissions, the higher the reduced gas, the higher the electric current generated (Rohid et al. 2022, Salamah & Cahyonugroho 2023). Based on this, researchers will need to analyze the effectiveness of the flow rate of the reduced gas solution on the concentration captured by comparing the types of NaOH and KOH absorbents. The results of the absorption that occurs will form a new solution that will be used as electrochemical-based electrical energy. The process was analyzed for its effect on pH, temperature, EC, and reduced gas concentration.

MATERIALS AND METHODS

Research on CO₂ gas absorption, uses a type of sampling method, alkalimetry. This type of method is needed by researchers to analyze, identify, evaluate, and interpret in the form of graphs to review the concentration of CO₂ gas that is removed from the absorbent solution. Based on a scientific article from (Oktarina et al. 2023, Robiah et al. 2021) as the basis for selecting variables in the research that has been conducted. The variables in this study consisted of the influence on pH, electrical conductivity (EC), temperature, and concentration of carbon dioxide gas that was removed. For the absorption process, the airflow rate used is fixed at a speed of 10 L.min⁻¹ and the type of absorbent used is in the form of NaOH and KOH, which has a concentration of 0.01 M at the initial concentration used. We used flow rate concentration with variations of 2, 4, and 6 L.min⁻¹. As for the CO₂ gas flow rate used with variations of 10, 20, and 30 L.min⁻¹. The absorption process runs for 20 min to determine the optimum point during the process. The results of the captured output will be analyzed for the concentration of dissolved CO₂ gas and the remaining absorbent that does not form a new solution during the absorption process.

Actual Sample Preparation

The samples used are NaOH and KOH absorbent solutions, which have a concentration of 0.01 M and have gone through a dilution process from solid to liquid. In the dilution

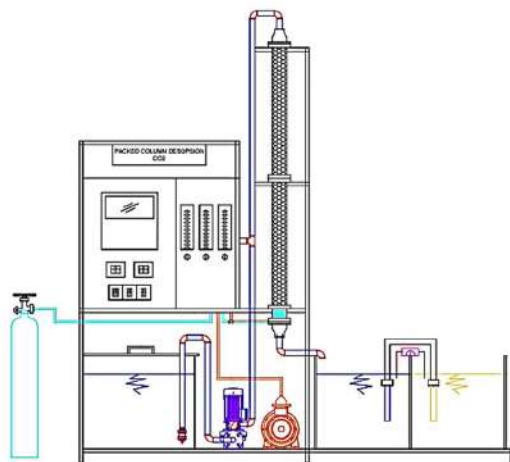


Fig. 1: Gas absorption column reactor.

process, in addition to absorbents, there is a dilution of the solution used for the alkalimetric method in the form of acid and wet titration using HCl solution as the neutralizer. HCl solution, which has a concentration of 32%, will be diluted to a concentration of 0.1 M. In addition, a dilution of the BaCl_2 solution is used to determine the remaining concentration of the solution that does not form a new solution. The concentration of BaCl_2 used is 0.1 M, which is added to the output sample from the absorption process. Furthermore, in the electrochemical analysis, it is used as an electrolyte solution in the form of CuSO_4 solution, which has a concentration of 0.1 M and has gone through a dilution process. In addition to preparing the absorbent and diluting the solution, the Gas Absorption Column reactor is prepared, which is the process of gas absorption that occurs in the column. The reactor used uses a size with the following dimensions:

- The gas cylinder used has a capacity of 2 m^3 which has a height of 1.2 m with a diameter of 0.14 m.
- The reservoir used to accommodate the absorbent solution in the *inlet* and *outlet* channels has a size of 0.73 m in the length of the tub, 0.6 m in the width of the tub, and 0.46 m in the height of the tub. The tub used has a water capacity of up to 150 L, which can accommodate the solution during the absorption process for 20 min.
- The absorption column called *the settling column*, is made of acrylic, which has a thickness of 5 mm, a length of 1.4 m, and a diameter of 2.5 inches. In the absorption column, there is a *solvent riching* that has a size of $10 \times 10 \text{ mm}$.
- The reactor is equipped with a centrifugal type of pump that can inject at a speed of $12 \text{ L} \cdot \text{min}^{-1}$, and there is an air compressor that has a tube capacity of $25 \text{ L} \cdot \text{min}^{-1}$.

- The reactor used has a total height of 2.17 m, with a length of 1.84 m and a width of 0.9 m.

The following reactor equipment used in reducing carbon dioxide gas is shown in Fig. 1.

Work Procedure in the Study

The carbon dioxide gas absorption process utilizes NaOH and KOH as absorbents to compare their effectiveness. The first step is to activate the reactor, starting with an air compressor and a water pump. Subsequently, the flow meter is adjusted for each flow rate, as determined by the variables mentioned above. The analysis is then carried out using the sampling method.

The absorption process was left undisturbed for 5 minutes to allow the reactor to stabilize. The sampling process was conducted at the outlet tube, collecting 200 mL of sample every 0, 5, 10, 15, and 20 minutes. During sampling, the reactor was monitored to ensure that the injected flow rate remained stable. Each sample was directly tested for parameters such as pH, temperature, conductivity (DHL), CO_2 gas concentration removed, and the residual solution that does not form a new compound. The test was conducted promptly to ensure accurate results.

The dissolved CO_2 content was analyzed using the alkalimetry method, which involves a wet acid reaction. This method consists of two stages: the first stage determines the initial concentration of dissolved CO_2 gas, while the second stage evaluates the remaining absorbent solution.

In the first stage, a 50 mL absorbent sample was placed into an Erlenmeyer flask, followed by the addition of 1-2 drops of phenolphthalein (PP) indicator. The indicator functions within a wet reaction. The sample was then titrated using HCl solution in a burette until the color transitioned

from pink to clear. The titration results were recorded as a reference point.

Next, titration was conducted on the outlet sample at 5-minute intervals. A 50 mL sample was used, and 1-2 drops of methyl orange (MO) indicator were added. The indicator functions within an acidic reaction, resulting in a color change to orange. The sample was titrated with HCl in a burette until it transitioned to a reddish-orange hue. The titration results were recorded to determine the concentration of trapped CO₂.

In the second stage of the alkalimetry method, a 50 mL sample was taken from the outlet reservoir. The solution was then supplemented with a CO₂ concentration titration value based on the initial absorbent titration, multiplied by 10% of the results obtained. The calculated results determine the volume of BaCl₂ solution required. Following this, methyl orange (MO) indicator was added until a color change occurred, and the solution was diluted with HCl in a burette until it turned reddish-orange. The titration results were recorded to determine the remaining concentration of the solution that does not form a new compound.

Parameter Measurement

In the absorption process, an analysis test was carried out on the parameters of pH, temperature, and DHL which were used to determine the effect of the absorbent solution that occurred in the *settling column* on the concentration of dissolved CO₂. Before the analysis of the equipment used to measure parameters in the form of pH meters, thermometers, and conductivity meters, calibration was first carried out so that the tools used gave accurate and precise results, in addition, the balance or deviation from the determination value will be indicated by the tool. In addition, FTIR (Fourier Transform Infra Red) analysis was carried out, which was used to identify the compounds contained in the sample by analyzing the functional groups of the compounds contained in it.

Electrochemical-Based Electrical Stress Measurement

The measurement of electrical stress was carried out on a sample resulting from the absorption process in the form of a bicarbonate solution obtained from CO₂ gas that binds to the absorbent solution. The samples taken will be analyzed with galvanized cell or volta cell groups. The voltaic cell/galvanized cell uses an oxidation reaction by using an anode as a negative electrode by using a copper plate and a cathode as a positive electrode by using a zinc plate, and there is a jagarm bridge that contains positive and negative ions to neutralize the solution. So that there is a difference in tendency that will cause electrical voltage currents.

RESULTS AND DISCUSSION

Effect of pH on Flow Rate Variation of Absorbent Solution and CO₂ Gas

In the absorption of CO₂ gas, some factors influence the absorption process, one of which is the pH concentration in the absorbent solution. The effect of pH will decrease from a strong base to a weak acid due to gas diffusion with the solution. The results are presented in Table 1.

Table 1 shows the results of the analysis of the influence of pH on the type of absorbent used. The table will be interpreted in the form of a graph to determine the elevation of each solution flow rate and CO₂ gas flow rate.

Fig. 2. proves that the effect on pH provides a significant change from the condition of the solution, which has strong wet properties, to weak acid due to the chemical reaction between CO₂ gas, which has acidic properties that meet with absorbent solutions that are strong wet (F. Huda et al. 2022). From the research results obtained, the concentration of the solution flow rate has an influence on the capture of CO₂ gas. This is because the smaller the flow rate is injected, the longer the residence time of the solution in the column, so gas capture increases. While the flow rate of the injected gas solution also affects the diffusion of CO₂

Table 1: pH concentration of each absorbent to the flow rate of the solution and CO₂ gas.

Type of Absorbent	Flow Rate of Absorbent [L.min ⁻¹]	Flow Rate of CO ₂ Gas [L.min ⁻¹]	Initial pH	Final pH
Absorbent NaOH	2	10	10	7.2
		20	10	6.8
		30	10	6.4
	4	10	10	7.4
		20	10	6.8
		30	10	6.4
	6	10	10	7.8
		20	10	6.9
		30	10	6.5
Absorbent KOH	2	10	9.5	6.9
		20	9.5	6.4
		30	9.5	6.2
	4	10	9.5	6.9
		20	9.5	6.4
		30	9.5	6.3
	6	10	9.5	7
		20	9.5	6.5
		30	9.5	6.3

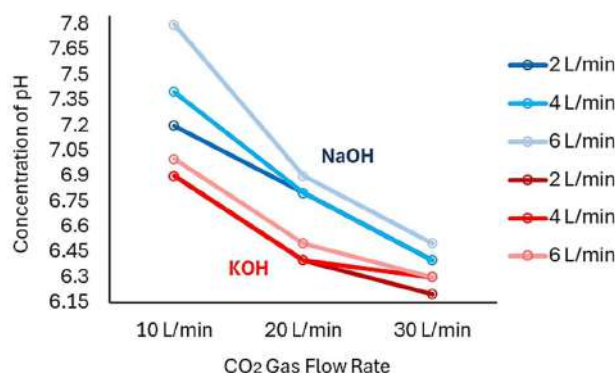


Fig. 2: Relationship between the effect of pH on the flow rate of solution and CO₂ gas from each absorbent.

in the absorbent. The more CO₂ gas is input, the higher the concentration of CO₂ captured (Dewi et al. 2024). From the results of research on each absorbent that has been accumulated, the effective flow rate is obtained at 2 L.min⁻¹ at the solution flow rate, while the effective CO₂ gas flow rate can be removed at a speed of 30 L.min⁻¹. For the type of absorbent used, it will affect the effectiveness of CO₂ that is desorbed. The effect of absorbent-type concentration is caused by chemical compounds that are ionized in solutions so that they can conduct the solution well in capturing CO₂ gas. The research shows that the effective absorbent is

Table 2: Temperature concentration of each absorbent against the flow rate of the solution and CO₂ gas

Type of Absorbent	Flow Rate of Absorbent [L.min ⁻¹]	Flow Rate of CO ₂ Gas [L.min ⁻¹]	Initial Temperature	Final Temperature
Absorbent NaOH	2	10	28,8	28,9
		20	28,5	28,8
		30	28,6	28,9
	4	10	28,3	28,5
		20	28,9	29,1
		30	27,4	27,7
	6	10	28,1	28,8
		20	28,9	29,2
		30	28,1	28,5
Absorbent KOH	2	10	27,5	27,9
		20	28,5	28,5
		30	28,0	28,2
	4	10	28,5	28,7
		20	27,6	27,8
		30	28,4	28,5
	6	10	29,1	29,0
		20	29,5	29,5
		30	29,5	29,6

obtained in NaOH, which uses a concentration of 0.01 M. Previous research shows that the higher the concentration of absorbent used, the more CO₂ is desorbed (Yuliani et al. 2023).

Effect of Temperature on Flow Rate Variation of Absorbent Solution and CO₂ Gas

In the absorption of CO₂ gas, some factors affect the absorption process, one of which is the temperature of the absorbent solution. The influence of temperature will increase due to the diffusion of gas with solution. The results are presented in Table 2.

Table 2 shows the results of the analysis of the influence of temperature on the type of absorbent used. The table will be interpreted in the form of a graph to determine the elevation of each solution flow rate and CO₂ gas flow rate.

Fig. 3 shows that the temperature has an influence on the concentration of CO₂ captured. The graph shows an increase from the initial temperature to the final temperature, so the greater the CO₂ gas injected, the higher the temperature produced, while the smaller the CO₂ gas injected, the lower the temperature obtained. This is due to the amount of CO₂ gas added from the reaction that occurs in the rich solvent. Based on scientific articles, the effect of temperature will correlate to the empirical equation that has been developed, explaining that an increase in temperature can increase the value of constant during the process of gas diffusion with solution (Parningotan & Dewi 2021). The increase in the constant value in the gas diffusion process will cause mass transfer between gas and liquid. From the research obtained, there are other factors that affect the temperature in the solution in the form of the influence of room temperature, which can provide changes in temperature in the solution so that the temperature obtained during the research process will experience the instability obtained (Ihsanpuro et al. 2023).

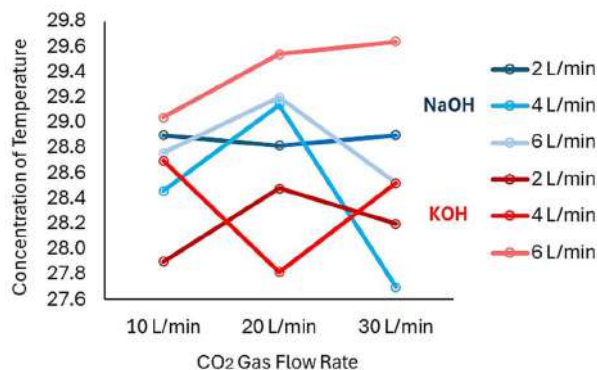


Fig. 3: Relationship between the effect of temperature on the flow rate of solution and CO₂ gas from each absorbent.

Effect of EC on Flow Rate Variation of Absorbent Solution and CO₂ Gas

In the absorption of CO₂ gas, some factors affect the absorption process, one of which is the EC of the absorbent solution. EC's influence will increase due to the diffusion of gas with solution. The results are presented in Table 3.

Table 3 shows the results of the analysis of DHL's influence on the type of absorbent used. The table will be interpreted in the form of a graph to determine the elevation of each solution flow rate and CO₂ gas flow rate.

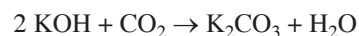
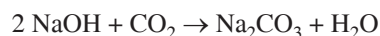
Table 3: DHL concentration of each absorbent to the flow rate of the solution and CO₂ gas.

Type of Absorbent	Flow Rate of Absorbent [L.min ⁻¹]	Flow Rate of CO ₂ Gas [L.min ⁻¹]	Initial DHL	Final DHL
Absorbent NaOH	2	10	0.206	0.225
		20	0.219	0.241
		30	0.213	0.239
	4	10	0.216	0.231
		20	0.208	0.228
		30	0.223	0.247
	6	10	0.222	0.234
		20	0.227	0.245
		30	0.228	0.248
Absorbent KOH	2	10	0.200	0.214
		20	0.211	0.227
		30	0.213	0.230
	4	10	0.214	0.225
		20	0.214	0.227
		30	0.213	0.228
	6	10	0.202	0.213
		20	0.202	0.214
		30	0.202	0.216

Based on Table 3 and Fig. 4, it is evident that electrical conductivity (EC) significantly influences the amount of desorbed CO₂ gas. During the CO₂ absorption process, an increase occurs in the absorbent solution from the initial reservoir to the final reservoir. A higher EC value indicates a greater amount of dissolved gas, whereas a lower EC value reflects less gas captured. This is attributed to the presence of absorbent material in the liquid phase that reacts with CO₂ gas, resulting in increased electrical conductivity. The effect of electrical conductivity depends on the total concentration of solutes ionized in the solution (Ramadhanti 2023). The mobility of dissolved ions correlates with their valence, thereby influencing the measured EC. Solutions containing ions exhibit higher conductivity due to the movement of electrons from the negative to the positive electrode (Arisukma et al. 2021). The study showed that the highest EC concentration was achieved with a solution flow rate of 2 L·min⁻¹ and a CO₂ gas flow rate of 30 L·min⁻¹. The optimum flow rate was determined by the difference between the initial and final concentrations, yielding a difference of 0.027 μmhos·cm⁻¹ in the NaOH solution and 0.017 μmhos·cm⁻¹ in the KOH solution.

Effect of Dissolved CO₂ Concentration on Flow Rate Variation of Absorbent Solution And CO₂ Gas

Based on the results of the laboratory analysis, it is evident that the type of absorbent significantly affects the concentration of dissolved CO₂ gas. Before conducting the analysis, the initial concentration of each absorbent was determined to serve as a reference point for assessing the amount of CO₂ gas captured. The following reaction equations were used in the analysis:



From the results of the initial concentration value, the dissolved CO₂ gas analysis is then carried out on the type of

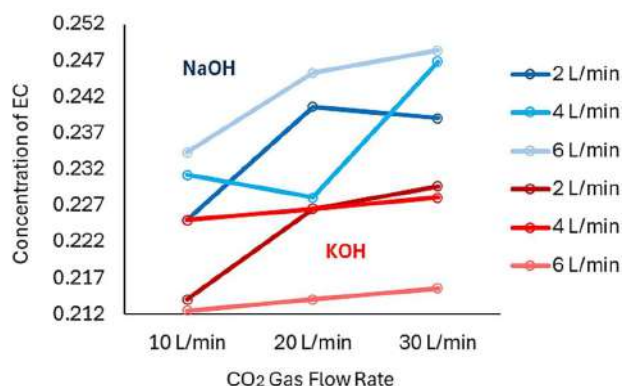


Fig. 4: Relationship between the effect of EC on the flow rate of solution and CO₂ gas from each absorbent.

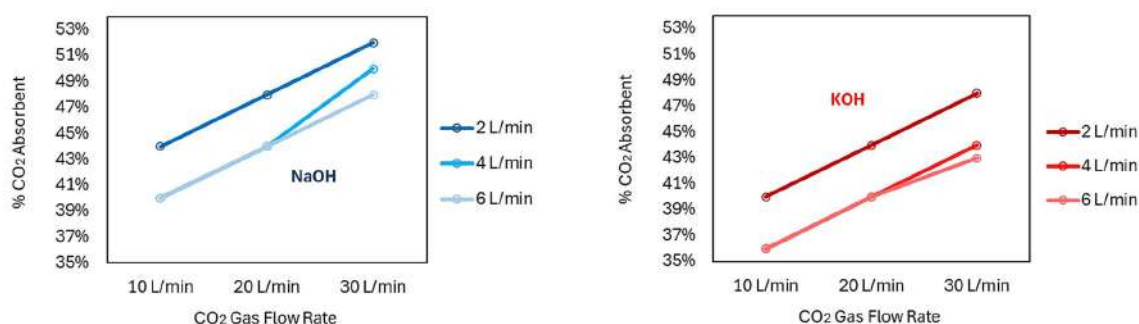
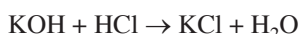
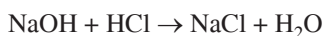
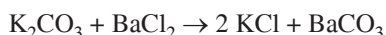
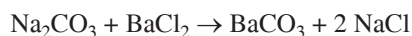


Fig. 5: Relationship between dissolved CO₂ gas from each absorbent.

absorbent used. The following is the reaction equation that occurs during titration, including:



In the second stage of titration, the analysis of the remaining absorbent that does not form a new solution during the absorption process is carried out by adding BaCl₂ solution so that the reaction will form as follows.



During the research process, the results of the analysis were obtained during the titration process. In addition, an analysis test of influence parameters on pH, temperature, and EC was carried out as supporting data in the research conducted (Nugroho et al. 2023, Adhi et al. 2022). The following are the results of the analysis that has been carried out on the flow rate of solution and gas to the concentration of dissolved CO₂.

Table 4 shows the results of the analysis of the effect of the concentration of dissolved CO₂. The table will be interpreted in a graph to show the elevation value resulting from the CO₂ concentration removed.

Table 4: Concentration of dissolved CO₂ gas from each absorbent.

Type of Absorbent	Flow Rate of Absorbent [L.min ⁻¹]	Flow Rate of CO ₂ Gas [L.min ⁻¹]	% CO ₂ Absorbent
Absorbent NaOH	2 L.min ⁻¹	10 L.min ⁻¹	44%
		20 L.min ⁻¹	48%
		30 L.min ⁻¹	52%
	4 L.min ⁻¹	10 L.min ⁻¹	40%
		20 L.min ⁻¹	44%
		30 L.min ⁻¹	50%
Absorbent KOH	2 L.min ⁻¹	10 L.min ⁻¹	40%
		20 L.min ⁻¹	44%
		30 L.min ⁻¹	48%
	4 L.min ⁻¹	10 L.min ⁻¹	36%
		20 L.min ⁻¹	40%
		30 L.min ⁻¹	44%
	6 L.min ⁻¹	10 L.min ⁻¹	36%
		20 L.min ⁻¹	40%
		30 L.min ⁻¹	43%

Based on Fig. 5, it can be seen that the effect of the solution flow rate and CO_2 gas will have an impact on the concentration of dissolved CO_2 , so flooding will occur. The flooding process occurs due to the collection of liquid in the absorption column that passes from the inlet column to the outlet column. The flooding process if it gets higher, will cause liquid hold-up so that the liquid bound in the room will increase (Fadlih & Megawati 2020). In addition, in the absorption process, there will be a mass transfer of substances from gaseous to liquid. This process uses counter-current flow. Counter current flow is the opposite flow direction so that there is a large fraction of contact or fluid friction. Counter-current flow events occur when an increasingly large absorbent load that has reached saturation point will experience an increase in CO_2 absorption so that the resulting solution will have a high CO_2 concentration (Saputra et al. 2023). There are supporting factors during the absorption process in columns that are influenced by the contact area in the column and the packing used. The effect of the contact area in the column will be able to increase the diffusion of gas into a solution. If the flow rate of the solution is in a small fluid, the contact time with the gas against the solvent will be longer. Thus, if the contact time experiences a difference in concentration, it can cause a driving force in the diffusion process that occurs in the fluid (Daiyan et al. 2020). The diffusion process of carbon dioxide gas which has a high fraction with an absorbent solution that has low levels of carbon dioxide, so that gas contact with the solution will cause a flow rate in the solution. In the absorption process, the flow used is turbulent or exceeds the Reynold number so that the greater the substance that will collide with the gas, can increase the absorption of gas in the solute. While packing gives influence in the contact area. Packing is used in a random position so that it will have low pressure on the gas used for the chemicals used (Maulizar et al. 2023, Gantina 2021).

Based on the type of absorbent used in the form of NaOH and KOH it shows the difference in desorbed concentration. This is evidenced by the results of the study above, showing NaOH compounds can remove 52% compared to KOH by 48% because chemical compounds have been fully ionized into hydroxide ions (OH^-) in liquid form. The process where the release of hydroxide ions in solution. The proton compound that has been received depends on the concentration of the solution used, by reviewing the covalent value of the bond. Meanwhile, based on each absorbent used it shows that the highest or most effective concentration in $\text{NaOH} > \text{KOH}$ solution. This proves that the concentration of NaOH can trap CO_2 gas better than KOH because chemical compounds are fully dissociated or ionized into hydroxide ions (OH^-) in solution. This means that all molecules have

a very good ability to release hydroxide ions to increase the concentration of OH^- in solutions. The strength in solution is determined by whether it is easy for a compound to accept protons, release OH^- ions, or contribute free electron pairs (Angraini & Sutjahja 2023). In terms of freedom, compounds based on ionization ability are influenced by covalent or ionic characters in their bonds (Haryanti et al. 2024). The covalent or ionic character of the bond is discussed in the Fajal rule. Fajal suggests that the smaller the size and the greater the strength of a cation, the greater the ability of the cation to cause anion to polarize. The easier the anion is polarized, the more the covalent character of a bond increases. If the bond is more covalent, the compound is more difficult to ionize (Chotimah et al. 2022). In addition, the selection of NaOH and KOH absorbent types because they have compounds in the form of high amines compared to other materials. Amine-type materials, namely high reactivity with CO_2 molecules, high thermal stability, and high absorption capacity, can absorb more CO_2 gas, and the materials used are easy to find and more affordable (Angraini & Sutjahja 2023).

Effect of Dissolved CO_2 Concentration on the Electrochemical-Based Electric Current Voltage

In the absorption of CO_2 gas, some factors affect the absorption process, one of which is the concentration of CO_2 gas in the absorbent solution. The effect of CO_2 gas concentration will increase if the higher the concentration

Table 5: Electrical Voltage of each absorbent to the flow rate of solution and CO_2 gas.

Type of Absorbent	Effective Flow Rate of Absorbent	Effective Flow Rate of CO_2 Gas	Electrical Voltage
	L.min^{-1}	L.min^{-1}	Volt
Absorbent Na_2CO_3	2	30	0,817
Absorbent K_2CO_3	2	30	0,798

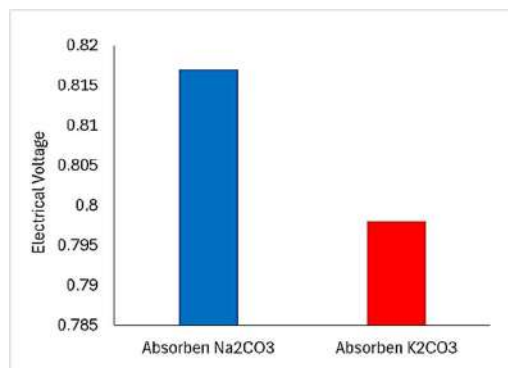


Fig. 6: The relationship between electrical voltage to the flow rate of solution and CO_2 gas from each absorbent.

received, the higher the electrical voltage produced due to the diffusion of the gas with the solution. The results are presented in Table 5.

Table 5 shows the results of the analysis of the influence of CO₂ gas that can produce an electrochemical-based electrical voltage on the type of absorbent used. The table will be interpreted in the form of a graph to determine the elevation of each solution flow rate and CO₂ gas flow rate.

The results from Fig. 6 prove that the solution resulting from the absorption process can produce an electric voltage of 0.817 volts in NaOH absorbent for an electric voltage of 0.798 volts from KOH absorbent. The voltage produced comes from the CO_2 that is absorbed in the solution. The higher the dissolved CO_2 , the higher the electric voltage produced, and the lower the desorbed CO_2 , the lower the electric voltage produced (Erans et al. 2022). The results of the research that has been carried out show that the process of emitting acidic CO_2 gas can be applied to electrical energy using electrochemical methods. The method utilizes two types of electrodes in the form of tumbaga plates as anodes (-) by using an electrolyte solution in the form of CuSO_4 solution and zinc plates as a cathode (+) by using a solution resulting from the absorption process in the form of K_2CO_3 solution. The conversion process begins with the capture of CO_2 gas from the Column Gas Absorption reactor, then the CO_2 gas acid content that spreads will occur a chemical process between the compounds that produce carbonate compounds. The higher the acidity level of a solution, the greater the number of protons produced. This will have an impact of mutual attraction between the electrons in the cathode and the protons in the anode so that electrical energy is formed during the process. CuSO_4 solution, which is an electrolyte solution that will undergo oxidation (release electrons). The difference in the tendency to oxidize produces artificial fruiting, which will result in a potential difference (electron driver) called the cell potential measured using a voltmeter (Ramadhanti 2023).

Based on the research results, technology is very promising as a solution for optimizing the use of available resources. The results of the production process in the oil and gas industry sector which has the potential to produce high CO₂ emissions. The results of emissions will be captured with this technology, which is converted into electrochemical-based electrical energy. The form of implementation in accordance with CCS technology has been carried out by the Indonesian government through the Minister of Energy and Mineral Resources Regulation Number 2 of the Year concerning Determination of Carbon Emissions and Storage, as well as Determination, Storage, and Utilization of Carbon in the Production Process of Hydrocarbons and Gas. Currently, there are 15 CCS projects in Indonesia spread across Sumatra, Java, Kalimantan and Papua. This technology will have an impact on the environment and economy because it reduces climate change due to the greenhouse effect and can increase economic value for industries obtained from electrical energy. However, there are challenges faced in the form of high investment costs in building infrastructure, and the lack of public awareness of Carbon Capture and Storage (CCS) in Indonesia is still relatively low. To overcome the limitations of the research, there needs to be support from the government, both financially and non-financially, as well as technological advances that can reduce the cost of CCS. In addition, to increase public awareness, wider socialization of the CCS concept is needed so that there is increased interest in CCS research in Indonesia, which is reflected in the new regulatory measures that have been issued to regulate short-term Research and Development (R&D) plans related to CCS. These regulations not only emphasize the technical aspects but also underline the importance of developing non-technical regulations as part of CCS development in Indonesia. (Putri et al. 2024).

The Results of the Analysis Using an Infrared Spectrophotometer

Sample testing from the results of the CO₂ gas absorption

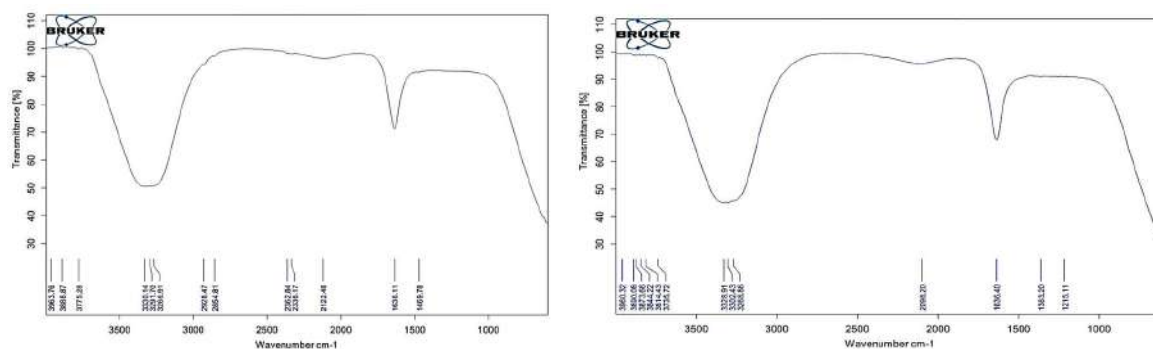


Fig. 7: Spectrogram results in (a) Na_2CO_3 solution and (b) K_2CO_3 solution.

Table 6: Absorption results on the FTIR spectrogram in each absorbent.

Uptake of Na ₂ CO ₃	Function group absorption (cm ⁻¹)	Bonding	Intensity
3330,14	3200-3600	O-H	Strong
1636,11	1690-1760	C=O	Strong

Uptake of K ₂ CO ₃	Function group absorption (cm ⁻¹)	Bonding	Intensity
3328,91	3200-3600	O-H	Strong
1636,40	1690-1760	C=O	Strong

process will be analyzed by an infrared spectrophotometer to determine the functional group of the molecular structure in the sample. The identification of the functional group will be interpreted in a graph that will be shown in the absorption ranges in Fig. 7.

The graph (Fig. 7) presents the results of the functional group analysis for each type of absorbent used. The graph is interpreted in Table 6 to identify the absorption characteristics of each absorbent.

The qualitative analysis based on the graph shows functional group absorption between 3200 and 3600 cm⁻¹, indicated by a broad, sloping elevation, corresponding to the O–H bond—typically associated with alcohols bonded to hydrogen. The functional group identified is consistent with the compound 1-pentanol. Additionally, absorption observed between 1690 and 1760 cm⁻¹, with a sharp and narrow elevation, corresponds to the C=O bond, indicative of ketone groups (alkanones), which are isomeric with aldehydes (alkanals). The functional group isomers detected are consistent with the compound 2-pentanone (Haryanti et al. 2024).

CONCLUSIONS

Based on the results of the study, it can be concluded that CO₂ gas reduction using different types of absorbents has a significant effect on CO₂ concentration. The findings indicate that the most effective absorbent was NaOH, which achieved an optimum solution flow rate of 2 L·min⁻¹ and a CO₂ gas flow rate of 30 L·min⁻¹, resulting in a reduction of CO₂ concentration by 52%. This effectiveness was supported by observable changes in pH, temperature, and electrical conductivity (EC).

Furthermore, the absorbed CO was utilized to generate electrochemical-based electrical energy, achieving an optimum voltage output of 0.817 volts. These results highlight the promising potential of this approach in reducing CO₂ emissions, particularly from the fossil fuel sector.

In support of emission reduction goals, the Indonesian government has implemented significant initiatives aimed at

decreasing greenhouse gas emissions. With a commitment to achieving net-zero carbon emissions by 2060, Indonesia has introduced strategies such as Clean Coal Technology (CCT) combined with Carbon Capture and Storage (CCS). These efforts align with the global paradigm shift toward energy conservation and zero-emission solutions to combat climate change.

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Isolation and Characterization of Phosphate-Solubilizing Bacteria from Compost and Open Dump Sites: Implications for Plant Growth

K. M. P. I. Jayathilake^{1,2}, P. M. Manage¹ and F. S. Idroos^{1†}

¹Centre for Water Quality and Algae Research, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka

²Faculty of Graduate Studies, University of Sri Jayewardenepura, Sri Lanka

†Corresponding author: F. S. Idroos; sumaiyaidroos@sci.sjp.ac.lk

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ABSTRACT

Phosphorus (P) is a vital macronutrient for plant growth. The bioavailability of P in soils is limited due to the insolubility of P. This study aimed to isolate and characterize Phosphate Solubilizing Bacteria (PSB) strains and determine their efficiency of phosphate solubilization and their effect on plant growth. pH, Electrical Conductivity (EC), Total Organic Carbon (TOC), organic nitrogen, and available phosphorus in soil were analyzed. Pikovskaya's Agar (PKV) was used to isolate PSB strains and identified through biochemical tests and 16S rRNA gene sequencing. The solubilization efficiency of the isolates was assessed in PVK broth supplemented with 0.5% tricalcium phosphate (TCP), and their effect on plant growth was evaluated in pot experiments. Mung bean (*Vigna radiata*) was selected as the experimental plant. Five *Bacillus* strains were identified, and the genotypic test was confirmed as *Bacillus pumilus* strain PRE14, *Bacillus altitudinis* 41KF2b, *Bacillus cereus* ATCC 14579, *Bacillus siamensis* strain KCTC 13613, and *Bacillus subtilis* strain NCIB 3610. All isolated strains were positive for the catalase test, gram staining, motility test, starch hydrolysis test, and spore staining test. The citrate utilization test and the spot test for indole production were negative for isolated PSB strains. *B. siamensis* strain KCTC 13613 showed the highest phosphate solubilizing efficiency with the maximum phosphorus concentration of 9.17 ± 0.07 mg/kg recorded after 6 days of incubation. Pot experiments revealed that potting media inoculated with *B. siamensis* strain KCTC 13613 exhibited the highest shoot length (mean increase of 16.77 ± 0.74 cm), root length (mean increase of 10.99 ± 0.41 cm), and wet weight (mean increase of 0.60 ± 0.08 g) compared to other isolated PSB strains. All isolated *Bacillus* spp. Strains demonstrated a significant difference ($n=9$, $P < 0.05$) in measured growth parameters compared to plants grown under the control potting media. Soil pH increased post-germination, with the lowest pH recorded at 7.40 ± 0.09 for media inoculated with *B. siamensis* strain KCTC 13613, indicating organic acid production. These findings reveal the potential of *Bacillus* spp. as bio-inoculants to promote sustainable agriculture by enhancing phosphorus availability and plant growth.

INTRODUCTION

Phosphorus (P) is an essential macronutrient and plays a vital role in various physiological and biochemical processes like energy transfer, photosynthesis, and nutrient uptake of plant growth (Khan et al. 2023, Jayathilake et al. 2024). Conventional agricultural practices mainly depend on the application of chemical fertilizers to meet the phosphorus demands of plant growth (Srivastav et al. 2024). However, the excessive use of chemical fertilizers results in serious negative impacts on the natural environment: soil and water pollution, disturbance in the microbial community of the soil, nutrient runoff causing eutrophication in aquatic life, etc. (Kumar et al. 2019, Hossain et al. 2022). Therefore, researchers turned their attention to alternative ways that would not pollute the environment and thus avoid other negative factors associated with chemical fertilizers (Ajmal et al. 2018, Jayathilake et al. 2024). The bioavailability of phosphorus in many agricultural

soils is limited due to its predominance in insoluble forms like phosphate minerals and organic compounds (Phytates, Humic substances, and Phospholipids). This limitation creates a significant challenge to agricultural productivity, and plants struggle to access an adequate P supply. The forms of P that are taken up by plants from soil solution are phosphate anions (mainly H_2PO_4^- and HPO_4^{2-}) (Motamedi et al. 2016). To address the issue of phosphorus availability while mitigating environmental risks, alternative approaches rooted in sustainable agricultural practices have gained prominence. Among such approaches, the use of Phosphate Solubilizing Bacteria (PSB) stands out as a very promising strategy. PSB plays a significant part in agroecosystems by converting insoluble soil phosphate into soluble forms (H_2PO_4^- and HPO_4^{2-}), consequently making sufficient phosphorus available for use as an essential plant nutrient. This conversion process enhances the availability of phosphorus, a vital nutrient for plant growth and development. By solubilizing phosphate, these bacteria make it more accessible to plants, thus promoting better nutrient uptake and ultimately leading to improved crop yields. This ability of PSB highlights their significance in sustainable agriculture practices aimed at enhancing soil fertility and productivity (Shome et al. 2022, Silva et al. 2023).

PSB is a group of microorganisms with diversity that can convert insoluble phosphorus to soluble forms through various mechanisms. These include organic acid production, proton extrusion, and enzymatic activity (Patel & Goswami 2020). The identification and characterization of these bacteria from different soil environments is very promising for enhancing phosphorus uptake in plants. Previous research has been able to show the potential of PSB not only in increasing available phosphorus in soils but also in promoting overall plant growth through improved uptake of other nutrients and soil health (Yadav et al. 2023). The benefits resulting from PSB-mediated phosphate solubilization are not only limited to the availability improvement of phosphorus. Inoculation studies with PSB have also reported positive effects on soil health parameters, nutrient cycling process, and promoting overall plant growth. By promoting nutrient uptake, improving soil structure, and reducing the reliance on chemical fertilizers, PSB contributes to the sustainability and resilience of agricultural systems, aligning with the principles of agroecology and ecological farming (Arjumend et al. 2022).

The isolation and characterization of PSB from different soil environments hold significant potential for enhancing phosphorus availability in agricultural soils (Janati et al. 2022). Compost and open dump sites represent unique

ecological niches rich in microbial diversity, including PSB, due to the decomposition of organic matter and the accumulation of nutrient-rich materials. Therefore, exploring these sites for the isolation of PSB offers an opportunity to identify novel strains with efficient phosphate solubilization capabilities. For their effective application as biofertilizers in sustainable agriculture, it is essential to understand the characteristics and behavior of isolated PSB strains. Biochemical tests that help evaluate metabolic activities, genotypic identification by techniques such as sequencing 16S rRNA genes, and evaluating phosphate solubilization efficiency under laboratory conditions are generally involved in efforts toward characterizing these isolates. These analyses provide valuable insights into the diversity, functionality, and potential agricultural benefits of the isolated PSB strains (Asghar et al. 2023, Dodino-Gutiérrez et al. 2024). PSB have attracted increasing attention for their role in improving phosphorus availability for legume plants and also for their wider application in other crops. Recent studies have already shown the potential of PSB in improving nutrient uptake, growth, and yield in both legume plants and nonlegume plants and fast-growing plants, illustrating their diversity and versatility in supporting various agricultural sectors (Biswas & Shivaprakash, 2021, Kumar et al. 2016, Angadi et al. 2017, Jayathilake et al. 2024). This study aims to isolate and characterize PSB strains from compost and open dump sites and determine the phosphate solubilization efficiency and their potential impact on plant growth. The research results are significant in terms of contributing to sustainable developments in agriculture by harnessing the potential benefit of soil-microbe-plant interactions to enhance nutrient cycling and agricultural production.

MATERIALS AND METHODS

Collection and Analysis of Physiochemical Properties of Soil Sample

Compost piles associated with soil and open dump soil in Karadiyana (6°48'51.8" N, 79°54' 17.0" E) were collected to isolate the phosphate-solubilizing bacteria. Soil pH, Electrical Conductivity (EC), total soil carbon, soil organic nitrogen, and soil available phosphorus were determined by standard protocols and methods (Walkley & Black 1934, AOAC 2000, Mussa et al. 2009).

Isolation of Phosphate Solubilizing Bacteria

Phosphate-solubilizing bacterial strains were isolated using Pikovskaya's Agar medium. 10 g of each soil sample was homogenized in 90 mL sterilized saline. A series of 10-fold

dilutions of the suspension were prepared for each sample. 100 µL of each concentration was spread directly onto the surface of Pikovskaya Agar medium plates (Ha & Chu 2020). Pure cultures of five isolated strains were labeled as PSB-A, PSB-B, PSB-C, PSB-D, and PSB-E.

Biochemical Analysis of Bacterial Isolates

Rhizospheric microbes were characterized by different biochemical methods, catalase test, gram staining test, motility test, starch hydrolyses test, spore staining, citrate utilization test, and spot test for assessment of indole production (Hamza et al. 2017). These biochemical tests aimed to confirm the potential of isolates for nutrient cycling and plant nutrition enhancement.

Genotypic Identification of Bacterial Isolates

The DNA extraction was performed using the Wizard Genomic DNA Purification Kit. The 16srRNA gene was amplified by Polymerase Chain Reaction (PCR) and subjected to sequencing at Macrogen, Korea.

Phylogenetic Tree Construction

The phylogenetic tree was constructed using MEGA software based on 16S rRNA gene sequences. Sequence alignment was performed using the CLUSTALW algorithm, and the tree was generated using the Neighbor-Joining method with 1,000 bootstrap replicates to assess branch reliability.

Phosphate Solubilization Efficiency of Bacterial Isolates

The phosphate solubilization activity test was conducted in 150 mL conical flasks containing 100 mL of PVK broth supplemented with 0.5% TCP (pH = 7). Exactly 1 mL of bacterial inoculant (10^7 CFU/mL) was inoculated into prepared broth media, while sterile distilled water served as the control. The experimental setup was performed in triplicate. The cultures were then incubated on a rotary shaker at 28°C and 130 rpm for 8 days. Aseptic aliquots of 1.5 mL of culture supernatant were collected on the 2nd, 4th, 6th, and 8th days. Following the centrifugation at 12,000 rpm for 2 minutes to remove suspended solids and mycelial fragments, 0.1 mL of each culture supernatant was taken to estimate the phosphorus released from TCP. The available soluble phosphate in culture supernatants was assessed using the Bray extraction method at 882 nm (Hui et al. 2011, Doilom et al. 2020).

Preparation of Potting Media

The growth medium for the pot experiment contained a blend of compost, coir dust, and soil in a 1:1:1 ratio and isolated bacterial strains. The bacterial inoculant was

prepared in sterilized distilled water. Subsequently, each pot was inoculated with 10 mL of bacterial inoculant (10^8 CFU/mL) from each isolate, ensuring consistent distribution of the strains throughout the sterilized media in the pots.

Determination of the Effect of PSB Strains on Mung Bean (*Vigna radiata*) Plant Growth

Plant growth parameters (shoot length and root length) were recorded to determine the effect of the selected isolates on plant growth. A control pot was treated with 10 mL of sterilized water. The pots were irrigated twice daily with 50 mL of water per day. The experiment followed a completely randomized block design with five replications per treatment. The potting media was kept sterile through aseptic techniques at the time of planting and irrigation to avoid microbial contamination from the outside environment. The physiochemical parameters of potting media (pH, electric conductivity, available phosphorus, and soil organic carbon) were determined before seed germination and after 30 days of seed germination (Walkley & Black 1934, Mussa et al. 2009, Pal et al. 2009).

Statistical Analysis

Data analysis and graphical representations were conducted using Microsoft Excel. Data from different treatments were calculated and statistically analyzed with a one-way analysis of variance (ANOVA) using Python 3.11 (VS Code).

RESULTS AND DISCUSSION

Bacterial strains were isolated from compost piles associated with soil and open dump site soil to determine the potential of phosphate-solubilizing bacteria (PSB) on plant growth. Fig. 1 represents the physicochemical properties of compost soil and open dump site soil samples. Both soil samples have shown acidic characteristics. The compost soil has a lower pH value compared to the open dump site soil. This acidic condition is optimal for the proliferation of PSB strains. Li et al. (2022) and Zhao et al. (2024) have shown that acidic conditions are more conducive to the growth of PSB strains. The collected soil samples have high concentrations of organic carbon, organic nitrogen, and soil moisture content. Previous studies have shown that organic carbon, organic nitrogen, and soil moisture content are vital for the growth of PSB and essential for their proper development (Atif et al. 2023, Wang et al. 2023).

Table 1 presents the isolated PSB strains. Five PSB strains were isolated and labeled as PSB-A, PSB-B, PSB-C, PSB-D, and PSB-E, all of which belong to the genus *Bacillus*.

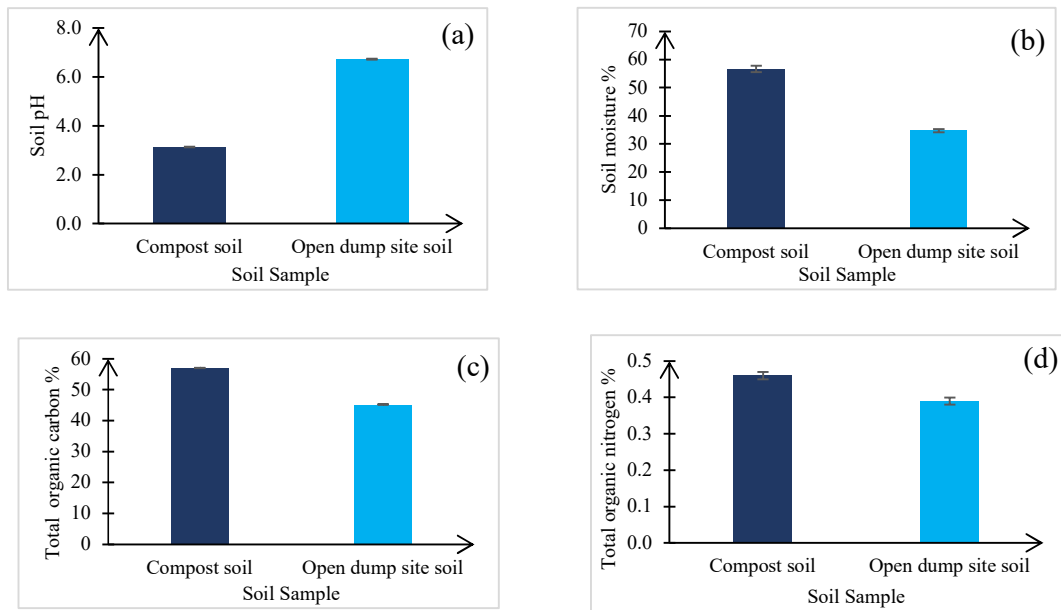


Fig. 1: Physiochemical properties of collected soil samples (a) soil pH, (b) soil moisture content, (c) total organic carbon, and (d) total organic nitrogen.

Table 1: Isolated PSB Strains.

Isolated Strains	Bacterial strains
PSB-A	<i>Bacillus pumilus</i> strain PRE14
PSB-B	<i>Bacillus altitudinis</i> 41KF2b
PSB-C	<i>Bacillus cereus</i> ATCC 14579
PSB-D	<i>Bacillus siamensis</i> strain KCTC 13613
PSB-E	<i>Bacillus subtilis</i> strain NCIB 3610

Genotypic identification confirmed that the five isolated PSB strains are *B. pumilus* strain PRE14, *B. altitudinis* 41KF2b, *B. cereus* ATCC 14579, *B. siamensis* strain KCTC 13613, and *B. subtilis* strain NCIB 3610. Table 2 illustrates the results of the biochemical analysis test for the isolated PSB strains. All isolated *Bacillus* species tested positive for the catalase test, Gram staining, motility test, starch

hydrolysis test, and spore staining test, while they tested negative for the citrate utilization test and the spot test for indole production.

The catalase test indicates the presence of the enzyme catalase, which is essential for breaking down hydrogen peroxide, a toxic byproduct of aerobic metabolism (Gebicka & Krych-Madej 2019). Gram staining confirms the cell wall structure characteristic of *Bacillus* species. The motility test reveals the ability of these bacteria to move, which is crucial for colonization and nutrient acquisition (Madigan et al. 2015). Starch hydrolysis demonstrates the production of amylase, allowing the bacteria to utilize starch as a carbon source. Spore staining identifies the presence of endospores, which are vital for bacterial survival under adverse conditions. The negative citrate utilization test indicates

Table 2: Biochemical analysis of isolated strains.

Bacterial strains	Biochemical test						
	Catalase test	Gram staining test	Motility test	Starch hydrolyses test	Spore Staining	Citrate Utilization Test	Spot Test for Assessment of Indole Production
<i>Bacillus pumilus</i> (PRE14)	+	+	+	+	+	-	-
<i>Bacillus altitudinis</i> (41KF2b)	+	+	+	+	+	-	-
<i>Bacillus cereus</i> (ATCC 14579)	+	+	+	+	+	-	-
<i>Bacillus siamensis</i> (KCTC 13613)	+	+	+	+	+	-	-
<i>Bacillus subtilis</i> (NCIB 3610)	+	+	+	+	+	-	-

that these strains cannot use citrate as their sole carbon source, and the negative indole production test shows they lack the enzyme tryptophanase necessary for breaking down tryptophan. These biochemical characteristics are critical for identifying and understanding the metabolic capabilities of the PSB strains (Hamza et al. 2017).

The phylogenetic tree (Fig. 2) shows the evolutionary relationships among phosphate-solubilizing *Bacillus* isolates, constructed through 16S rRNA gene sequencing and MEGA software. This analysis includes strains *Bacillus pumilus* (PRE14), *Bacillus altitudinis* (41KF2b), *Bacillus siamensis* (KCTC 13613), *Bacillus subtilis* (NCIB 3610), and *Bacillus cereus* (ATCC 14579). These species have been documented for their efficient phosphate-solubilizing capabilities, beneficial for agricultural applications as biofertilizers (Tahir et al. 2013, Cherif-Silini et al. 2016, Ait-Ouakrim et al. 2023). In the phylogenetic tree, *Bacillus pumilus* (PRE14) is closely clustered with *Bacillus altitudinis* (41KF2b), suggesting they share recent common ancestry and may exhibit similar functional traits in phosphate solubilization. *Bacillus siamensis* (KCTC 13613) and *Bacillus subtilis* (NCIB 3610) form a secondary cluster, indicating moderate genetic divergence from the first cluster. *Bacillus cereus* (ATCC 14579) branches off earlier, indicating a greater evolutionary distance and potentially different ecological roles or mechanisms in phosphate solubilization. These clustering patterns reinforce previous research on *Bacillus* spp. as effective phosphate solubilizers, crucial for enhancing nutrient availability in agricultural soils. The close relationship between *B. pumilus* and *B. altitudinis* aligns with findings by Ait-Ouakrim et al. (2023) on efficient phosphate solubilization by *Bacillus* strains, while the functional diversity within this genus supports its broad application in biofertilizer development. Previous studies have shown that phylogenetic analysis of soil microbes underlines their multifunctional roles in

sustainable agriculture by emphasizing that they promote plant growth through ammonia production, siderophore synthesis, and phosphorus solubilization (Bramhachar et al. 2027, Verma & Saharan 2020).

Fig. 3 illustrates the solubilized phosphorus concentrations after 2, 4, 6, and 8 days of incubation in PVK broth inoculated with PSB isolates. *B. siamensis* strain KCTC 13613 exhibited the highest phosphate solubilizing efficiency on the 6th day of incubation compared to the other isolated PSB strains. The other *Bacillus* strains showed the highest available phosphorus levels on the 2nd day of incubation, with a gradual decrease in phosphorus concentration thereafter. Fig. 4 shows the pH values of TCP-containing PVK broth inoculated with PSB isolates after 2, 4, 6, and 8 days of incubation. The lower pH value is attributed to the formation of organic acids during the incubation period. PSB strains solubilize insoluble phosphorus (PO_4^{3-}) into plant-available phosphorus (H_2PO_4^- , HPO_4^{2-}) through the production of organic acids during the phosphate solubilizing process (Aruna et al. 2024). This plant-available phosphorus is crucial for proper plant growth and nutrient availability, emphasizing the role of PSB in enhancing soil fertility and promoting agricultural productivity (Yadav et al. 2023).

Fig. 5 represents the variation of growth parameters in Mung bean plants. Mung beans were selected due to their numerous advantages, including ease of cultivation, short growth cycle, wide availability, adaptability to various environments, and sensitivity to nutrient levels (Hou et al. 2019). The potting media inoculated with *B. siamensis* strain KCTC 13613 exhibited the highest shoot length, root length, and wet weight of the grown plants. This indicates the significant role of *B. siamensis* strain KCTC 13613 in promoting plant growth through the solubilization of insoluble phosphorus. Additionally, potting media inoculated with *B. pumilus* strain PRE14, *B. altitudinis* 41KF2b, *B. cereus* ATCC 14579, and *B. subtilis* strain NCIB 3610 showed significant

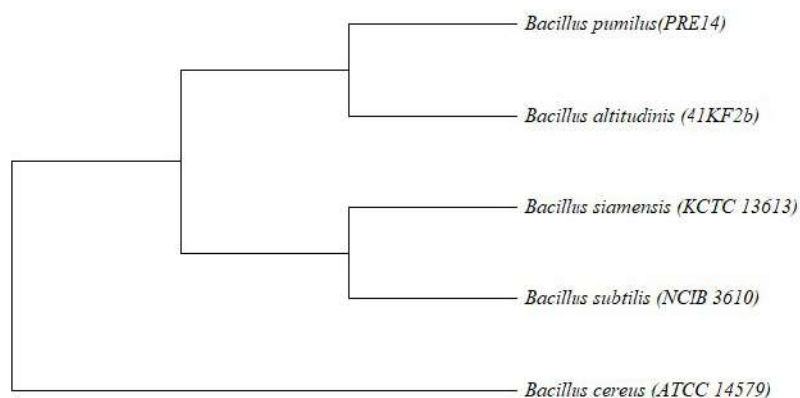
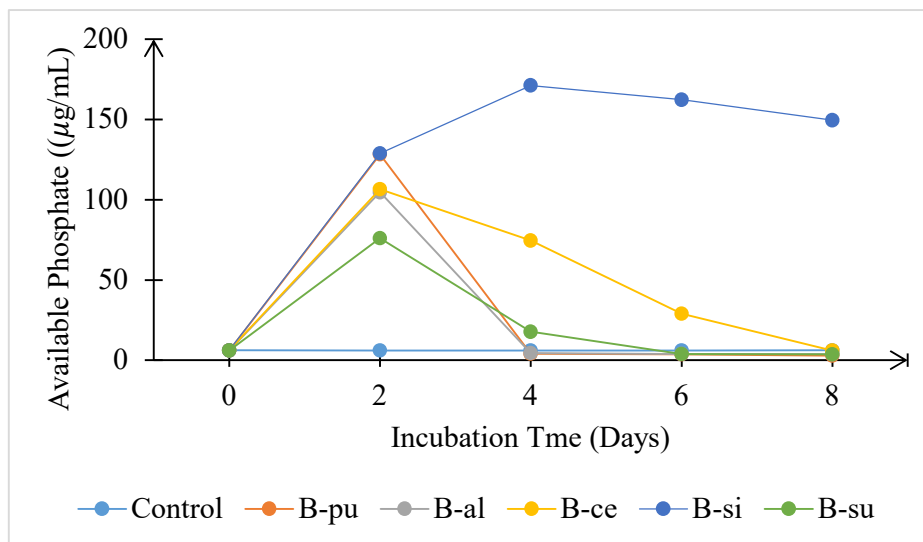
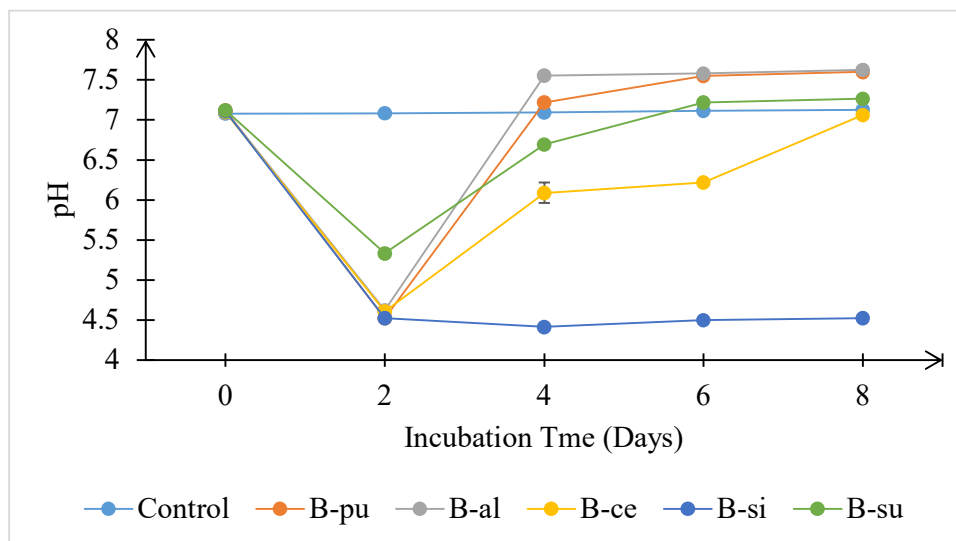


Fig. 2: The evolutionary relationships among isolated phosphate-solubilizing bacteria.



(Note: B-pu- *Bacillus pumilus* strain PRE14, B-al- *Bacillus altitudinis* 41KF2b, B-ce *Bacillus cereus* ATCC 14579, B-si - *Bacillus siamensis* strain KCTC 13613, B-su- *Bacillus subtilis* strain NCIB 3610)

Fig. 3: Solubilized P concentrations after 2, 4, 6, and 8 days of incubation in PKV broth inoculated with PSB isolates.



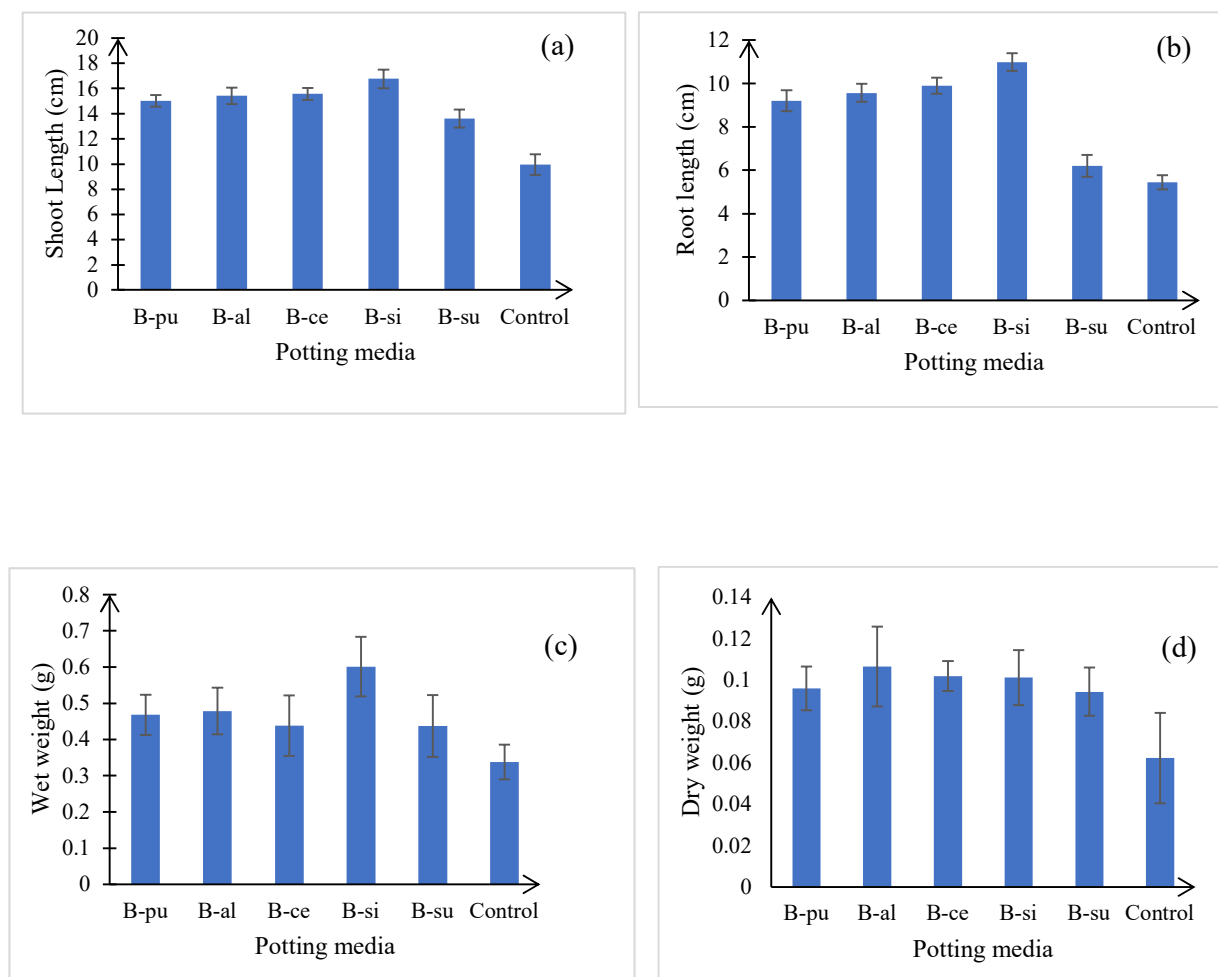
(Note: B-pu- *Bacillus pumilus* strain PRE14, B-al- *Bacillus altitudinis* 41KF2b, B-ce *Bacillus cereus* ATCC 14579, B-si - *Bacillus siamensis* strain KCTC 13613, B-su- *Bacillus subtilis* strain NCIB 3610)

Fig. 4: pH values of TCP containing PVK broth inoculated with PSB isolates after 2, 4, 6, and 8 days of incubation.

differences ($p < 0.05$) in the measured growth parameters (shoot length, root length, wet weight, and dry weight of the grown plants). The involvement of these strains in enhancing plant growth can be attributed to their ability to increase nutrient availability, particularly phosphorus, which is crucial for various physiological and biochemical processes in plants.

Table 3 presents the variation in physicochemical parameters of the potting media before seed germination (initial) and after 30 days of seed germination.

The initial pH value of the potting media was acidic. After 30 days of seed germination, the pH value of the potting media inoculated with PSB strains became basic. Specifically, the potting media inoculated with *B. siamensis* strain KCTC 13613 had a lower pH value (7.40 ± 0.09) compared to the media inoculated with other PSB strains and the control. This shift in pH suggests the metabolic activity of PSB strains, which can alter soil pH through the production of organic acids and subsequent phosphate



(Note: B-pu- *Bacillus pumilus* strain PRE14, B-al- *Bacillus altitudinis* 41KF2b, B-ce *Bacillus cereus* ATCC 14579, B-si - *Bacillus siamensis* strain KCTC 13613, B-su- *Bacillus subtilis* strain NCIB 3610)

Fig. 5: Variation of growth parameters of Mung Bean (*Vigna radiata*) plants (a) shoot length, (b) root length, (c) wet weight, and (d) dry weight.

Table 3: The variation of physiochemical parameters of potting media before seed germination (Initial) and after 30 days of seed germination.

Parameter	Before seed germination	After 30 days of seed germination					
	Initial	B-pu	B-al	B-ce	B-si	B-su	Control
pH	6.83 ± 0.02	7.62±0.07	7.52±0.05	7.66±0.11	7.40±0.09	8.00±0.06	8.23±0.08
EC [$\mu\text{S}\cdot\text{cm}^{-1}$]	1040.67±7.51	128.77±9.18	134.83±3.13	124.73±3.33	125.40±1.75	116.97±2.01	115.23±1.26
AP [$\text{mg}\cdot\text{kg}^{-1}$]	1.74±0.01	3.33±0.13	2.19±0.07	3.07±0.09	9.17±0.07	2.79±0.06	1.90±0.11
OC%	7.73±0.13	6.06±0.21	7.07±0.20	5.71±0.15	6.69±0.28	4.35±0.48	7.01±0.08

solubilization (Patel & Goswami 2020). The initial EC of the potting media was high ($1040.67 \pm 7.51 \mu\text{S}\cdot\text{cm}^{-1}$) compared to the media after 30 days of seed germination. The EC values of the media inoculated with PSB strains were lower, indicating improved nutrient utilization by the plants. Initially, the potting media had low available phosphorus

($1.74 \pm 0.01 \text{ mg}\cdot\text{kg}^{-1}$). After 30 days, the media inoculated with *B. siamensis* strain KCTC 13613 showed the highest available phosphorus level ($9.17 \pm 0.07 \text{ mg}\cdot\text{kg}^{-1}$), highlighting the strain's efficiency in solubilizing phosphorus. Other PSB-inoculated media also showed significant differences ($p < 0.05$) in available phosphorus compared to the control.

The initial organic carbon percentage of the potting media was $7.73 \pm 0.13\%$. Post germination, the media inoculated with PSB strains exhibited noticeable changes in organic carbon content, generally showing lower values compared to the initial media. This reduction could be attributed to microbial activity and plant uptake, indicating an active nutrient cycling process facilitated by the PSB strains.

The positive outcomes in catalase, Gram staining, motility, starch hydrolysis, and spore staining tests highlight physiological characteristics in the isolated *Bacillus* species. Catalase activity is critical for degrading hydrogen peroxide, thereby reducing oxidative stress on plants. Gram-positive staining indicates the structural integrity of bacterial cell walls, which is crucial for stability and persistence in the rhizosphere (Paray et al. 2023, Ghorpade et al. 2023). Motility facilitates bacterial movement towards plant roots, enhancing colonization and improving nutrient uptake efficiency. Starch hydrolysis capability suggests proficiency in carbohydrate metabolism, providing energy for bacterial growth and activities that support plant nutrition. Spore formation ensures survival during unfavorable conditions, enabling prolonged interactions with plants. These characteristics suggest that the isolated *Bacillus* species possess mechanisms that promote plant growth by enhancing nutrient availability, stress tolerance, and root colonization. In spite of testing negative for the citrate utilization test and the spot test for indole production, which are associated with nutrient solubilization and plant hormone modulation, respectively. The absence of citrate utilization suggests potential limitations in organic acid production, which could impact the solubilization of insoluble phosphates in soil. However, positive results for phosphate solubilization efficiency indicate that these strains can lower pH levels and increase available phosphorus, suggesting alternative pathways or microbial interactions that facilitate nutrient release beneficial for plant uptake. The lack of indole production capability implies a deficiency in auxin synthesis, which is crucial for root development and growth stimulation. However, the observed enhancement in plant growth parameters suggests that these strains may utilize other mechanisms, such as direct phosphate solubilization or synergistic interactions with soil microbes, to effectively promote plant growth. Moreover, previous studies have demonstrated that the isolated *Bacillus* spp. Possess significant potential for nitrogen fixation and nutrient solubilization that plants require (Saha et al. 2023, Novello et al. 2024). However, the absence of field trials limits the ability to evaluate scalability and applicability on-site. Moreover, the effect of PSB application could not be studied for long-term impacts on soil health as well as crop productivity. Future studies should focus on large-scale field trials, testing PSB efficacy across various types of soils and

climatic conditions, and investigating problems associated with commercial production and distribution.

These findings demonstrate the potential of utilizing *Bacillus* spp. as bio-inoculants to promote sustainable agriculture through improved phosphorus availability and enhanced plant growth.

CONCLUSIONS

This study successfully isolated and identified five phosphate-solubilizing bacterial (PSB) strains from compost and open dump sites in Karadiyana, which were identified as *B. pumilus* strain PRE14, *B. altitudinis* 41KF2b, *B. cereus* ATCC 14579, *B. siamensis* strain KCTC 13613, and *B. subtilis* strain NCIB 3610. The biochemical and genotypic analyses confirmed the efficacy of these strains in solubilizing phosphate under acidic soil conditions, which are conducive to their growth and activity. The phosphate solubilization efficiency varied among the isolates, with *B. siamensis* strain KCTC 13613 exhibiting the highest phosphate solubilizing activity. This strain demonstrated the greatest ability to lower the pH of the growth medium, indicating significant organic acid production, which is essential for phosphate solubilization. Furthermore, the pot experiment with mung bean plants revealed that inoculation with PSB strains significantly enhanced plant growth parameters, including shoot length, root length, and biomass. Particularly, *B. siamensis* strain KCTC 13613 significantly improved the availability of phosphorus in the potting media, leading to better plant growth outcomes compared to other strains and the control. The study also observed a shift in soil pH towards basicity and a reduction in electrical conductivity and organic carbon content post-germination, suggesting active microbial involvement in nutrient cycling and soil fertility enhancement. The focus of future research should shift toward evaluating the efficiency of PSB on different soil types under field conditions and their compatibility with other microbial consortia for further optimization of applications under different agricultural systems. These findings highlight the potential of utilizing *Bacillus* spp. as bio-inoculants to promote sustainable agriculture through improved phosphorus availability and enhanced plant growth.

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Spatiotemporal Changes of the Forest Cover in Northeastern Ghat Zone of Odisha, India Using Multi-Year Landsat Data

Fawaz Parapurath¹, Rashmirekha Senapati², Argha Ghosh², Bama Shankar Rath^{3†} and Deepti Verma⁴

¹Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India

²Department of Agricultural Meteorology, Odisha University of Agriculture and Technology, Bhubaneswar-751003, Odisha, India

³Department of Agronomy, Odisha University of Agriculture and Technology, Bhubaneswar-751003, Odisha, India

⁴AICRPAM Unit, ICAR-Central Research Institute for Dryland Agriculture, Santosh Nagar, Hyderabad-500059, Telangana, India

†Corresponding author: Bama Shankar Rath; bsrath816@gmail.com

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ABSTRACT

Forests are essential to the terrestrial ecosystem, which supports a sustainable way of life and economy for people. As a result of their ability to capture atmospheric carbon dioxide and mitigate its worldwide consequences, forests are crucial for halting climate change. In light of this context, the current study's objective is to assess the changes in Land Use & Land Cover (LULC) and forest cover across the North Eastern Ghat Zone (NEGZ) of Odisha, India, from 1990 to 2020. Firstly, multi-year preprocessed Landsat data at ten-year intervals (1990, 2000, 2010 and 2020) were collected using cloud computing Google Earth Engine (GEE) platform, and the entire region was divided into five separate classes based on the Normalized Difference Vegetation Index (NDVI) thresholds viz., Very Dense Forest (VDF), Moderately Dense Forest (MDF), Open Forest (OF), and Non- Forest Land (NFL). Through the use of Supervised & Unsupervised techniques of classification, five main LULC categories were also established, viz., Agriculture, Barren Lands, Forest, Settlements, and Water Bodies. The results infer that the forest cover was reduced by 20%, wherein a gradual decrease in the VDF area by 14.21% of the NEGZ was significant during the study period. Unlike the VDF dynamics, the OF coverage showed a slight increase of 4.56% of NEGZ. On the contrary, the settlement area increased by about 130%. However, this study could infer that the expansion of settlements due to population hike is the primary driver of deforestation and forest fragmentation (because the population growth and increased settlements accounted for 97% and 93% of the variability in forest cover). Additionally, it was found that the variation in the forest cover could explain 45% variability of the mean air temperature as indicated by the coefficient of determination. Therefore, by placing special focus on the aforementioned findings and conclusions, we may conclude that the current study may contribute to research on forest management, climate change mitigation, and sustainable development.

INTRODUCTION

Forests are recognized as one of the most critical land use categories, which play an indispensable role in the terrestrial ecology. According to FAO (2000), forests are those land cover classifications that cover an area of at least 0.5 hectares with a potential canopy cover and tree height of at least 10% and 5 meters, respectively. Since forests serve as the foundation for organic carbon synthesis and regulate the water cycle, they influence the climate of an area. Even in the absence of anthropogenic climate forcing, rapid increases in the frequency of extreme weather events pose significant challenges (Subba Rao et al. 2024). Thus, forests have a major role in the sustainable human existence and economy (Shimelis 2017).

From the hydrological perspective, forests can recycle rainwater effectively into the atmosphere because of their higher rate of evapotranspiration when

compared to other ecosystems. Moreover, forests weaken the hydrological cycle by reducing plant evapotranspiration, downwind precipitation, and cloud formation. Within forests, trees further contribute to the cloud formation by emitting volatile organic compounds, which act as a component for the condensation nuclei (Duku & Hein 2021). As deforestation rates have risen, they are currently at risk of rapid decline (Pielke Sr et al. 2011, Malhi et al. 2022, Gaonkar et al. 2024), contributing significantly to the global loss of biodiversity (Vieira et al. 2008, Giam 2017), resulting in decreasing rainfall and increasing temperature (Lawrence & Vandecar 2015, Lejeune et al. 2015).

Large-scale deforestation disturbs the carbon cycle and the Earth's radiative equilibrium because more CO₂ and methane are released into the atmosphere (Lawrence et al. 2022). It also influences the wind turbulence and rainfall pattern variation. So, when forests are converted into other land use classes, the aberrations in rainfall patterns become more profound. The effects of deforestation can be observed at scales ranging from watersheds to continents, and changes in the forests of one country or watershed can impact rainfall in other nations or watersheds as well. Therefore, forest cover represents a significant driver of global and local climate change (Prevedello et al. 2019).

According to the Forest Survey of India (FSI) (2011), the total forest cover accounts to be 712,249 km² (71.22 Mha), representing 21.67% of the country's total land area. Among the states, the forests in Odisha are numerous, diverse, multi-layered, and dense, with an actual forest cover of 48,903 km², which constitutes 7.1% of the nation's total forest cover and 31.4% of the state's total geographical area, respectively (Forest Survey of India 2011). Globally, forest acreage has shrunk by 3% (4128 Mha - 3999 Mha) from 1990 to 2015. However, it is observed that the forest cover is likely to decline at an alarming rate, whereas in some areas, forest cover is increasing at a very slow pace. Therefore, monitoring forest resources and biodiversity is highly necessary for ensuring the balance of the food chain and various biogeochemical cycles.

To develop effective forest management policies and practices (Forkuo & Frimpong 2012), it is crucial to obtain accurate land use and land cover (LULC) information (Manandhar et al. 2009). LULC data is vital for understanding human impact on natural landscapes because the changes in LULC reflect how ecosystems are altering their capacity to provide services to human society now and in the future (Senapati et al. 2024). Therefore, understanding LULC changes and identifying transformation hotspots are highly necessary for urban planners and environmentalists (Tiwari et al. 2024). However, the traditional methods for

monitoring land use and land cover are time-consuming, labour-intensive, and cost-ineffective. Moreover, these methods are insufficient for obtaining large-scale and continuous observations of the Earth's surface (Satyawan & Hooda 2014). Whereas, the recent advancements in satellite-based remote sensing provide an unparalleled opportunity for observing and monitoring the Earth's surface at fine spatial resolutions and frequent intervals (Sawaya et al. 2003, Mulder et al. 2011, Ghazaryan et al. 2018, Ghosh et al. 2019, 2022, 2024, Mugo et al. 2020, Ashutosh & Roy 2021, Abebe et al. 2022).

The most common use of satellite-based remote sensing is LULC change detection, which can now be done with precision using the Google Earth Engine (GEE) platform. GEE is a cloud-based geospatial analysis tool (Gorelick et al. 2017) that uses the Simple Non-Iterative Clustering (SNIC) algorithm to facilitate the efficient grouping of similar pixels and identification of potential individual objects (Achanta & Susstrunk 2017). Several researchers have generated various indices using remote sensing techniques to assess and monitor the forest ecosystems in terms of integrity, fragmentation, and transformation (Kunwar et al. 2020, Sharma et al. 2020). Among the indices, the Normalized Difference Vegetation Index (NDVI) is the most commonly used index for monitoring forest cover in any region. NDVI's sensitivity to canopy cover provides insights about the spatial variations in forest density (Parapurath et al. 2020). Further, medium-resolution multispectral optical satellites like the Landsat series have proven their efficiency for monitoring the forest cover over time. (Devi et al. 2021, Islam et al. 2021, Thakur et al. 2021).

During the period from January 1, 2015, to February 5, 2019, a staggering 4,968.48 hectares of forest land in Odisha were diverted for non-forestry purposes under the provisions of the Forest Conservation Act, 1980. Despite the ecological significance of the North-Eastern Ghat Zone, which forms part of the biodiverse Eastern Ghats and hosts an array of endemic plant and animal species, it is necessary to evaluate the deforestation dynamics that threaten its biodiversity and environmental stability. Taking all these factors into consideration, the present study was conducted to assess the changes in land use & land cover and forest cover dynamics in the North Eastern Ghat Zone of Odisha over the last three decades (1990-2020).

MATERIALS AND METHODS

Study Area

The districts of Kandhamal, Rayagada, Gajapati, and Ganjam are all part of Odisha's North Eastern Ghat Zone. The region

spans 27,913.32 km², with latitudes 18.75°N to 20.69°N and longitudes 82.87°E to 85.18°E (Fig. 1). It represents around 35% of Odisha's total forest cover. This area has a hot, humid, sub-humid environment with an average of 1597 mm of rainfall per year. The most common soil types in this region are red soil, mixed red and black soil, lateritic alluvial soil, and brown forest soil.

Data

Landsat time series data were employed to map the land use & land cover, and forest cover. The pre-processed multi-year Landsat data were collected at 10-year intervals spanning from 1990 to 2020, using the Google Earth Engine (GEE), through Java scripting (Table 1). However, a field survey was conducted in the study area during 2020 to collect the ground truth data using the stratified random sampling approach for the accuracy assessment of forest cover classification. Additionally, the daily mean air temperature at 2 metres height over the NE Ghat Zone was collected from the Google Climate Engine platform. For this purpose, the ERA5 (Fifth generation ECMWF [European Centre for Medium-Range

Table 1: Date of acquisition of Landsat data from 1990-2020.

Year	Acquisition date	Satellite and sensors	Spatial resolution
1990	25.12.1990	Landsat 5 TM	30 m
2000	02.01.2000	Landsat 5 TM	30 m
2010	20.12.2010	Landsat 5 TM	30 m
2020	17.11.2020	Landsat 8 OLI	30 m

Weather Forecasts] atmospheric reanalysis) daily aggregates, provided by the Copernicus Climate Change Service (C3S, 2017), were utilized in this study.

Computation of NDVI

The Normalized Difference Vegetation Index (NDVI) was calculated for each year to evaluate the spatial variations in forest cover density. Its values range from -1 to +1, with higher and lower values indicating denser and sparser vegetation, respectively (Ghosh et al. 2019). NDVI for a given year was calculated using the raster calculator menu in QGIS 3.14 software.

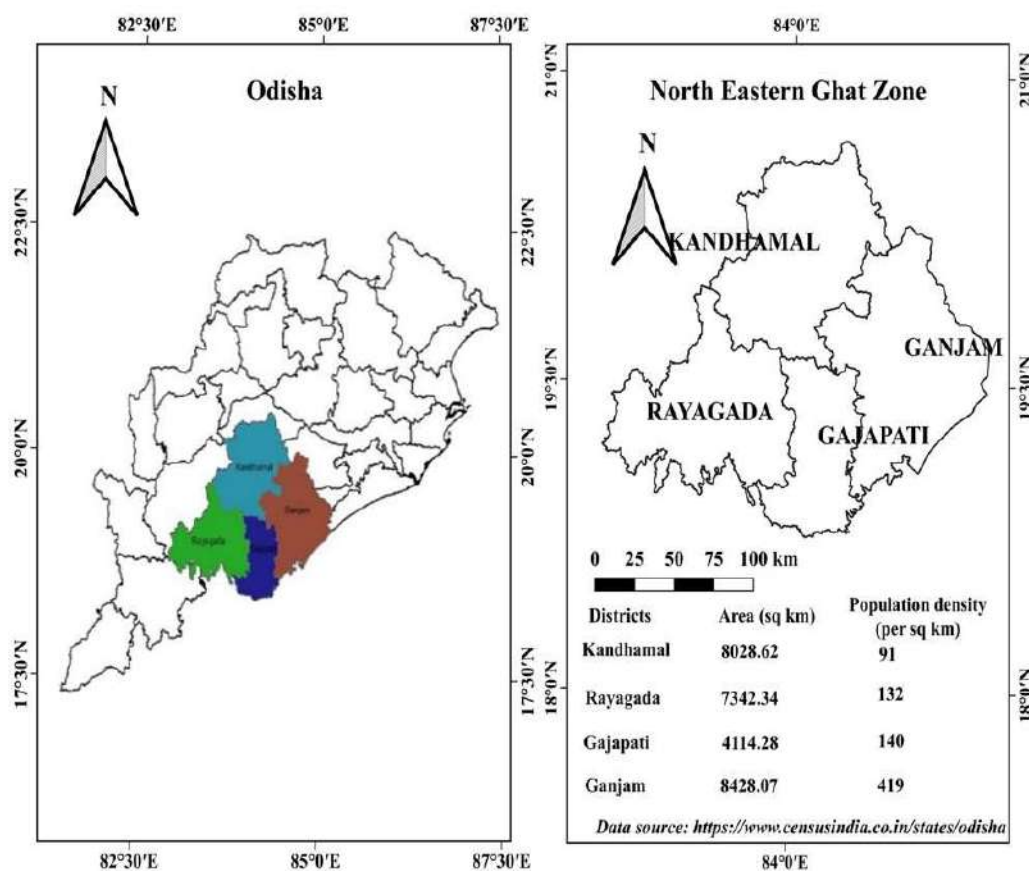


Fig. 1: Geographical location of the North Eastern Ghat Zone of Odisha.

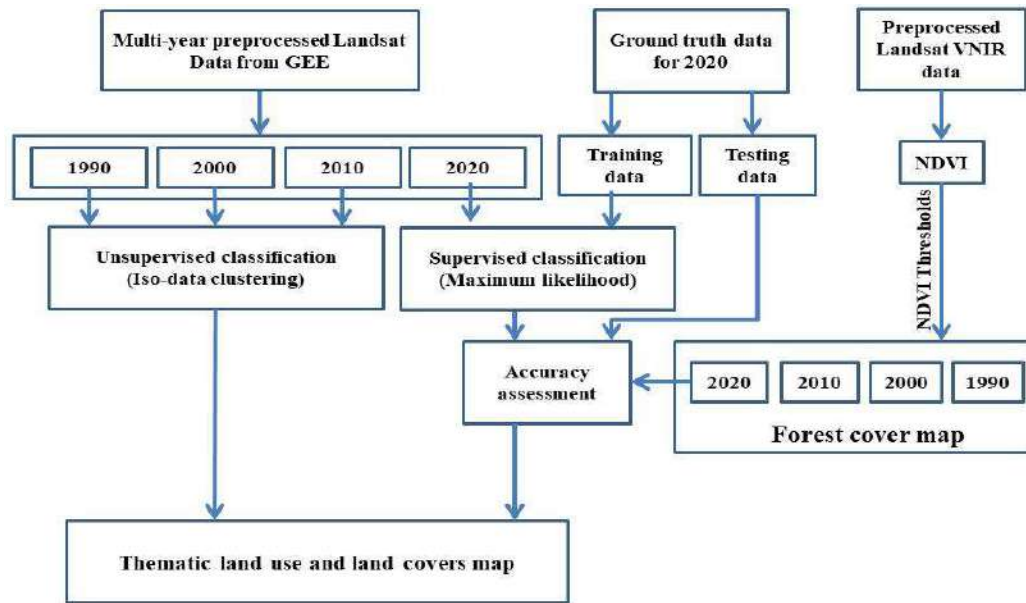


Fig. 2: Steps for LULC and forest cover mapping.

To calculate NDVI, the following formula (Rouse et al. 1974) was used.

$$NDVI = \frac{R_{NIR} - R_R}{R_{NIR} + R_R} \quad \dots(1)$$

Where, R_{NIR} = Reflectance in the near-infrared (NIR) band and R_R = reflectance in the red band.

Preprocessing and Classification

The pre-processed Landsat TM and OLI data were obtained from the Google Earth Engine (GEE) for additional processing and categorization, including the visible bands (Blue, Green, and Red) and the Near Infrared (NIR) bands. The final pre-processed Landsat data for 1990, 2000, and 2010 were subsequently categorized using the unsupervised classification approach (iso-data clustering). However, in 2020, a maximum likelihood supervised classification method was used with the System for Automated Geoscientific Analysis (SAGA) 6.4.0 software.

The entire study area was classified into five LULC classes, viz. Agriculture, Barren land, Forest, Settlements, and Water bodies (Table 2). Similarly, the study area was also classified into four different forest cover classes based on the NDVI thresholds (Hartoyo et al. 2021, Islam et al. 2021). These thresholds were fixed through field observations and available literature. Apart from this, the characteristics of different forest cover classes are also detailed (Table 3). Finally, the layout of LULC maps and forest cover maps was generated using QGIS 3.14 (open-source GIS). The workflow of this study, encompassing satellite data

processing and classification steps, is depicted in a flow chart (Fig. 2).

Accuracy Assessment

Confusing matrices were created to verify the forest cover and Table 2: Description of the LULC classes.

Land use classes	Description
Agriculture	Cropping lands with crops
Barren Land and Rocks	Unused lands, uncultivated lands, and hills
Forest	Dense and less dense vegetation
Settlements	Residential and commercial concrete structures and roads
Water Bodies	Ponds, lakes, canals, and rivers

(Senapati et al. 2024)

Table 3: Description of the forest cover classes.

Forest cover classes	Description	NDVI thresholds
Very Dense Forest (VDF)	Very dense vegetation (Tall statured trees with high canopy cover)	0.8 to 1
Moderately Dense Forest (MDF)	Moderately dense vegetation (Medium height trees with moderate canopy)	0.6 to 0.79
Open Forest (OF)	Less dense vegetation (short trees or plants with high spread and less canopy cover)	0.4 to 0.59
Non-Forest land (NFL)	No vegetation	<0.4

(Source: Parapurath et al. 2020, El-Gammal et al. 2014)

LULC classifications. These matrices contain the accuracy of the user for every class in the rows and the accuracy of the producer for every class in the columns. The total classification accuracy was calculated using the diagonal values in the matrices. However, the ground truth data and field survey were only accessible for 2020; therefore, it's crucial to remember that the accuracy evaluation was only done for that year.

The user's, producer's, and overall accuracy were calculated using the following formulae.

User's accuracy =

$$\frac{\text{Total number of corrected pixels}}{\text{Total number of pixels in the particular row}} \times 100 \quad \dots(2)$$

Producer's accuracy =

$$\frac{\text{Number of corrected pixels}}{\text{Total number of pixels in the particular column}} \times 100 \quad \dots(3)$$

Overall accuracy =

$$\frac{\text{Number of pixels in the diagonal cells of the error matrix}}{\text{Total number of pixels in the error matrix}} \times 100 \quad \dots(4)$$

Kappa coefficient, a more reliable measure of classification accuracy, was calculated using the following formula (Stehman 1996).

$$KS = \frac{N \sum_{h=1}^q \hat{N}_{hh} - \sum_{h=1}^q N_h \hat{M}_h}{N^2 - \sum_{h=1}^q N_h \hat{M}_h} \quad \dots(5)$$

Where N is the total number of observations; $\hat{N}_{hh} = \frac{N_h}{n_h} n_{hh}$; $\hat{N}_{hh}$ is an unbiased estimator of N_{hh} ; N_h is the row total

Trend Analysis of Mean Air Temperature

To determine the mean monthly air temperature trend over the study period, ERA5 daily mean air temperature data were used to perform the non-parametric Mann-Kendall (MK) test (Mann 1945, Kendall 1975) at four significance levels ($p = 0.001, 0.01, 0.05$, and 0.1). In this study, a macro-enabled spreadsheet program (MAKESENS) was utilized to carry out the Mann-Kendall test. Moreover, the rate of change in monthly mean air temperature was computed using Sen's nonparametric method (Sen 1968).

Determination of the Relationship of Forest Cover Dynamics with Population Dynamics and Air Temperature

To determine the influence of population growth and settlement expansion on forest cover dynamics, a simple correlation study was conducted. Additionally, the study examined the impact of forest cover loss on air temperature

through correlation analysis. For this purpose, the decadal mean air temperature was calculated and correlated with the total forest area from 1990 to 2020.

RESULTS

Spatiotemporal Changes of the LULC

Supervised classification of the multi-year Landsat TM and OLI images yielded the land use and land cover dynamics from 1990 to 2020. Fortunately, a clear decreasing trend was observed in the forest cover as against the gradual increasing trend in the settlement areas. The forest cover was reduced by 20% from 1990 (14910.96 sq.km) to 2020 (11924.76 sq.km). On the contrary, the settlement areas increased by about 130% (Table 4). During the last decade, agricultural lands and barren lands observed a significant reduction in their coverage by 26% and 36% respectively. However, the area under waterbodies remained almost the same.

Forest Cover Dynamics

The forest cover classification was done for the NE Ghat Zone of Odisha at 10 years (1990, 2000, 2010, and 2020) interval and the results so obtained have been presented in Fig. 3. The spatial and temporal changes in different forest cover classes (VDF: Very Dense Forest, MDF: Moderately Dense Forest, OF: Open Forest) and Non-Forest lands (NFL) have been given in Fig. 4 (a & b). The figures detail that the area covered by the NFL gradually increased from 13302.36 sq.km to 15988.56 sq.km over 1990-2020. Moreover, a gradual decrease in the VDF cover was noticed during the study period, from 26.85% to 12.64% of the total geographical area of the NE Ghat Zone (Table 5). The maximum reduction in the VDF cover occurred between 2010 and 2020 (7495.16 sq.km to 3528.54 sq.km). Although the MDF depicted a decreasing pattern but the rate of decrease was very less compared to the VDF. Unlike the VDF dynamics, the OF observed a rise in its coverage by 42% over the study period.

Accuracy Assessment

In order to evaluate the accuracy of the classification, an error matrix or confusion matrix was developed. The accuracy assessment for the 2020 LULC classification showed that both producers' and users' accuracy were more than 80% for each land use class (Table 6). The overall accuracy yielded a value of 87.5% for the LULC classification. Furthermore, the kappa coefficient, which measures the agreement between the predefined producer ratings and the user-assigned ratings, was determined to be 0.84.

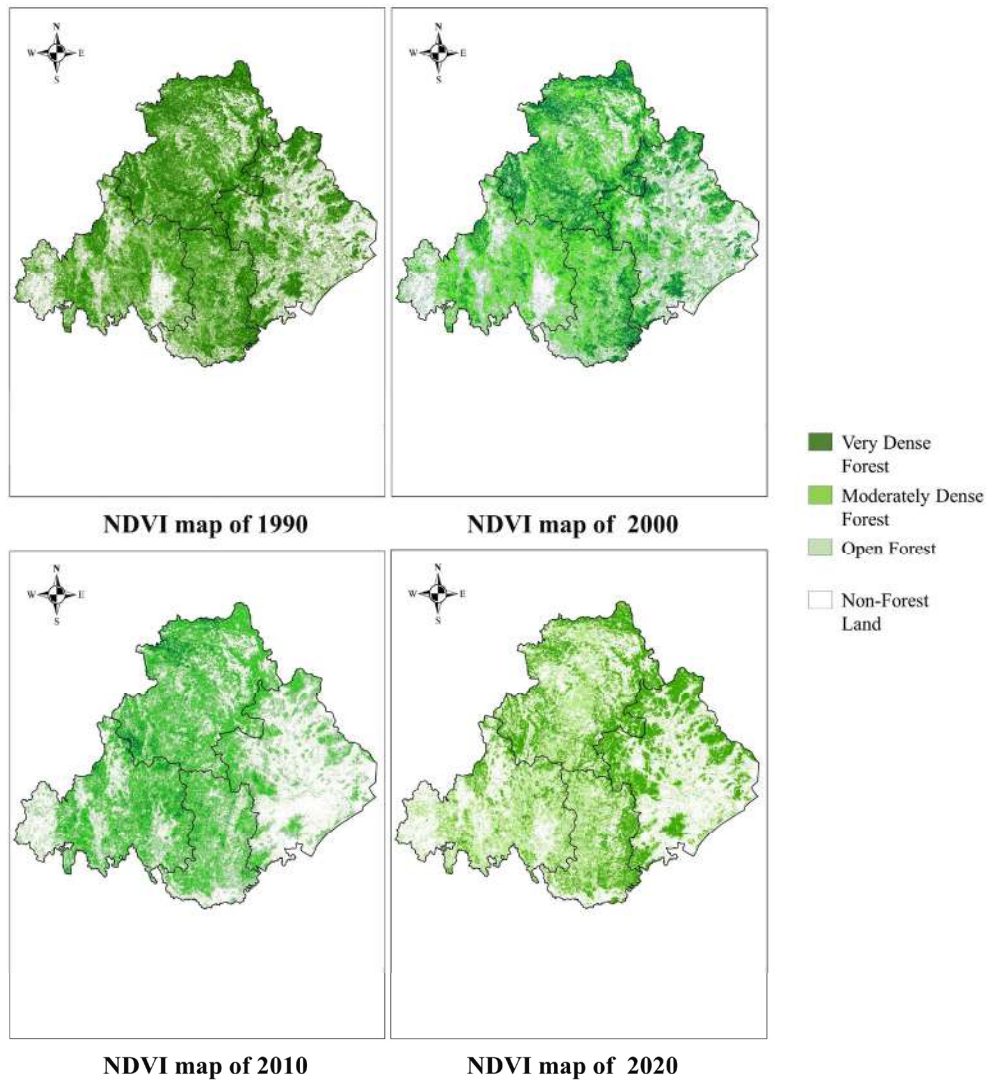


Fig. 3: Forest cover maps of the North Eastern Ghat zone of Odisha over the study period.

Table 4: LULC change detection over 1990 to 2020.

Land use classes	Area in 1990 [sq.km]	Area in 2000 [sq.km]	Changes over 1990 to 2000 [sq.km]	Area in 2010 [sq.km]	Changes over 2000 to 2010 [sq.km]	Changes over 1990 to 2010 [sq.km]	Area in 2020 [sq.km]	Changes over 2010 to 2020 [sq.km]	Changes over 2000 to 2020 [sq.km]	Changes over 1990 to 2020 [sq.km]
Agriculture	5368.88	5606.94	238.06	5417.47	-189.47	-48.59	4001.93	-1415.54	-1605.01	-1366.95
Barren land	2012.85	2100.91	88.06	2084.08	-16.83	71.23	1326.62	-757.46	-774.29	-686.23
Forest	14910.96	14008.44	-902.52	13395.55	-612.89	-1515.41	11924.76	-1470.79	-2083.68	-2986.02
Settlements	3902.51	4477.5	574.99	5302.58	825.08	1400.07	8984.46	3695.88	4470.96	5045.95
Water Bodies	1718.12	1719.53	1.41	1713.64	-5.89	-4.48	1711.55	-2.09	-7.98	-6.57
Total	27913.32	27913.32	0.00	27913.32	0.00	0.00	27913.32	0.00	0.00	0.00

Table 5: Forest cover change detection from 1990 to 2020.

Forest cover classes	Area in 1990 [sq.km]	Area in 2000 [sq.km]	Changes over 1990 to 2000 [sq.km]	Area in 2010 [sq.km]	Changes over 2000 to 2010 [sq.km]	Changes over 1990 to 2010 [sq.km]	Area in 2020 [sq.km]	Changes over 2010 to 2020 [sq.km]	Changes over 2000 to 2020 [sq.km]	Changes over 1990 to 2020 [sq.km]
VDF	7495.16	6311.55	-1183.61	4820.32	-1491.23	-2674.84	3528.54	-1291.78	-2783.01	-3966.62
MDF	4380.93	4583.84	202.91	4943.39	359.55	562.46	4090.46	-852.93	-493.38	-290.47
OF	3034.87	3113.05	78.18	3631.84	518.79	596.97	4305.76	673.92	1192.71	1270.89
NFL	13002.36	13904.88	902.52	14517.77	612.89	1515.41	15988.56	1470.79	2083.68	2986.2
Total	27913.32	27913.32	0.00	27913.32	0.00	0.00	27913.32	0.00	0.00	0.00

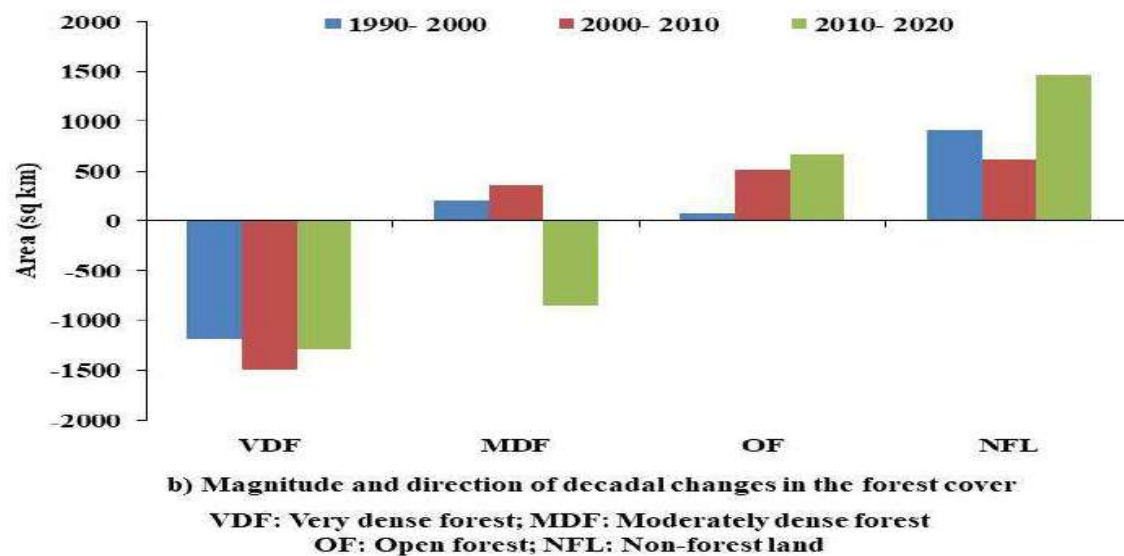
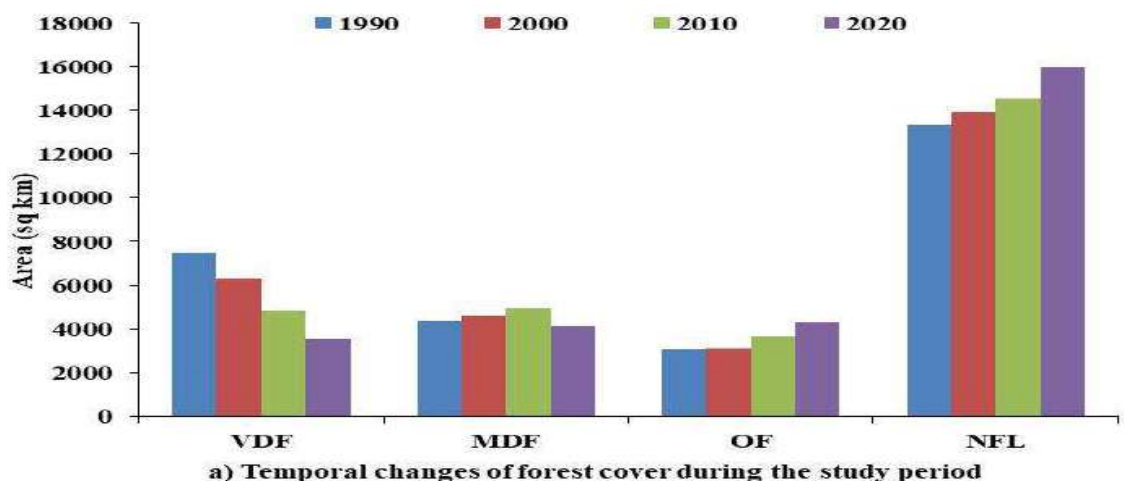


Fig. 4: a) Temporal changes of forest cover during the study period; b) Magnitude and direction of decadal changes in the forest cover.

The user's accuracy of different forest cover classes varied from 82% for NFL to 92% for VDF, while the producer's accuracy ranged from 81% for MDF to 97% for

NFL (Table 6). The overall accuracy and kappa coefficient were determined to be 87% and 0.82, respectively. The higher value of the kappa coefficient indicates a substantial

Table 6: Confusion matrices for LULC classification and forest cover classification for the year 2020.

Land use and land cover classification									
Classes	Agriculture	Barren Lands	Forest	Settlements	Water Bodies	User's sum	UA [%]	OA [%]	k
Agriculture	32	0	1	3	0	36	88.89	87.50	0.84
Barren Lands	1	26	3	0	0	30	86.67		
Forest	4	0	40	1	0	45	88.89		
Settlements	0	0	2	35	0	37	94.59		
Water Bodies	0	5	2	3	42	52	80.77		
Producer's sum	37	31	48	42	42	200			
Producer's accuracy (%)	86.45	83.87	83.34	83.34	100				
Forest cover classification									
Classes	VDF	MDF	OF	NFL	User's sum	UA (%)	OA [%]	k	
VDF	49	4	0	0	53	92.45	87.00	0.82	
MDF	7	53	0	0	60	88.33			
OF	2	5	39	1	47	82.98			
NFL	0	3	4	33	40	82.5			
Producer's sum	58	65	43	34	200				
Producer's accuracy (%)	84.48	81.54	90.7	97.06					

level of agreement between the predefined producer ratings and the user-assigned ratings, reflecting the reliability of the classification results.

Mean Air Temperature Trend from 1990 to 2020

The non-parametric Mann-Kendall (MK) test and Sen's slope estimator were applied to ascertain the monthly mean air temperature trends across the study area. It is evident from the result that the temperature in each month increased except in January, as indicated by the Test Z values (Table 7).

Table 7: Trend statistics of mean air temperature over the North Eastern Ghat Zone from 1990 to 2020.

Months	Test Z ^a	Q ^b
January	-0.986	-0.013
February	0.238	0.002
March	0.238	0.003
April	0.136	0.002
May	0.578	0.005
June	0.884	0.018
July	0.850	0.008
August	2.413*	0.017
September	2.244*	0.015
October	1.360	0.015
November	0.850	0.011
December	2.176*	0.035

*Significance level, $p = 0.05$; a = Mann-Kendall trend statistics; b = Sen's slope

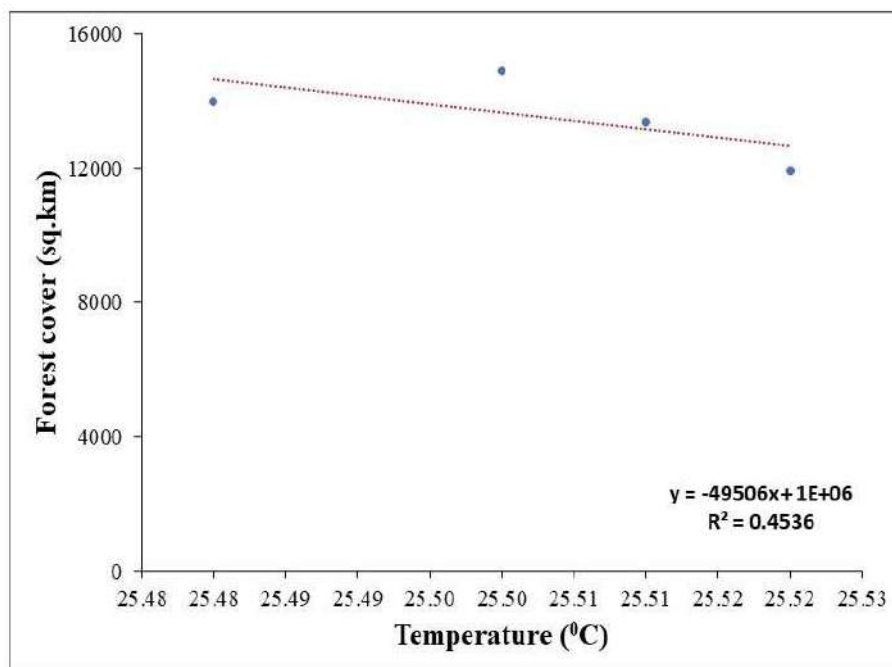
Significant ($p = 0.05$) rising trends in mean air temperature were observed for August (Test $Z = 2.413^*$), September (Test $Z = 2.244^*$), and December (Test $Z = 2.176^*$). The maximum rate of increase in mean air temperature, at 0.035°C per year, was noted in December, followed by 0.018°C per year in June, and 0.017°C per year in August. However, the mean air temperature in January displayed a decreasing trend with a decline of 0.013°C per year, although this trend was not statistically significant.

Forest Cover Dynamics with Population Growth and Settlements

The correlation coefficients showed that settlements and population expansion were negatively correlated with forest cover (Table 8). Remarkably, there was a considerable negative association between the dynamics of the forest cover and the dynamics of the population ($r = -0.985$) and settlements ($r = -0.963$). Majorly, the rise of settlements brought on by population increase is the main cause of deforestation and forest fragmentation. However, the coefficient of determination ($R^2 = 0.971$ for population and $R^2 = 0.9271$ for settlement areas) showed that the growth

Table 8: Correlation matrix among forest cover dynamics, population growth, and increased settlement areas.

Parameters	Population	Settlements [sq.km]	Forest cover [sq.km]
Population	1		
Settlements	0.908	1	
Forest cover	-0.985	-0.963	1



Correlation coefficient (r) between forest cover dynamics and mean air temperature: -0.674

Fig. 5: Relationship of forest cover dynamics with mean air temperature.

of the population and the expansion of settlements were responsible for 97% and 93% of the variability in forest cover dynamics, respectively.

The effect of forest cover change on the mean air temperature was shown by the correlation study (Fig. 5). The results revealed a negative correlation between forest cover dynamics and mean air temperature, as depicted by the correlation coefficient ($r = -0.674$). Specifically, the variation in forest cover could explain 45% of the variability in mean air temperature, as demonstrated by the coefficient of determination ($R^2 = 0.4536$).

DISCUSSION

The present study addressed a noticeable and consistent decreasing trend in total forest cover change over the last three decades. A clear decreasing trend of total forest cover was observed. The continuous loss of forested areas was mostly brought on by the expansion of roads, mining, industrialization, agriculture, and other land-development activities (Mishra et al. 2022). Moreover, the degradation of forest lands due to mining activities was also highlighted in the study area by Dutta et al. (2015). Notably, between 2000 and 2020, there was a significant reduction in agricultural land, attributed to rapid population growth. As a result, urban regions within the study area experienced significant long-term expansion.

Over the last three decades, the area covered by very dense forests has consistently decreased, while open forests and non-forest land have expanded. Due to deforestation, it was clear that certain types of forest cover, such as very dense forests, have a steadily declining trend. The escalating trends of population growth and urbanization have led to significant encroachment and exploitation of resources in forested regions. Consequently, the forest density and canopy cover transitioned from being extremely dense to sparsely dense.

Further, the trend analysis of mean air temperature unequivocally demonstrated an increase in temperature over the study area in the past decades. The rise in monthly mean temperature can be attributed to the escalation in the number of hot days and daily maximum temperature. According to Kothawale et al. (2010), the study region experienced an increase in the number of hot days at a rate of 2.0 days per decade. Gouda et al. (2017) also studied the adverse impacts of LULC change, forest fragmentation, urbanization, and industrialization on air temperature in the state of Odisha. The findings highlighted a 0.25 % increase in urbanization over the state during the period from 2005 to 2015. Warming trends across several districts of the state were also documented by Panda & Sahu (2019).

The study becomes more significant because the dynamics of forest cover showed a negative correlation

with temperature, indicating an inverse relationship between deforestation and warming. Various researchers have consistently reported such a negative association between deforestation and temperature. For instance, Li et al. (2016) utilized satellite data to quantify the potential and actual impacts of forest loss on land surface temperature (LST). Their study demonstrated that deforestation significantly contributes to the warming, particularly in tropical regions. Prevedello et al. (2019) further supported this finding, stating that deforestation results in consistent warming at both tropical and temperate regions, whereas afforestation leads to a more cooling effect. Apart from this, Lawrence & Vandecar (2015) identified the rise in land surface temperature with the concordant decrease in vegetation, leading to warmer days. Therefore, all these findings underscore the significant role of forest cover in influencing the local and regional temperatures.

Although mining and associated activities in the study zone and across the entire Odisha state have been observed to negatively impact the ecosystem and forest cover (Temper & Martinez-Alier 2013), this impact has led to significant tribal protests in various regions of Odisha, including the NE Ghat Zone (Kumar 2014). Thus, the administration and policymakers have to pay attention to the plight of the residents and work towards developing management techniques to address the situation promptly (Hota & Behera 2019, Mishra et al. 2022). In these areas, sustainable development necessitates striking a compromise between environmental preservation and economic concerns.

On the contrary, certain regions in India exhibit notable afforestation rates, underscoring the success of focused greening initiatives. Pujar et al. (2022) conducted a landmark study, utilizing approximately one million geo-tagged afforestation records across the Indian subcontinent. The findings highlighted afforestation hotspots concentrated in Telangana, followed by Andhra Pradesh, with peak activity zones observed in central Telangana. This surge in afforestation activity reflects a strategic administrative emphasis since 2013–14, driven by citizen-centric greening programs and district-level performance optimization. Telangana and Andhra Pradesh emerged as leaders in asset creation, achieving 6.3 lakh and 2.8 lakh completed assets, respectively. The spatial analysis of afforestation activities under the Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) presents an unprecedented opportunity to understand the geographical distribution and impact of these initiatives across India. However, states with a strong commitment to greening have demonstrated exceptional planning and execution capabilities, achieving remarkable afforestation milestones. Nevertheless, the potential for replicating and scaling these successes remains

significant. Afforestation, as a cornerstone of sustainable greening efforts, thrives on robust community participation and demands precise monitoring and assessment using advanced GIS-enabled tools and methodologies.

CONCLUSIONS

Over the past thirty years, urban areas expanded rapidly, contributing to a concerning decline in forest cover. The study zone experienced environmental warming, which was related to deforestation due to growing urbanization and population. So, immediate attention from the policymakers and planners is crucial to address the alarming decrease in forest cover over the NE Ghat Zone of Odisha. Therefore, actions should be taken promptly to counter the loss of very dense forest and forest fragmentation, as these pose potential threats to the environment and, consequently, to human livelihoods.

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Geospatial Analysis of Hydrological Responses and Surface Runoff Patterns in the Saroor Nagar Urban Watershed

Shiva Chandra Vaddiraju^{1,2†}  and Reshma Talari³ 

¹Department of Civil Engineering, National Institute of Technology, Tadepalligudem, Andhra Pradesh, India

²Department of Civil Engineering, Maturi Venkata Subba Rao (MVSR) Engineering College, Hyderabad, Telangana, India

³Department of Civil Engineering, National Institute of Technology, Andhra Pradesh, India

†Corresponding author: Shiva Chandra Vaddiraju; shivachandra135@gmail.com

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ABSTRACT

A useful technique for forecasting a watershed's hydrological response and creating effective management plans for its water resources is hydrological modeling. Due to the ongoing rapid urbanization since the formation of the Telangana State, the primary goal of this investigation is to use geospatial techniques to simulate rainfall-runoff processes in the Saroor Nagar urban watershed, Telangana, for the years 2008, 2014, and 2020. The rainfall-runoff process is simulated using the Hydrologic Engineering Centre's Hydrologic Modeling System (HEC-HMS) model. Three distinct techniques are utilized to simulate the infiltration loss, the transformation of surplus rainfall into surface runoff, and the flow routing of the channel reach: the Soil Conservation Service-Curve Number (SCS-CN) approach, the SCS unit hydrograph technique, and the Muskingum routing approach. The discharge data from the Hussain Sagar catchment is taken into consideration during the calibration and validation of the proposed model using the regionalization method due to the lack of gauging in the watershed. The model's performance is evaluated by employing the coefficient of determination (R^2) as well as the Nash-Sutcliffe Efficiency (NSE). The HEC-HMS model analysis indicates that between 2008 and 2020, the simulated peak discharge increased from 44.4m³/s to 57.1m³/s. During calibration, R^2 and NSE are 0.88 and 0.75, correspondingly; during validation, they are 0.83 and 0.89. The study's conclusions unequivocally show that the suggested model can faithfully replicate basin stream flow and that it can be used as a guide for wise water resource management in the watershed.

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INTRODUCTION

Hydrological investigations are essential for effectively managing the water resources that are currently available. It is expected that the concerns with water resources will intensify in the future. Hydrological models can enhance our understanding of the availability of water resources and the processes that occur within river basins worldwide (Gebre 2015). Govindaraju et al. (2024) used the SCS-CN method to estimate the surface runoff of the Kurumballi watershed. Hydrological models must be developed in order to assess the water availability in basins and prepare for future environmental changes. The primary obstacle to meeting the fundamental needs of all individuals lies in the sustainable governance of the finite reservoir of freshwater resources on our planet. Failure to effectively manage water resources could have detrimental consequences for society, potentially leading to droughts or floods. The rainfall-runoff model is a significant hydrological model that analyzes the correlation between precipitation and the resulting surface runoff, considering the impact of different physical characteristics of the watershed (Choudhari et al. 2014, Ramly & Tahir 2015). Water resource experts have spent decades examining the relationship between structure complexity and streamflow prediction from rainfall events (Oloche et al. 2010). HEC-HMS is

essential for estimating runoff across diverse hydrological contexts. Its adaptability makes it suitable for applications in flood forecasting and water resource management (Basu et al. 2021).

The HEC-HMS model allows for both lumped and distributed parameter” modeling (Paudel et al. 2019). The model incorporates various input data, such as rainfall and land use, enabling detailed runoff simulations (Paredes et al. 2020). HEC-HMS uses multiple mathematical models to evaluate dendritic watershed hydrological functions. The control specifications, the meteorological model, and the basin model are the 3 inputs that constitute the HEC-HMS model. To accurately forecast the behavior of each of these components within a hydrological system, it is imperative to utilize a specific set of parameters. Each basin element supplies the element with its downstream counterpart to enhance river water flow and construct a dendritic network (Agrawal 2005). Throughout the simulation run, the boundary conditions of the river basin are taken into account by the meteorological model. It includes data on the amount of precipitation and evapotranspiration that fall within the watershed. In essence, control specifications provide the time step, start and end dates, and duration of the simulation (Scharffenberg & Fleming 2006). “All input data has been stored in time-series and paired-value formats in the HEC Data Storage System.

The HEC-HMS model calculates gross water volume and peak flow for each basin component (Agrawal 2005). Namara et al. (2020) used HEC-HMS to simulate rainfall-runoff processes in the Awash Bello watershed. The model performed well, with $R^2 = 0.867$, $NSE = 0.855$ during calibration $R^2 = 0.863$, and $NSE = 0.739$ during validation. Paudel et al. (2019) showed how simple it is to analyze as well as comprehend the hydrological response along with the prediction of the upper Marshyangdi River basin, thereby demonstrating the “suitability of the HEC-HMS model as a hydrological model. Patil et al. (2019) utilized the HEC-HMS model to forecast the rainfall-runoff relationship in the upper basin of the Godavari River. Their findings demonstrated that urbanization was the cause of the increased runoff volumes. Sarminingsih & Rezagama (2019) employed the HEC-HMS model to study the relationship between rainfall and runoff in the Garang watershed.

Tassew et al. (2019) successfully simulated streamflow in the Lake Tana Basin, Ethiopia, employing the HEC-HMS model. Mokhtari et al. (2016) simulated rainfall-runoff in the Algerian Wadi Cheliff-Ghrib watershed using the HEC-HMS model. Their simulation demonstrated how the HEC-HMS model might be employed in hydrological” modeling. The continuous rainfall-runoff model yielded very satisfactory

results when compared to field discharge measurements in Kaffas & Hrisanthou’s (2014) study of a watershed in the Greek Kosynthos River. Singh & Ranjan (2022) revealed that the Punpun basin achieved better results from monsoonal rainfall-runoff models. The monthly model was found to be more effective than the daily and monsoonal models. “Nadeem et al. (2022) utilized the HEC-HMS model to replicate floods in the Hazara watershed in Pakistan. The model generated an increasing alert for flood prediction.

Hamdan et al. (2021) employed the HEC-HMS to replicate the A-Adhaim River’s discharge between 2015 and 2018 and determined that the model performed satisfactorily. Verma et al. (2022) successfully simulated the rainfall-runoff processes in the Upper Sabarmati River using the HEC-HMS model. HEC-HMS is particularly effective in urban watersheds, where rapid runoff presents unique challenges (Verma & Kumar 2023). Its integration with Geographic Information Systems (GIS) enhances spatial analysis and visualization capabilities (Brahman et al. 2022). Recent advancements in parameter optimization have improved model calibration and reliability (Yadav et al. 2022).” Hyderabad, the capital of Telangana state, is close to the study area of Saroor Nagar.

Over the past few years, there has been significant growth in both the infrastructure and technological landscape of Hyderabad. The main cause of this issue is insensitive urbanization practices that disregard the improvement of drainage systems, insufficient maintenance, as well as improper disposal of solid waste into drainage systems. Nevertheless, modifications to the initial terrain have also reduced the linkages between soil and water, consequently amplifying the challenge of controlling and assimilating the erratic rainfall that results in urban flooding. Table 1 displays the actual and normal rainfall details of the study area during the last two decades. The investigation area encountered abnormally high precipitation during the specified time periods, in contrast to the average monthly rainfall established by historical data. Our research aims to utilize the HEC-HMS model to analyze the impact of changes

Table 1: Historical rainfall data for the study area.

Month/Year	Actual rainfall during that month [mm]	Normal rainfall during that month [mm]	Source
August 2000	413.7	176.9	Chief Planning Officer, Ranga Reddy District
August 2008	508.4	176.9	
September 2016	456.4	121.5	
September 2019	232.9	121.5	
October 2020	465.6	86.5	
July 2021	371.3	141	

in Land Use and Land Cover (LULC) on the amount of surface runoff in the Saroor Nagar area between 2008 and 2020.

STUDY AREA

The investigation is conducted in the Saroor Nagar watershed, which is located in the state of Telangana. The study area is 42 km² and lies between latitudes 17°16'59" N and longitude 78°29'25" E and between latitudes 17°21'47" N and longitude 78°33'50" E. There is a range of 411 to 556 meters in altitude. The study area is residential. The usual temperature range is between 34 and 36 degrees Celsius. 725.8 mm of normal annual rainfall is expected. The study area experienced a rise in annual precipitation from 1054.8 mm in 2008 to 1478.6 mm in 2020. Given the process of urbanization and the impact of climate change, it is important to determine the possibility of surface runoff in the study area, and Fig. 1 depicts the study area map.

MATERIALS AND METHODS

The LULC map, the Digital Elevation Model (DEM), the Soil map, along with meteorological data are needed for the modeling. Rainfall data is collected from the chief planning officer, district collectorate of Ranga Reddy, and LULC maps were taken from previous research (Vaddiraju & Talari 2022a, 2023, Vaddiraju et al. 2023). Discharge data is collected from The Greater Hyderabad Municipal Corporation. Cartosat 1 DEM is collected from The National Remote Sensing Agency, and the FAO soil database is used to extract the soil map.

Preparation of LULC and Soil Map

The LULC maps prepared by Vaddiraju & Talari (2022b) have been used in the present study. Hydrologic soil groups (HSGs) have been divided into A, B, C, & D on the basis of factors such as rainfall, runoff, and infiltration (Derdour et al. 2018). Group A is distinguished by a significant rate

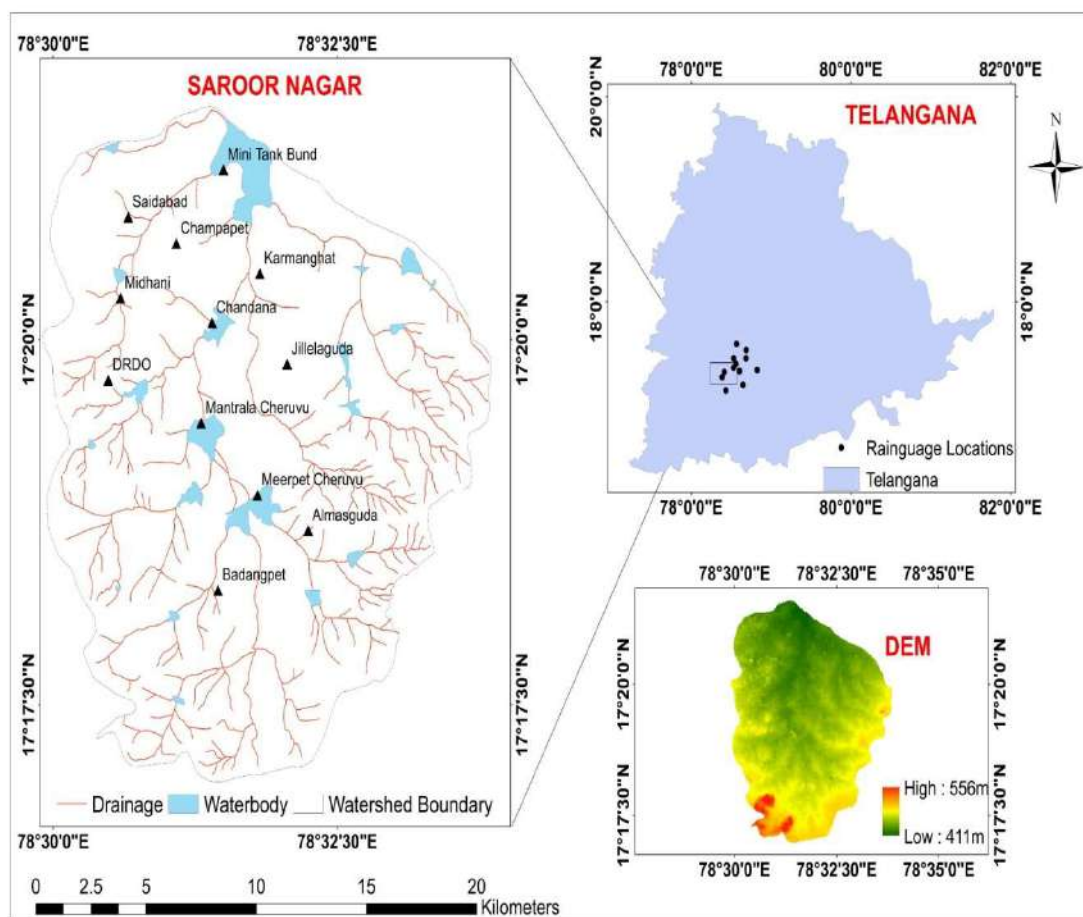


Fig.1: Study Area Map.

of infiltration and a limited capacity for runoff. Group B is composed of silt loam soil that has a coarse texture and moderate infiltration capacity. Group C soils, characterized by moderately fine textures, exhibit slow rates of infiltration as well as a moderate level of water transmission. These soils are typically classified as clay loam. In contrast, Group D soils have a high tendency for runoff and show limited infiltration. The study area is comprised exclusively of Group C soil.

Creation of Curve Number Map

The curve number (CN) of the study region is influenced by soil type and land use. Its value spans from 30-100. A lower CN value signifies a diminished runoff coefficient, while a higher value signifies an elevated runoff coefficient. CN values are assigned to different combinations of HSG and LULC using NRCS tables. Then, using the spatial join tool in ArcGIS, the CN maps are prepared for respective years. To calculate the mean CN for each sub-basin, employ Equation 1:

$$CN_{Avg} = \sum_{i=1}^n \frac{CN_i * A_i}{A_{Total}} \quad \dots(1)$$

Where, i denotes the number of subbasin

A_i denotes the area of the specific subbasin

and A_{Total} denotes the total area of the basin.

Hydrological Modelling using the HEC-HMS Model

The hydrological parameters of the basin were assessed by simulating the physical characteristics of each sub-basin. The SCS-CN method was utilized to quantify precipitation losses, chosen for its relevance and the availability of local data (Yu et al. 2017, Tassew et al. 2019). Curve Numbers (CN) for each sub-basin were calculated to facilitate accurate estimates of infiltration.

SCS Unit Hydrograph Transformation

The SCS-UH approach" was utilized to compute the discharge at the specified outlet. This approach requires two things: lag time as well as the percentage of impervious area in the basin (Barman & Bhattacharya 2020, Tassew et al. 2019). Subramanya (2013) states that the basin lag time is equal to 0.6 times the concentration time. For each sub-basin, calculations are performed to determine the proportion of imperviousness, lag time, concentration time, composite CN, potential soil storage, and initial abstraction.

Stream Flow Routing

The stream flow at the basin's outlet is determined using the straightforward and low-input Muskingum method (Song et al. 2011, Tassew et al. 2019). The input parameters K

and x in the Muskingum method are essential for effective flood routing. The parameter x , which ranges from 0 to 0.5, represents the proportion of the reach's length affecting the flow. K can be calculated by dividing the reach length (x) by the average flow velocity. Notably, when x equals 0.5, the channel exhibits both minimum and maximum attenuation.

Calibration and Validation of the Model

The watershed must be gauged for validation and calibration purposes. Because the Saroor Nagar watershed is not monitored by hydrometric stations, different approaches must be used to calibrate and validate the models. The regionalization method can be used to forecast hydrological model parameters for ungauged basins (Bardossy 2007). Watersheds with comparable hydrological treatments typically have similar basin characteristics, allowing hydrological parameters from various watersheds to be transferred. The Hussain Sagar Lake catchment, which is at 14 km, is considered a donor catchment, and its discharge values are transferred to the Saroor Nagar watershed based on the simple area ratio method for validation purposes. This method uses the ratio of the areas of the two watersheds to adjust the reference parameters, assuming that hydrological responses are proportional to watershed size. Trial-and-error calibration was used to obtain the correct values for each parameter that produced the best fit between the observed and predicted hydrographs. The calibration period in this study was from June 2014 to October 2014, and the validation period was from June 2020 to October 2020. The proposed HEC-HMS model's performance was assessed using the coefficient of determination and Nash-Sutcliffe Efficiency. Equation 3 depicts the NSE coefficient, which measures the predictive capability of the hydrological "model.

$$NSE = 1 - \frac{\sum_{t=1}^T (Q_{m,t} - Q_{o,t})^2}{\sum_{t=1}^T (Q_{o,t} - \bar{Q}_o)^2} \quad \dots(3)$$

Where, $Q_{(o,t)}$ = Observed flow at t time

$\bar{Q}_o$ is the mean of observed flow

and $Q_{(m,t)}$ = Simulated flow at t time ,

In a" linear regression setting, a metric called the coefficient of determination (R^2) is used to assess a model's ability to predict an outcome. Equation 4 illustrates how to "express R^2 :

$$R^2 = \frac{\sum_{i=1}^n (O_i - \bar{O})(S_i - \bar{S})}{\sum_{i=1}^n (O_i - \bar{O})^2 \sum_{i=1}^n (S_i - \bar{S})^2} \quad \dots(4)$$

Where, O_i = Observed flow at i time,

$\bar{S}$ = Mean of simulated flow at i time

S_i = Simulated flow at i time,

and $\bar{O}$ = Mean of observed flow at i time,

RESULTS AND DISCUSSION

LULC Maps

The LULC maps for the years 2008, 2014, and 2020 prepared by (Shiva Chandra and Reshma, 2022) were used in the present study. The LULC of the Saroor Nagar watershed consists of built-up, waterbody, vegetation, and barren classes. The LULC maps show that there is a continuous increase in the built-up area from 2008 to 2020, which means there is a situation that increases the runoff due to more impervious areas. Table 2 displays the statistics for LULC. Fig. 2 shows LULC maps for different years.

Soil and Curve Number Maps

The hydrological soil group map and the LULC maps from 2008, 2014, and 2020 are employed to create the CN map of the basin for the respective years. Fig. 3 displays the corresponding year's CN maps, and Table 3 shows HSG, CN, and Weighted CN values of the watershed.

HEC-HMS Model Development

The Saroor Nagar Watershed's seasonal flow is simulated by the model, which was created using HEC-HMS software. Seven sub-basins have been identified within the study area, along with the basin's outlet. The basin model is depicted in Fig. 4 and Fig. 5–11 display each sub-basin attribute regarding CN, lagtime, initial abstractions, and impervious area, respectively. The analysis of sub-basin characteristics

Table 2: Watershed LULC class distribution.

Class Name	2008 Area [%]	2014 Area [%]	2020 Area [%]
Built-up	41.8	54.4	64.9
Waterbody	1.3	1.5	1.9
Vegetation	23.9	23.5	25.3
Barren	32.8	20.8	7.9

reveals a decline in initial abstractions and lagtime between 2008 and 2020. Simultaneously, there is a consistent rise in curve number and imperviousness, indicating the possibility of increased runoff.

Simulation of Runoff in the Saroor Nagar Urban Watershed Using HEC-HMS

After the basin characteristics are calculated, the CN, initial abstractions, imperviousness, and lagtime are fed into the HEC-HMS module for each sub-basin, and the model is executed to simulate the runoff of 2008, 2014, and 2020. Due to the absence of observed discharge data for the Saroor Nagar tank, the model is calibrated using the observed discharge data of the Hussain Sagar catchment using the regionalization method. The discharge values recorded in the Hussain Sagar catchment are transferred to the Saroor Nagar watershed using the simple area ratio technique. The discharge values from the donor catchment are scaled based on the area ratio of the donor and receiving catchment. The daily observed data from June 1, 2014, to October 31, 2014, is used to calibrate the model, and the daily noted data from

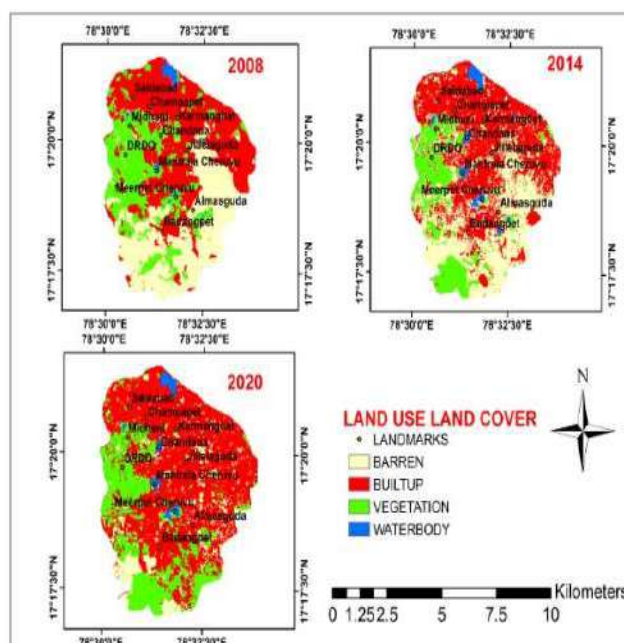


Fig. 2: LULC Maps.

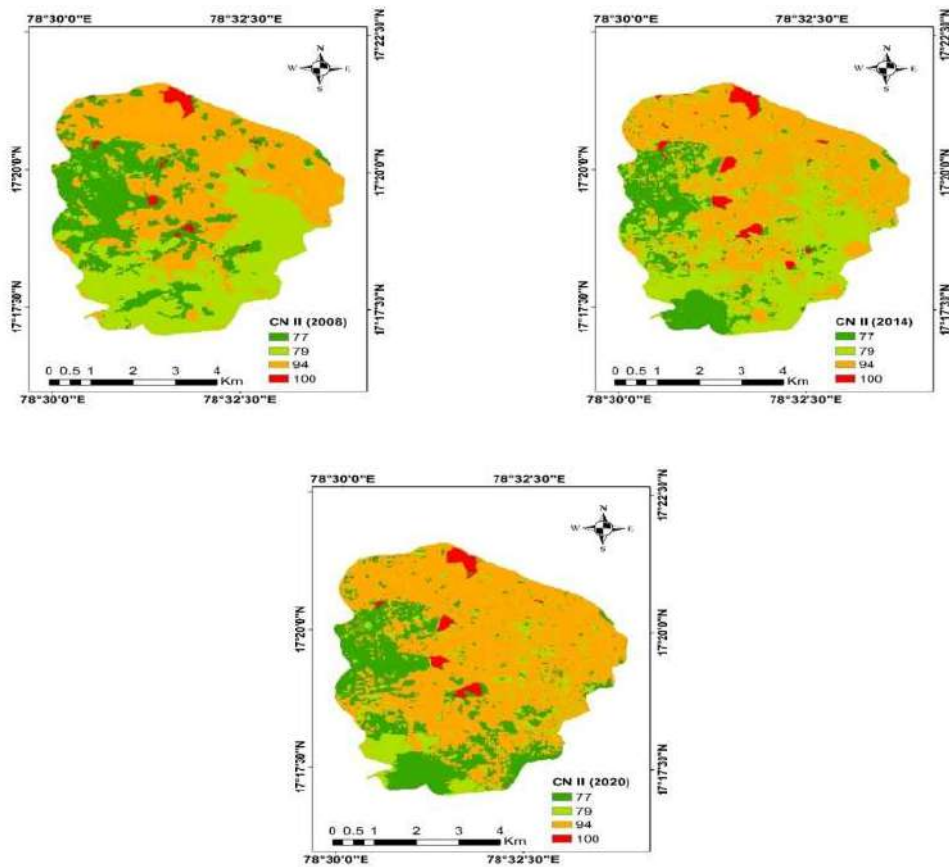


Fig. 3: CN Maps.

Table 3: Saroor Nagar Watershed LULC, HSG, and CN “Distribution.

Year	LULC	HSG	Curve Number (CN)	% of Area	(% of Area) X (CN)	Weighted Curve Number
2020	Built-up	C	94	65	6110	89 (CN II)
	Waterbody	C	100	2	200	
	Vegetation	C	77	25	1925	
	Barren	C	79	8	632	
2014	Built-up	C	94	54.5	5123	87 (CN II)
	Waterbody	C	100	1.3	130	
	Vegetation	C	77	23.5	1809	
	Barren	C	79	20.7	1635	
2008	Built-up	C	94	41.8	3929	85 (CN II)
	Waterbody	C	100	1.4	140	
	Vegetation	C	77	24	1848	
	Barren	C	79	32.8	2591	

June 1, 2020, to October 31, 2020, is used to validate the model. The calibration is done by considering SCS Curve Number (Curve Number Scale Factor), SCS Curve Number (Initial Abstraction Scale Factor), Muskingum – K, and

Muskingum x as the calibration parameters. Initially, the model is simulated by taking values of 1, 1, 0.5, and 0.2 for SCS Curve Number (Curve Number Scale Factor), SCS Curve Number (Initial Abstraction Scale Factor),

Table 4: Saroor Nagar Watershed Calibration “Parameters.

Element	Parameter	Initial Value	Optimized Value
All Sub-basins	SCS Curve Number – Curve Number Scale Factor	1.00	0.9
All Sub-basins	SCS Curve Number - Initial Abstraction Scale Factor	1.00	0.9
All Reaches	Muskingum – K	0.5	0.1
All Reaches	Muskingum – x	0.2	0.1

Table 5: Model Output and Performance.

Year	Simulated Discharge		Observed Discharge		NSE	R ²
	Peak [m ³ .s ⁻¹]	Volume [1000 m ³ .s ⁻¹]	Peak [m ³ .s ⁻¹]	Volume [1000 m ³ .s ⁻¹]		
2008	44.4	36570	Data Not Available	Data Not Available	Data Not Available	Data Not Available
2014	10.3	14428	13.9	8238	0.75”	0.88
2020	57.1	54269	51.4	41490	0.89	0.83



Fig. 4: Basin Model of the study area.

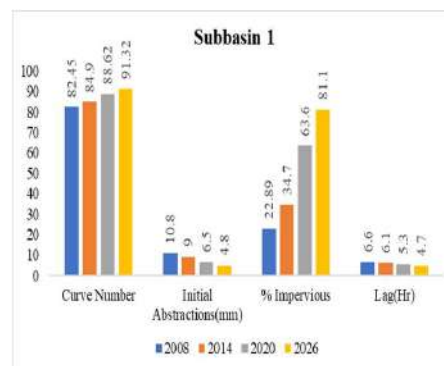


Fig. 5: Basin Characteristics of Sub-basin 1.

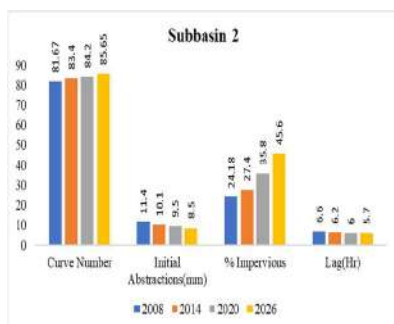


Fig. 6: Basin Characteristics of Sub-basin 2.

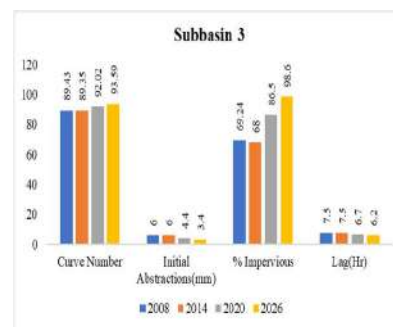


Fig. 7: Basin Characteristics of Sub-basin 3.

Muskingum – K, and Muskingum x, respectively. Later, based on trial-and-error methods, optimized values are found, and simulations are run to estimate the surface runoff. The initial and optimized values of calibrating parameters are presented in Table 4. The simulated discharge data for 2020 is validated using the optimized values that were acquired during the calibration process.

The NSE as well as R² are employed to determine the performance of the proposed HEC-HMS model. The model demonstrates strong performance, as indicated by the R² & NSE values obtained during the calibration as well as validation phases, which are 0.88 & 0.83, and 0.75 & 0.89, accordingly. The analysis shows that between 2008 and 2020, the simulated peak discharge

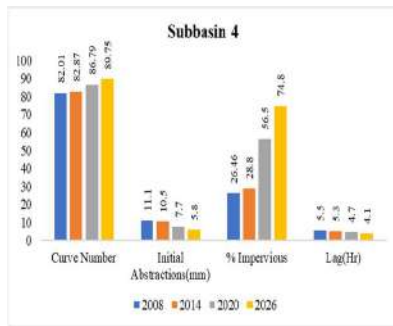


Fig. 8: Basin Characteristics of Sub-basin 4.

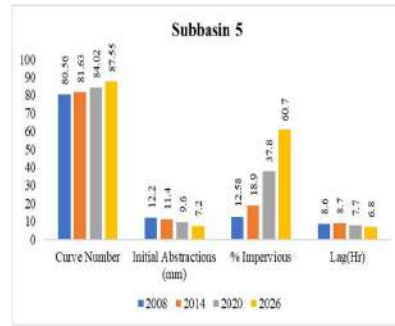


Fig. 9: Basin Characteristics of Sub-basin 5.

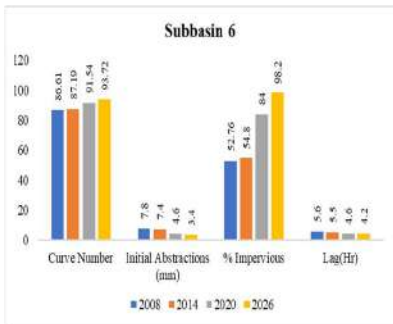


Fig. 10: Basin Characteristics of Sub-basin 6.

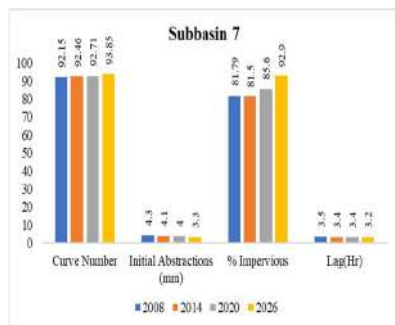


Fig. 11: Basin Characteristics of Sub-basin 7.

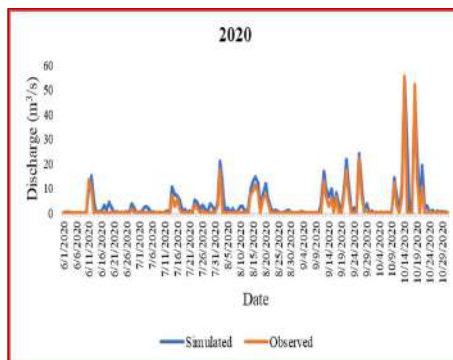
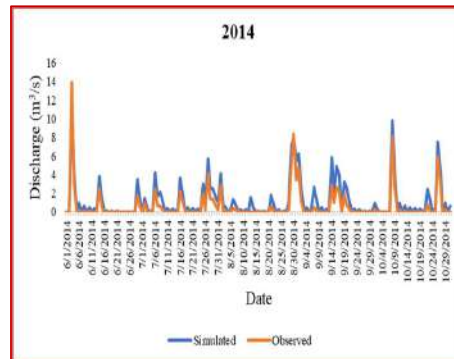
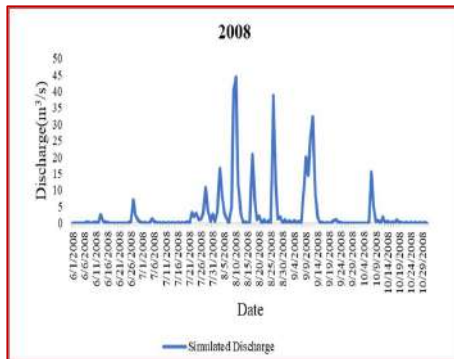


Fig. 12: HEC-HMS Simulation results of 2008, 2014, and 2020.

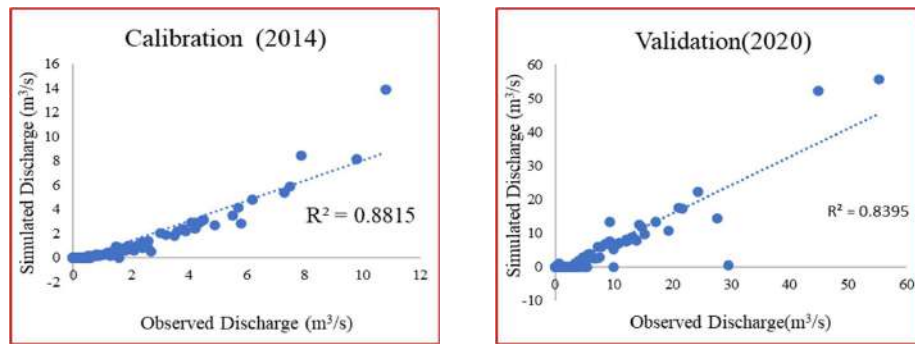


Fig. 13: Linear regression of simulated and observed discharge during calibration and validation period.

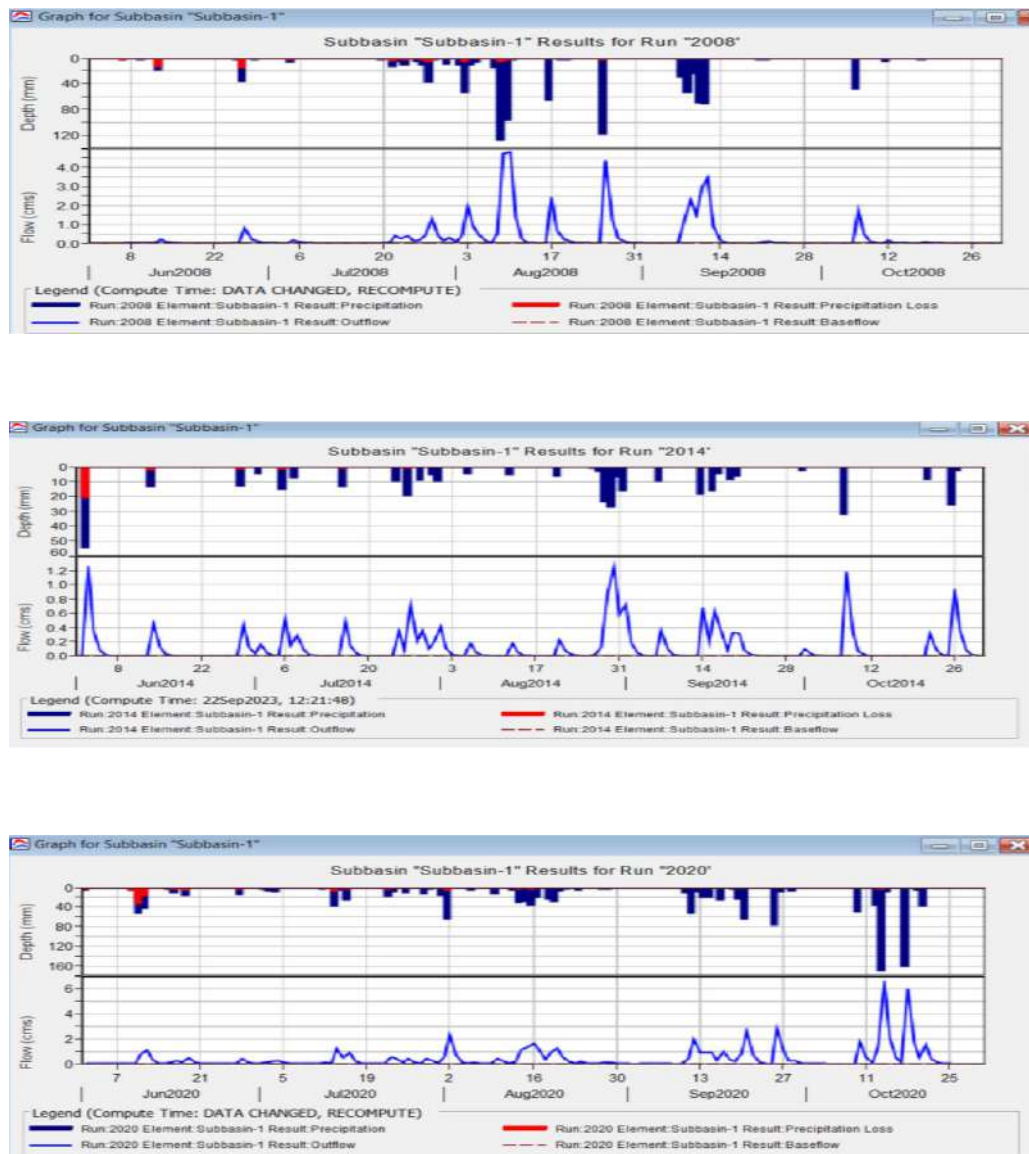


Fig. 14: Simulation details of sub-basin 1 for the years 2008, 2014, and 2020.

improved from $44.4 \text{ m}^3 \cdot \text{s}^{-1}$ to $57.1 \text{ m}^3 \cdot \text{s}^{-1}$, and the discharge volume increased from 36.57 MM^3 to 52.26 MM^3 . The simulated and observed discharge were lower in 2014, as the study area witnessed less rainfall of only 523 mm as opposed to 725 mm of normal annual rainfall. During the validation period of 2020, the simulated peak discharge is $57.1 \text{ m}^3 \cdot \text{s}^{-1}$, whereas the observed discharge is $51.4 \text{ m}^3 \cdot \text{s}^{-1}$. Table 5 presents specifics of observed and simulated discharges as well as model performance. Fig. 12 displays the details of the simulation for different years. Fig. 13 shows the linear regression of the observed as well as simulated discharge during the calibration and validation years. Fig. 14 shows the details of some sub-basin simulations.

CONCLUSIONS

This study effectively utilized the HEC-HMS model to simulate the rainfall-runoff processes in the Saroor Nagar urban watershed of Telangana from 2008 to 2020, highlighting the impact of rapid urbanization on surface runoff. The model demonstrated strong performance with calibration R^2 and NSE values of 0.88 and 0.75 and validation values of 0.83 and 0.89, respectively. Analysis revealed an increase in peak discharge from $44.4 \text{ m}^3 \cdot \text{s}^{-1}$ in 2008 to $57.1 \text{ m}^3 \cdot \text{s}^{-1}$ in 2020, correlating with an increase in built-up areas and associated impervious surfaces. The LULC analysis showed a significant rise in urban land use, contributing to heightened runoff volumes. The study underscores the importance of integrating hydrological models in urban planning and water management strategies, especially in areas prone to flooding. It advocates for sustainable land management practices and infrastructure improvements to mitigate flooding risks. Additionally, the use of regionalization techniques for calibration in ungauged watersheds proved effective. These findings provide crucial insights for local authorities to enhance flood preparedness and water resource management. Future studies should focus on continuous monitoring and adaptive management strategies to address ongoing changes in land use and climate.

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Bioaccumulation of Heavy Metals in Catfish (*Hemibagrus nemurus*) at the Downstream of Citarum River, Indonesia

Farrel Edrea Muhammad[†]  and Tati Suryati Syamsudin

School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia

[†]Corresponding Author: Farrel Edrea Muhammad: farreledreamuhammad@gmail.com

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ABSTRACT

Rivers are highly dynamic systems. Rapid population growth and the development of industrial areas around rivers significantly influence water quality. This research aims to analyze the heavy metal content in both water and catfish in the lower reaches of the Citarum River. Five locations were purposively selected: Station 1 (26.87 m asl), Station 2 (18.35 m asl), Station 3 (22.5 m asl), Station 4 (22.5 m asl), and Station 5 (22.5 m asl). At each site, physical and chemical water parameters (temperature, pH, depth, brightness, current speed, and DO), as well as concentrations of heavy metals (Hg, Pb, and Cd), were measured. Catfish were collected using stocking nets and fishing rods from the five stations, with assistance from local fishermen. Sampling was conducted over 14 consecutive days, from June 20 to July 4, 2022. The bioaccumulation of metals in both water and catfish was analyzed and calculated using the bioaccumulation factor (BAF) formula. The results showed that the accumulation of Hg in catfish was higher than that of Pb and Cd. Spatially, Pb accumulation was highest at Station 5 (4 times that of the water concentration) and lowest at Station 2 (2 times that of the water). Cd accumulation was highest at Station 5 (5 times that of the water) and lowest at Station 2 (3 times that of the water). Hg accumulation was highest at Station 1 (6 times that of the water) and lowest at Station 4 (2 times that of the water).

INTRODUCTION

Industrial activities around the river produce pollutants that have an impact on river ecosystem pollution. Waste streams from industry produce an accumulation of chemical pollutants, one of which is heavy metals. Heavy metals in waste enter waters and contaminate aquatic organisms such as fish, which serve as the main source of protein for humans (Briffa et al. 2020, Walker et al. 2012).

Heavy metals are highly toxic pollutants and are difficult to decompose naturally in the environment. Due to their non-biodegradable, bioaccumulative, and toxic nature, heavy metals have become one of the major problems in the environment (Sharifuzzaman et al. 2015). Heavy metals can contaminate water and concentrate in organisms living in waters and pose direct or indirect risks to humans and ecosystems (Wang et al. 2018). Heavy metal pollutants are inherent in our daily lives. It is a negative consequence that we get, and heavy metals have a huge non-biodegradable impact on all types of pollution that should be avoided (Sani et al. 2022).

Heavy metals cannot be transferred directly into the food chain but move to higher-level consumers through bioaccumulation processes. Among the many pollutants entering the waters, river pollution due to metals has become an international concern as they are toxic and persistent in the aquatic environment for longer periods with increased accumulation at trophic levels (Ibrahimi & Taherianfard 2011). Metals present in the water will accumulate in fish tissues due to the enrichment of pollutants that make the relative contamination of



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organisms to their environment. Bioaccumulation of high concentrations of heavy metals in fish is one of the effects of food absorption, and then organisms at higher trophic levels have a higher accumulation of heavy metals (Borga 2008). Metal accumulation at higher trophic levels results in biomagnification in food webs. Biomagnification is the result of the uptake of contaminants from food leading to concentrations higher up the food chain (Drouillard 2008). The consumption of aquatic organisms such as fish that contain large amounts of heavy metals will accumulate in body tissues and then move to the next trophic level, then gradually pass on to humans (Monsefrad et al. 2012). Then, higher concentrations of metals beyond safe limits affect serious diseases and high mortality rates in humans (Ali et al. 2019).

Fish is one of the most consumed animals and serves as a carrier for heavy metal transfer to humans (Shrivastava et al. 2011). The accumulation of heavy metals in fish depends on the amount of heavy metals present in the aquatic environment, the absorption pathway of heavy metals, the time of exposure of fish to heavy metals, and the type and size of fish (Noël et al. 2013).

Catfish is a type of fish that lives in rivers and lakes, one of which is found in the lower Citarum River. Catfish is one of the commodities with high economic value in Indonesia and is popular among domestic consumers, as well as in the Southeast Asia region (Handoyo et al. 2010). Catfish is a

carnivorous fish, with the main food of adult catfish being small fish and insects (Tang 2003).

The downstream Citarum River, which has suffered ecosystem damage due to pollution and land degradation occurring around the watershed, has decreased the quality of the aquatic environment. The degraded environmental quality due to heavy metal pollutants then causes the aquatic organisms living in the ecosystem, such as catfish, to become contaminated. If the consumption of fish from the downstream Citarum River continues to occur, it will endanger the health of humans who consume it. Therefore, the purpose of this study is to analyze the accumulation of heavy metals in catfish in the lower Citarum River and to analyze the heavy metal content in the water of the lower Citarum River.

MATERIALS AND METHODS

Study Area

The Citarum River is one of the rivers in West Java Province stretches from Gunung Wayang (2182 m asl) to the estuary of Java Sea (Muara Gembong). As the longest (297 km) and largest river in West Java Province, the Citarum River passes through agricultural areas, residential and industrial areas (Mustika & Sofyan 2017). The management of the Citarum River (BBWS) divided the river into three segments that were upper, middle, and lower parts (downstream).

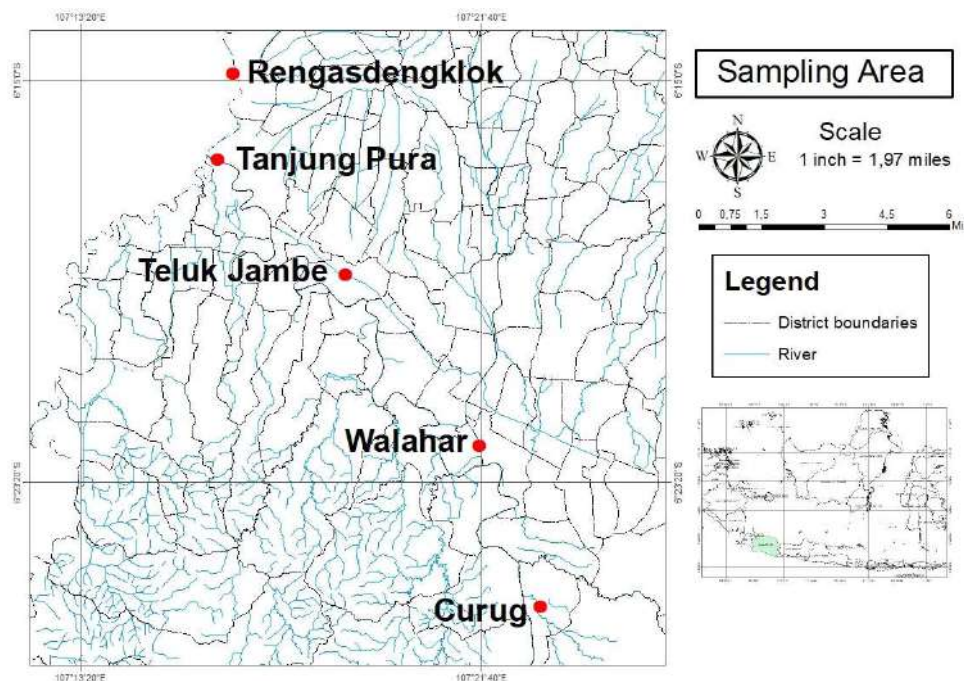


Fig. 1: Sampling locations in the lower parts of the Citarum River.

Based on the preliminary survey and land use, five locations were determined along the downstream river as sampling sites (Fig. 1), that were:

1. Curug Dam (Station 1), situated in the upper parts of the downstream Citarum River (6°26'48.62" S, 107°22'43.50" E; 26.87 m asl). The dam is used for hydropower and irrigation water for the Karawang area and surrounding. The water flow passed through industrial areas such as the cotton industry (5.3 km), silicon industry (3.3 km), and textile industry (2.9 km).
2. Walahar (Station 2) is a dam (6°23'00.8" S, 107°21'39.9" E; 18.35 m asl) that serves as a source of water for the local population and rice fields. In the area, there is a food and beverage industry (2.7 km), a plastic industry (1.7 km), and residential areas (145 m).
3. Teluk Jambe (6°19'36.76" S, 107°18'43.68" E; 22.5 m asl). This site (Station 3) is adjacent to residential areas (77.8 m), a manufacturing area (1.2 km), and a paper industry area (750 m).
4. Tanjung Pura (6°19'36.76" S, 107°18'43.68" E; 22.5 m asl). This site (Station 4) is the confluence of the Cibeet River and the Citarum River and passes through to residential areas (70 m).
5. Rengasdengklok (6°14'44.4" S, 107°16'32.5" E; 22.5 m asl). This site (Station 5) has passed through the textile industry area (1.9 km), the plastic industry area (1.7 km), and many residential areas (116 m).

Environmental Conditions

At each station, the physical and chemical parameters (temperature, pH, depth, brightness, and dissolved oxygen) were measured in situ at three sites (the right, middle, and left bank) of the river. One liter of water was taken by Lamotte water sampler and stored in HDPE plastic bottles. Sampling was conducted in the morning (07.00) and afternoon (16.00). These activities were conducted every day from June 20 to June 24, 2022, successively.

The Total of Suspended Solid (TSS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and heavy metal levels (Hg, Pb, and Cd) of water were measured following the Standard Method for the Examination of Water and Wastewater 23rd Edition (Baird et al., 2017). Water quality standards refer to the decree of the World Health Organization (WHO of 2022) on standard water for drinking water and the Indonesia Ministry of Environment (IME No. 22 of 2021) on standard water for recreation facilities, aquaculture, agriculture, and other designations. Fish quality standards refer to the Indonesian National Standard (INS 7387 of 2009).

Fish Collection

The catfish were collected using stocking nets and fishing rods from 5 stations assisted by local fishermen. Sampling efforts were carried out for 14 consecutive days from June 20 to July 4, 2022. The fish obtained were preserved in a box filled with ice. Identification and the body size measurement (length and weight) were measured in the laboratory (Saenin 1968).

Heavy Metal Accumulation Analysis

Heavy metal concentrations (Hg, Pb, and Cd) in fish and water samples were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES), following the method outlined by Baird et al. (2017). A 100 mL sample was homogenized, and 15 mL of HNO₃ was added. The mixture was heated in an electric bath until the volume was reduced to 25 mL and then cooled. The sample was filtered using Whatman No. 42 filter paper, and distilled water was added to bring the volume to 50 mL. The sample was then homogenized and transferred to a vial for further analysis. The fish sample (muscle tissue) was dried in an oven at 100–105 °C and prepared according to the USGS Test Method B-9001-95. One gram of dried fish muscle was placed in a beaker, followed by the addition of 10 mL of HNO₃ and 2 mL of H₂O₂. The sample was heated in an electric bath for 2 hours, then cooled and filtered using Whatman No. 42 filter paper into a 25 mL volumetric flask. HNO₃ was added to bring the volume up to 25 mL.

To quantify the bioaccumulation of metals from water to biota, the bioaccumulation factor (BAF) was calculated as the ratio of heavy metal concentration in fish to that in water, following the formula proposed by Borga (2008).

$$BAF = \frac{C_{or}}{C_w}$$

Where C_{or} is the heavy metal concentration in organisms and C_w is the heavy metal concentration in water.

Data Analysis

Data on water physicochemical parameters were analyzed using PCA to characterize the five stations by XLSTAT 2023. The relationship between heavy metals in fish and the water was analyzed using MANOVA by SPSS version 26. The relationship between metal content and fish body size was analyzed using Pearson correlation analysis by Microsoft Excel and the interpretation refers to Sugiyono (2017).

RESULTS

Aquatic Environmental Conditions

The environmental conditions of the sampling sites varied.

The water depth varied between 0.8m at station 2 (Walaha Dam) to the deepest 3.81m at station 1 (Curug Dam) (Fig. 2: A). The lowest temperature was 28.58°C at station 2 (Walaha Dam), and the highest was 35.56°C at station 4 (Tanjung Pura) (Fig. 2B). The current velocity was between 7.14 m.s⁻¹ at station 1 (Curug Dam) and 0.31 m.s⁻¹ at station 3 (Adiarsa) (Fig. 2C). The light penetration in water was 28 cm at station 4 (Tanjung Pura) up to 14.87 cm at station 2 (Walaha Dam) (Fig. 2D).

At all stations, the pH is almost neutral. At stations 1, 3, 4, and 5, the pH was 6, and at station 2 (Walaha Dam) was 6.33 (Fig. 2E). The dissolved oxygen (DO) varied between 6.2 mg.L⁻¹ at station 4 (Tanjung Pura) and 8.11 mg.L⁻¹ at station 1 (Curug Dam) and station 2 (Walaha Dam) (Fig. 2F). The lowest total solid suspended (TSS) was

11 mg.L⁻¹ at station 1 (Curug Dam), and the highest was 396.6 mg.L⁻¹ at station 2 (Walaha Dam) (Fig. 2G). The lowest biological oxygen demand (BOD) was 5.4 mg.L⁻¹ at station 1 (Curug Dam), and the highest was 92 mg.L⁻¹ at station 2 (Walaha Dam) (Fig. 2 F). The lowest chemical oxygen demand (COD) was 11.6 mg.L⁻¹ at station 1 (Curug Dam), and the highest was 265.1 mg.L⁻¹ at station 2 (Walaha Dam) (Fig. 2G).

The metal content of the five study sites varied. The lowest Hg was 0.0007 mg.L⁻¹ at station 2 (Walaha Dam), and the highest was 0.0024 mg.L⁻¹ at station 4 (Tanjung Pura). The same phenomenon was found for Cd, where the lowest concentration of Cd was 0.0007 mg.L⁻¹ at station 2 (Walaha Dam), and the highest concentration o was 0.0024 mg.L⁻¹ at station 4 (Tanjung Pura). However, in the

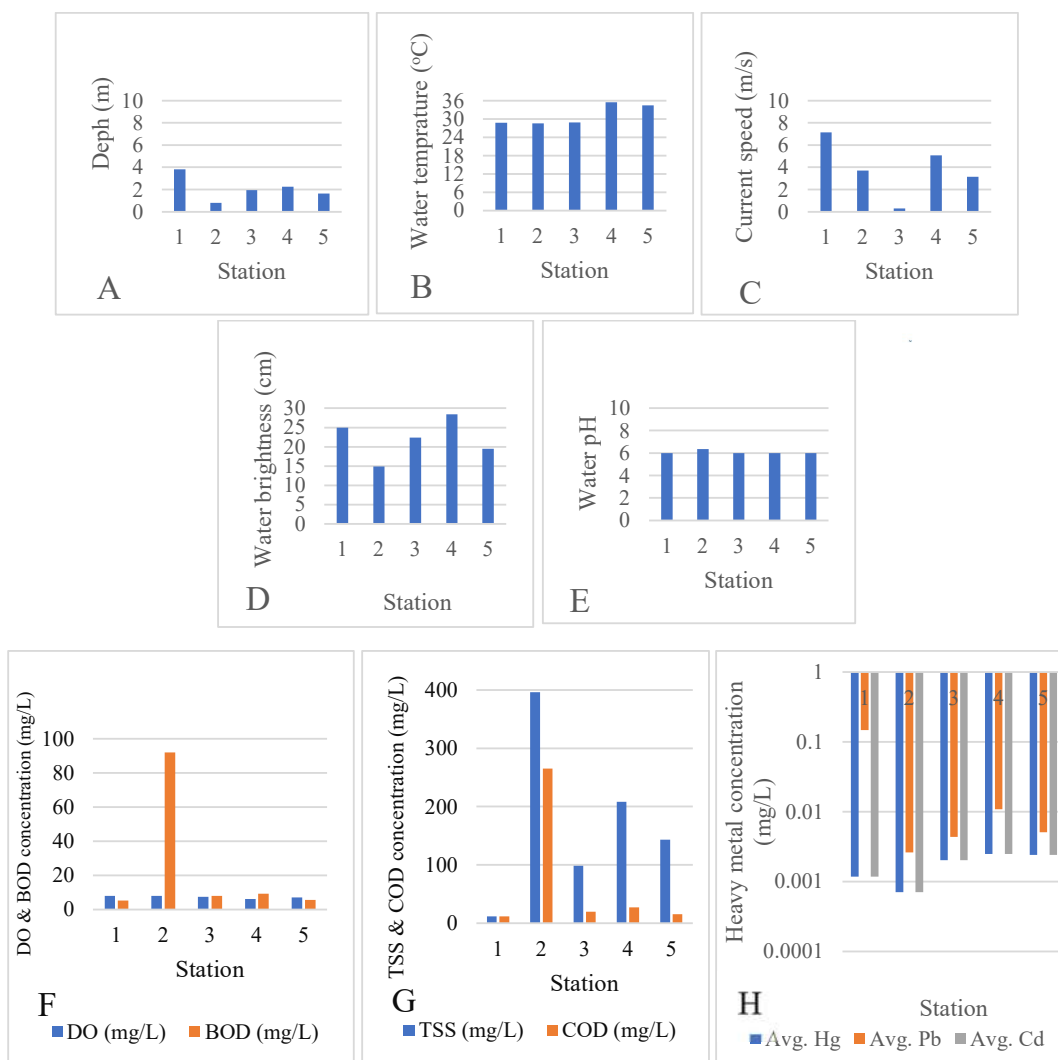


Fig. 2: Average environmental conditions at all study sites at Citarum downstream.

case of Pb concentration at station 2 (Waluhar Dam), the concentration of Pb was the lowest at 0.0026 mg.L^{-1} , and the highest concentration of Pb was 0.1480 mg.L^{-1} at station 1 (Curug Dam) (Fig. 2H).

Spatially, the environmental condition of each station influenced the water condition of the Citarum downstream. For example, station 1 (Curug Dam) is characterized by the depth, current speed, and Pb concentration. Station 2 (Waluhar Dam) is characterized by pH, BOD, COD, and TSS. Station 3 (Adiarsa) is characterized by light penetration and depth. Station 4 (Tanjung Pura) is characterized by the water temperature, light penetration, and the concentration of Hg and Cd. At station 5 (Tunggak Jati), characterized by water temperature, brightness, concentration of Hg and Cd.

This result was showed by the Biplot of axes F1 and F2 is 60.40%, (Fig. 3).

Heavy Metal Accumulation

The results show that the catfish downstream of Citarum River accumulate heavy metals of Hg, Pb, and Cd in high concentrations in their body ($\text{BAF} > 1$). The concentration of Hg in catfish was 4.9 times higher than in the water, the Pb was 4.1 times, and the Cd up to 4.3 times (Table 1). The national standard of Pb concentration (WHO of 2022) for Pb in the river was more than a threshold, and in the Citarum River, was exceeded the standard quality. The concentration of Hg and Cd are still below the standard of water quality. However in the catfish, the Hg and Pb concentrations have

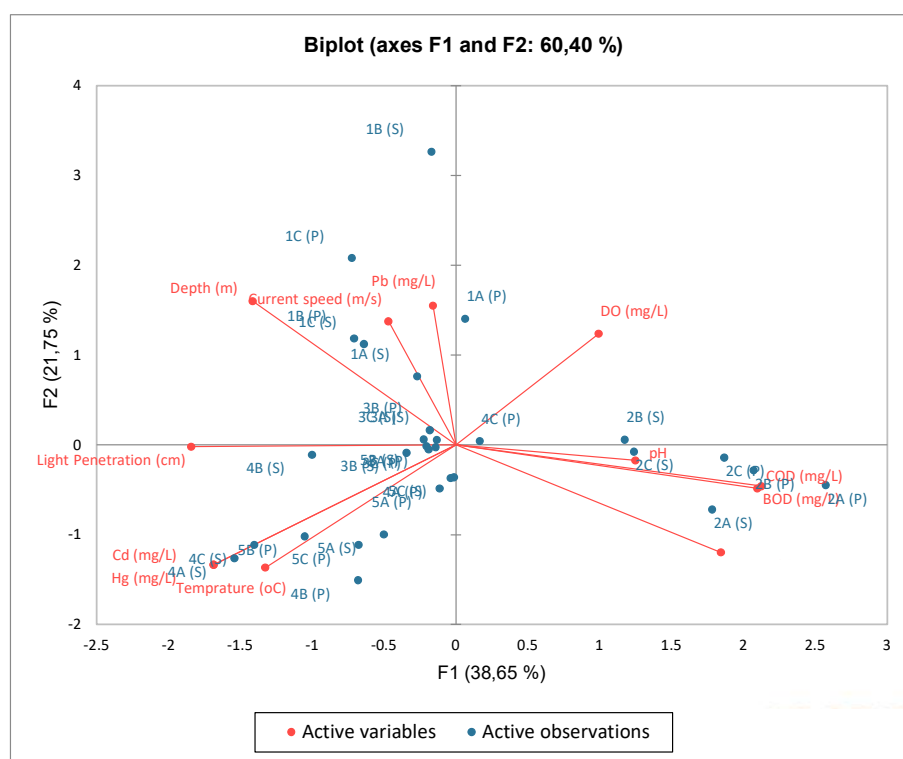


Fig. 3: Environment Characteristics at five stations at Citarum downstream.

Table 1: Average of BAF at 5 stations and metal content in water and fish.

Heavy metal	Average Bioaccumulation Factor (BAF)	Description	Average metal content		Quality standard	
			Water [mg.L^{-1}]	Fish [mg.L^{-1}]	Water [mg.L^{-1}] From WHO	Fish [mg.L^{-1}] From INS
Mercury (Hg)	4,9	>1	0,0017	1,26*	0,006	0,5
Lead (Pb)	4,1	>1	0,0341*	0,31*	0,01	0,3
Cadmium (Cd)	4,3	>1	0,0017	0,01	0,003	0,1

*: Exceeds water quality standards (WHO of 2022 & INS No. 7387 of 2009)

exceeded the threshold value, and the Cd concentration is still below the standard threshold value set by Indonesia National Standard (INS) No. 7387 of 2009 (Table 1).

Spatially, at station 1 (Curug Dam), the Pb metal content in water was higher than Hg and Cd metals (Fig. 2). At station 2 (Walahaar Dam), the Pb metal content of water was higher than Hg, and Cd metals (Fig. 2). At station 3 (Adiarsa), Pb metal content in water was higher than Hg and Cd metals (Fig. 2). At station 4 (Tanjung Pura), there was an increase in all three metals when compared to station 3, but the ratio remained the same, i.e., the Pb metal content in the water was higher than the Hg and Cd metals (Fig. 2). At station 5 (Tunggak Jati), the content of the three aquatic metals decreased when compared to station 4, but the ratio remained the same, i.e., the content of Pb metal in water was higher than Hg and Cd metals (Fig. 2). The Pb metal content that is higher than Hg and Cd metals in five stations is suspected in the environment around the station many industries are suspected of discharging Pb waste more dominantly than Hg and Cd metals.

The accumulation of heavy metals in catfish living in Station 1 (Curug Dam), station 2 (Walahaar Dam), station 3 (Adiarsa), and station 5 (Tunggak Jati) accumulated Hg metal higher than Pb and Cd metals (Fig. 4). However, it was different with individual catfish in station 4 (Tanjung

Pura), which accumulated Pb metal higher than Hg and Cd metal. The accumulation of the three metals is in line with the metal content of the water, which is higher in Pb than in Hg and Cd. It is suspected that this is due to the confluence of water from the Citarum and Cibeet Rivers, which causes more waste input.

In the accumulation of heavy metals in catfish that live at station 1 (Curug Dam), station 2 (Walahaar Dam), station 3 (Adiarsa), and station 5 (Tunggak Jati) accumulate higher Hg metal compared to Pb and Cd (Fig. 4). However, this was different from individual catfish at station 4 (Tanjung Pura), which accumulated higher levels of Pb than Hg and Cd. The accumulation of these three metals is in line with the metal content in water, where Pb metal is higher than Hg and Cd metals. This incident occurred allegedly because the confluence of water from the Citarum and Cibeet Rivers created more waste input.

Relationship Between Environmental Conditions and Heavy Metals in Catfish

The results of the analysis between metal concentrations in catfish and aquatic metals show that there is an influence of aquatic heavy metals on the heavy metals contained in catfish. In the analysis between fish body weight and heavy metals in the body of catfish, there were varying correlations: Hg

Table 2: MANOVA Test results and correlation of heavy metals in water and fish.

Heavy metal	Wilk's Lambda test	Correlation Calculation Results	Pearson correlation	Correlation level
Mercury (Hg)	0.004	0.065	0.00-0.199	Very low
Lead (Pb)		0.939	0.80-1.000	Very high
Cadmium (Cd)		0.211	0.20-0.399	low

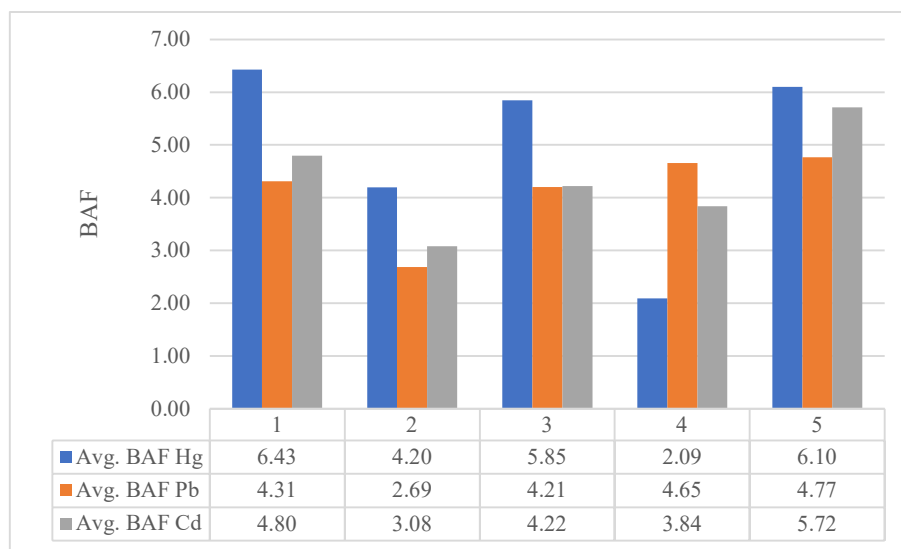


Fig. 4: Accumulation of heavy metal of catfish in five stations.

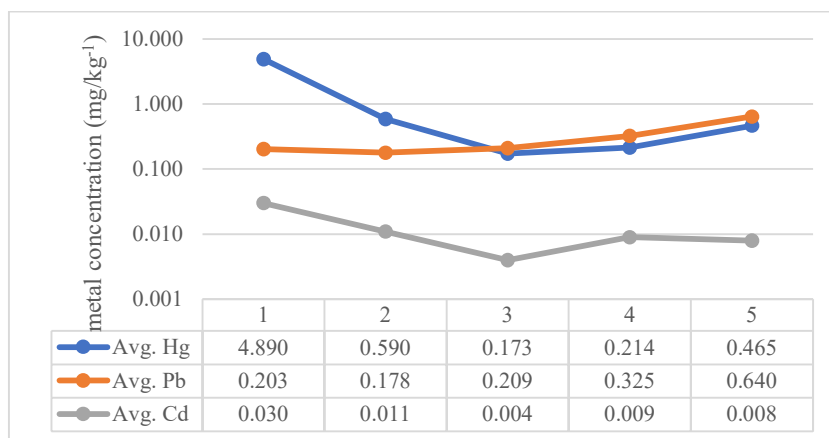


Fig 5: Heavy metal content in catfish.

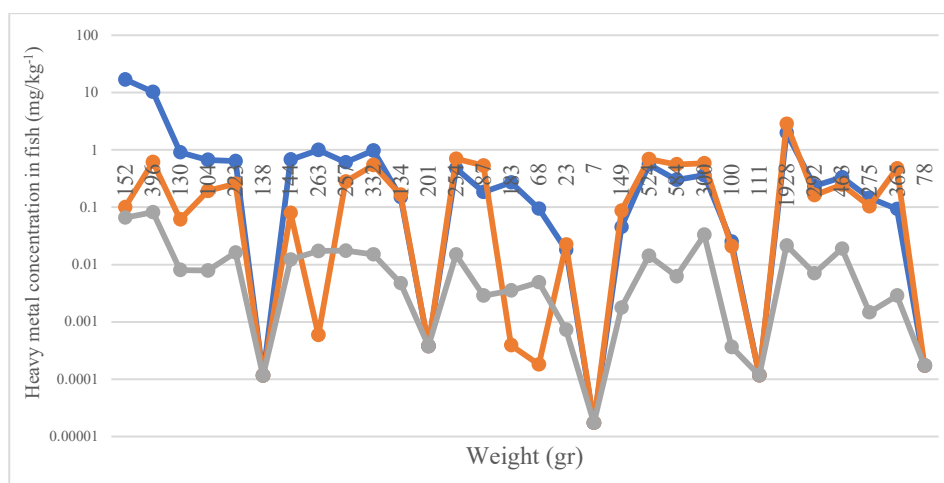


Fig 6: Comparison between body weight and heavy metals.

metal had a very low correlation, Pb metal had a very strong correlation, and Cd metal had a low correlation. (Table 2).

The average Hg metal content in individual fish at five stations ranged from 0.173-4.897 mg.kg⁻¹. The average Pb metal content in individual fish at five stations ranged from 0.178-0.640 mg.kg⁻¹. The average Cd metal content in individual fish at five stations ranged from 0.004-0.030 mg.kg⁻¹ (Fig. 5).

The Comparison between the body size of the catfish and the metal contained in the fish muscle has different results for each metal. In general, it can be seen that if the body size of the catfish is larger, the metal content in its body is higher compared to the smaller catfish (Fig. 6).

DISCUSSION

The downstream Citarum River has poor water quality when

monitoring DO, TSS, BOD, and COD parameters based on Indonesia Ministry of Environment No. 22 of 2021. A decline in environmental quality can occur due to factors including domestic activities, agriculture, livestock, and industrial activities located around river basins. Analysis of the River Pollution Index shows that domestic and industrial pollution contribute equally to the decline in the quality of the Citarum River (Marganingrum et al. 2013).

The Citarum River experiences severe water pollution and has become one of the most polluted rivers in the world (Cavelle 2013). Not only from domestic waste pollution but also industrial pollution, the results show that metal pollution in water such as Hg and Pb contained in Citarum River water has exceeded the threshold, and Cd is still below the threshold of water quality standards WHO (2022). Meanwhile, based on the findings of other research in different locations. Arief et al. (2012) stated that the concentration of Pb and Cd metals

in the water in the upstream Citarum watershed had exceeded the quality standard threshold value. Eka et al. (2016), based on the results of data collection on the concentration of the heavy metal Cd in the upstream Citarum River from 2008 to 2014, the highest concentration occurred in 2013 at 0.136 mg.L⁻¹ and the lowest in 2012 at 0.007 mg.L⁻¹.

Meanwhile, findings from other studies conducted in different locations support these results. Arief et al. (2012) reported that the concentrations of Pb and Cd in the upstream Citarum watershed had already exceeded the quality standard threshold. Similarly, in the present study, the levels of Hg and Pb in fish exceeded the maximum limits set by INS 7387 of 2009, whereas Cd levels remained below the threshold. Another study by Taufik et al. (2012) also found that fish inhabiting the upper Citarum River generally contained Pb and Cd levels that surpassed quality standards.

The metal content in catfish is influenced by the concentration of heavy metals present in the water. The enrichment of heavy metals in aquatic environments can lead to water contamination and directly affect the organisms living within these ecosystems. The distribution of metal elements in river systems is often dynamic, making fish valuable indicators for bioaccumulation and pollution assessment in aquatic ecosystems (Stankovic et al. 2014). Metals introduced into the environment tend to accumulate in aquatic organisms, eventually leading to pollution throughout the food chain (Ali & Khan 2018).

Based on the research findings, catfish is a species capable of accumulating heavy metals at high concentrations. The accumulation of metals in catfish can occur through absorption via the gills as well as through the digestive tract, ultimately leading to deposition in muscle tissue. Dietary factors also contribute significantly to metal accumulation, as catfish are carnivorous and feed on smaller fish and insects (Tang 2003).

Metal accumulation in fish can occur through the ingestion of contaminated food sources, many of which, such as invertebrate organisms, contain mercury, lead, and cadmium. Heavy metal pollutants can enter fish through multiple pathways, including food consumption, gill absorption, incidental ingestion of sediment particles, and direct uptake from the surrounding water (Handy & Poxton 1993, Vegh & Volpedo 2020).

The trophic position of catfish also contributes to the higher accumulation of heavy metals in their bodies. As metal concentrations increase along higher trophic levels, catfish are more likely to exhibit elevated levels due to biomagnification within the food chain. Catfish, being predators in the Lower Citarum River, occupy a higher trophic position compared to many other aquatic organisms, which further enhances their potential for heavy metal accumulation.

Metal accumulation in fish is not limited to muscle and bone tissues; it is influenced by both the biological characteristics of the fish and various environmental factors (Campbell et al. 2005). Environmental variables such as physicochemical and biological conditions, including pH, temperature, exposure duration, and habitat complexity, play a significant role. In addition, species-specific traits such as size, sex, reproductive cycle, feeding habits, and swimming behavior also affect bioaccumulation (Zeitoun & Mehana 2014, Pragnya et al. 2020). Key fish-related factors include age, body size (weight and length), behavioral traits, and physiological mechanisms, such as homeostatic regulation and biochemical processes (Ali & Khan 2018).

The presence of these influencing factors suggests that catfish possess physiological capabilities that allow for the absorption of metals from their aquatic environment. However, when the detoxification processes in catfish are disrupted, metal accumulation in their bodies can remain high. This may be attributed to the efficiency of excretion mechanisms in fish, where adult individuals generally excrete toxins more effectively than juveniles, thereby reducing the likelihood of metal transfer and accumulation through the food web (Nfon et al. 2008).

The results of the correlation analysis for each type of metal indicate that the relationship between heavy metal concentrations and fish growth can vary depending on the specific metal, fish species, and tissue type. For Hg and Cd, the data suggest that catfish body size does not have a significant influence on the concentration of these metals in the body, indicating that body size is not a primary factor in the accumulation of Hg and Cd. In contrast, a strong correlation was observed between catfish body size and Pb concentration, suggesting that body size is a key factor influencing the accumulation of Pb in catfish.

The presence of heavy metals in aquatic environments leads to increased metal accumulation in fish, which poses a serious health risk to humans who consume contaminated fish. Cadmium toxicity has been associated with kidney dysfunction, bone defects, osteomalacia, and lung cancer (Johri et al. 2010). Mercury is highly toxic to living organisms; its exposure can result in blindness, brain damage, kidney damage, mental retardation, and paralysis (Fu & Wang 2011). Lead accumulation in the human body can cause a range of adverse effects, including fatigue, anemia, brain damage, kidney dysfunction, and central nervous system impairment (Watt et al. 2000).

CONCLUSIONS

Based on the research results, Hg was found to have the highest concentration in catfish compared to Pb and Cd.

Individual fish at Station 1 (Curug Dam) contained the highest level of Hg, at 4.89 mg/kg. Similarly, individual fish at Station 2 (Walaha Bend) also showed elevated Hg levels at 0.56 mg/kg. In contrast, individuals at Station 3 (Adiarsa) showed a different pattern, with catfish containing a higher concentration of Pb at 0.20 mg/kg. Station 4 (Tanjung Pura) showed similar results, with Pb concentrations in individual fish reaching 0.32 mg/kg. At Station 5 (Tunggak Jati), Pb levels in fish were also elevated at 0.64 mg/kg.

Based on BAF calculations for catfish, the highest accumulation of Hg was observed at Station 1 (Curug Dam), while the lowest was at Station 4 (Tanjung Pura). The highest Pb accumulation occurred at Station 5 (Tunggak Jati), and the lowest at Station 2 (Walaha Bend). Similarly, the highest Cd accumulation was also at Station 5 (Tunggak Jati), and the lowest at Station 2 (Walaha Bend).

In catfish, the accumulation of Hg was nearly five times (4.9 times) that of its concentration in water, Cd was accumulated 4.3 times more than in water, and Pb was 4.1 times higher than its water concentration. Among the three heavy metals, catfish were found to accumulate more Hg than Cd and Pb. The high concentration of metals in the water contributes directly to the increased accumulation of these metals in catfish tissue.

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Using Artificial Intelligence Algorithms and Spatial Analysis of *Agaricus bisporus* in the Wilderness Near Lake Milh (Al-Razzaza), Iraq

Estabraq M. Ati¹, Rana F. Abbas¹, Abdalkader Saeed Latif², Reyam Naji Ajmi^{1†} and Oday Abdulhameed Jeewan³

¹Department of Biology Science, Mustansiriyah University, POX 46079, Baghdad, Iraq

²Polymers Research Unit, College of Science, Mustansiriyah University, Baghdad, Iraq

³Ministry of Environment, Technical Directorate, Baghdad, Iraq

†Corresponding author: Reyam Naji Ajmi; reyam80a@uomustansiriyah.edu.iq

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ABSTRACT

Advanced applications of artificial intelligence and geographic information systems (GIS) techniques are used to monitor plant growth across their vegetation seasons using morphological parameters. This research presents novel measurements to determine the concentrations of elements such as carbon (C), nitrogen (N), hydrogen (H), lead (Pb), and cadmium (Cd) in the mushroom "*Agaricus bisporus*" and in the surrounding soil and air. These data are spatially analyzed to contribute to long-term predictions of pollution index and future ecosystem risks. Pollution and element accumulation in the mushroom, soil, and surrounding air were monitored using data accompanied by a geographic map. Pollution was assessed by transforming the system and adopting a methodology that integrates traditional methods with artificial intelligence, aiming to address the challenges with greater efficiency and accuracy. Input parameters were used to develop models using artificial intelligence and statistical methods to detect metal accumulation, and monitor carbon, hydrogen, nitrogen, and seasonal changes. The response of plants to heavy metals (lead and cadmium) in soil and air and their impact on their growth and development, were analyzed. The techniques showed a significant reduction in the error rate when using fungi as an indicator to predict dietary heavy metal concentrations, as the accuracy of artificial intelligence was remarkable in estimating the concentration of elements and their transfer from soil to plant. The integration of artificial intelligence, machine learning, and GIS technologies enhances environmental management, as it provides the ability to monitor, predict, and provide sustainable assessments. This study provides insights to improve plant growth, reduce pollution, and support long-term food security at a lower cost and with greater accuracy in assessing environmental impacts.

INTRODUCTION

Karbala is located on this route together with Anbar governorate, where Lake Al-Razzaza (also known as Lake Milh) is located, and is the second largest lake in Iraq, and is influenced by the Euphrates River. Measured at about 1810 km² and can store 26 billion m³ of water, and the maximum height is 40 m above sea level. The Mycoflora is essential for maintaining the region's delicate ecosystem in Iraq, but is still researched inadequately (Ati et al. 2022a); Delineating fungi found in Lake Al-Razzaza and fungi living on palm trees (Al-Joboury 2022). Nitrogen, carbon, and hydrogen are among the elements that when studied help in explaining the processes that occur in the environment naturally (Al-Qaraghuli et al. 2021) they found that in Iraq after 2003 due to population increase and rise in the level of domestic, agricultural and industrial pollution especially in the middle and the south, this country is tending towards pollution crisis. In the case of the Karbala desert, there is confusion and technical difficulties in the management of the saline water channel, and the actual water demand has increased in the last few years (Al-Zuhairi et al. 2018).

Numerous mathematical models and artificial intelligence techniques, including artificial neural networks, along with various analytical methods, are employed

for forecasting the quantities of the specified elements (Alengebawry et al. 2021). These models incorporate multiple layers of interference data and an output layer that accurately focuses on heavy metals (HMs). In a study, the WT-MT-SAE and WTMSAE algorithms were used for deep feature extraction from fluorescence spectroscopic data that identified Pb in a sample of lettuce leaves, revealing that WT-MT-SAE performed better than WT-SAE (Alengebawry et al. 2024).

The GA-BPNN, which combines genetic algorithms with backpropagation neural networks, shares a similar architecture and parameters with BPNN and was used alongside BPNN to detect mercury in a soil sample analyzed with a spectrometer. The findings indicate that GA-BPNN yielded important results in the study (Ati et al. 2022b). In a different study, researchers aimed to identify copper and cadmium in rice leaves using AAS supported by BPNN. The findings indicated that BPNN accurately represented concentration levels in comparison to the statistical model (Arshad et al. 2015). MLP and RBN were employed to detect Cu, Ni, and Pb in river water samples with the assistance of AAS, where MLP successfully identified Ni and RBN was effective for Cu and Zn, while the ANFIS model was used to detect Pb. In a prior study, both BPNN and general neural regression net algorithms (GRNN) were used to find Cu, Fe, Mg, and zinc in wastewater using AAS data, yielding poor results for Fe (Arshad et al. 2015).

Mercury, iron, zinc, and lead were detected through artificial neural networks (ANN) and Multiple Linear Regression (MLR) using Vis-IR spectroscopy data from soil samples, but the effectiveness of these algorithms was predictable (Balali-Mood et al. 2021). In mining soil analysis, Pb was identified using ICP-AES along with multiple linear regression and partial least squares regression (Blessner). The PLSR method produced more precise results compared to the identified PLSR MLR (Balaram 2019). Another investigation involved lead detection in mining soil using AAS and space-based hyperspectral imaging, analyzed through ANN and Fuzzy Neural Network (FNN). In this context, the FNN demonstrated more accurate outcomes than the ANN algorithm. Also, techniques such as the successive Projection Algorithm (SPA), information preservation variables (IRIV), Extreme Learning Machine (ELM), Support Vectors SVM, and GRNN were used to detect Cu and Pb using ICP-OES on maize leaf samples. Thus, the estimation of Cu and Pb was more accurately masked by IRIV as opposed to SPA and GRNN (Fadhel et al. 2019, Gholami et al. 2024).

This research was conducted near Al-Razzaza Salt Lake in the Karbala Governorate, using data from the wild plant *Agaricus bisporus* found near Al-Razzaza Salt Lake, Iraq.

The study employed artificial intelligence algorithms and spatial analysis to evaluate pollution levels and create future prediction maps using the interpolation method for these factors, treating the mushroom as a natural bio-indicator of environmental conditions.

MATERIALS AND METHODS

Study Zone, Elemental Examination and Data Organization

Samples of air and fungus were gathered from the Al-Razzaza area. Soil samples of 500 grams each were taken from different sites, with five replicates collected from each site located 1000m away from the sampling areas. These soil samples were then placed in sterile bags. Following their placement in glass Petri dishes with sterile distilled water for 3-5 h, they were subsequently dried in an oven at 80°C for 48 h. The next step involved preparing it for weighing by filtering it through a steel sieve with a pore diameter of 2 mm. 250 grams were transferred into Teflon containers, then 6 mL of 65% HNO₃ and 3 mL of 37% HCl were added. Next, 5 mL of deionized water and 2 mL of 48% HF were included. The digestion samples were prepared using a Berghof-MSW2 microwave. After the process, the samples were transferred into 50 mL sterilized Falcon tubes using ultrapure water and a Blue Ribbon filtration Whatman filter. In order to achieve the total volume, the size was set at 50 mL. The concentrations were measured in milligrams per kilogram of dry weight using the PerkinElmer-Optima 7000DV Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) device according to the study by Arshad et al. (2015).

Soil Quality Index

The soil quality index shows that the parameters like nitrogen (N), hydrogen (H), and single carbon (CO) exist in the soil. The quantity of these pollutants in the soil quality during the monitoring period is shown, and the level of pollutants is presented in the form of a bar graph. This brings us to the result depicted in the chart below, which reveals that the most characteristic pollutant is carbon monoxide. From this, we can say that the main pollutant released by industries is carbon monoxide (Balaram 2019, Ahmed et al. 2020).

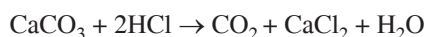
Air samples, as per Huang et al. (2023), filter paper used in the test particles in the ambient air, were collected by a fine dust sampler. Every last bit of it was sucked through the weighed filter paper before weighing the paper once again. The device was operated continuously twenty-four hrs a day, and the mass concentration was estimated by the weight of the material collected per volume. The richness of the air from which the samples were taken, metals were

extracted using an acid digestion method in order to isolate and extract heavy metals present in suspended particles of the collected samples and filter papers. Small pieces of filter paper were taken into a conical flask and weighed, and then added with 20 mL of cone. H_2SO_4 or 10 mL of cone. HNO_3 . The next process was to heat the mixture on the hot plate for a period of 4 h. The filtration and dilution method was used to quantify heavy metals with the help of a photometric analytical ICP-ES device with a sensitivity of up to ppm.

Carbon, N, H by ELEMENTRAC CSd

ELEMENTRAC CSd provides method details of the determination of organic carbon, nitrogen, and hydrogen in the soil and plant samples using the 'Thermal Scientific Flash EA 1112 analyzer.' Carbon and nitrogen contents in all samples were determined both before and after acidification.

Acidification is carried out on all types of soil and plant samples, related ELEMENTRAC CSd (QA, QC): VIO 002: The correct storage and preparation of samples [PRP 003: Soil sample preparation and handling] VOI 003 The correct storage and preservation of samples [PRP 004 Plant sample preparation and handling]. Elemental Analysis is a process that analyses which involves the complete combustion of the sample within a high-temperature reactor coupled with accurate and precise determination of the elemental gases produced with the help of a thermal conductivity detector to determine carbon and nitrogen present in organic form, Carbon, hydrogen and nitrogen estimations are carried out both before and after the removal of carbonates from the soil sample. Carbonates are extracted from the weighed samples into Ag capsules by flooding the samples with an aqueous acid solution. The carbonates are released as CO_2 through the following chemical equation: according (Ohyama et al. 2021).



Development and Setup of Neural Networks Along with Spatial Analysis

An artificial neural network consists of an input layer (hidden layers) and an output layer. The input layer consists of neurons, known as covariates, and the output layer contains neurons called dependent variables. There are no connections between neurons within the same layer, but they are densely connected to adjacent layers through synapse-like structures. In this way, the data gathered from the surrounding area is utilized for training the neural network in order to forecast the continuous values of the dependent variable. The entire sample of data was divided into three subsets, known as test data, to make reliable predictions. The cluster starts with random weights and then uses concentrations from previous

tests. The back propagation neural network algorithm with gradient ratios is used to update the concentrations to reduce error.

The software SPSS 27 was utilized to achieve the necessary data-driven model during the calculations, with the Multi-Layer Perceptrons (MLP) selected as the subunit and 1 chosen as the number of hidden layers. The hidden layer is set to have 2 neurons, and the sigmoid function is used as the activation function for both the hidden and output layers (Gholami et al. 2023). The internal structure of the MLP is created with 4 input variables, with the height (Pb, Cd) set as independent variables in each calculation, and the number of input variables individually represented by (C, H, N).

For each instance of the elements linked to the MLP architecture, the training, test, and rejection data were distributed at 70%, 20%, and 10%. The number of units in the input layer for each variable is determined by the algorithm, starting at 0.4 and using momentum of 0.9 with the squared error function when running the gradient descent algorithm. The algorithm processes all the data at once and then continues to utilize the stored data until a stopping criterion is reached, such as the maximum number of epochs for the contour maps that are initialized to read spatial distribution semantics (Uchimiya et al. 2020).

RESULTS AND DISCUSSION

Findings have indicated the presence of essential elements (nitrogen, carbon, hydrogen) in the soil and air of Al-Razzaza. Besides, the total elements (lead and cadmium) were also analyzed, with the results outlined in Tables 1 and 2.

Results indicated that the environmental components (N, C, H) of air and soil are receptors for pollutants in large quantities from various sources. Thus, they can be used in determining the origin of pollution, its characteristics, and the environmental factors that may be exposed to pollutants resulting from climate change, such as dust storms in most seasons of the year, rains, and thunder. The results indicate that the environmental components (N, C, H) of air and soil are receptors for pollutants in large quantities from multiple sources, and thus can be used to identify the source of pollution, its characteristics, and environmental factors exposed to pollutants resulting from climate change, such as dust storms in most seasons of the year, rain, and thunder, based on the longitude and latitude lines captured by GPS/Geko 201, a graphic map was determined for spatial analysis of all variables of the study areas based on the affected natural environmental and biological factors, and the graphic images obtained were considered the input variables as indicators indicating small points distributed in the data so that the

Table 1: Amounts of (N, C, H, Pb, and Cd) in *Agaricus bisporus* and the soil of the Al-Razzaza.

Code	Soil	C%	H%	N%	Cd ppm	Pb ppm	E: Longitude	N: Latitude
1*	AbS	8.68	8	2.17	1.81	0.29	42.243872	32.031541
1	AbS	8.06	9	3.13	0.93	0.12	42.252347	32.032871
1	AbS	8.99	10	3.07	1.35	0.15	42.254373	32.025972
2**	S	29.24	21.18	6.95	5.9	1.9	42.242483	32.026501
2	S	25.9	18.97	7.93	6.9	1.5	42.246169	32.021261
2	S	27.02	18.88	7.96	7	1.6	42.254928	32.021451

1* Mean concentration between the concentration of elements in *Agaricus bisporus* and with surrounding soil

2** Mean concentration in soil only, AbS: *Agaricus bisporus* with Soil, S : Soil only

Table 2: The amount of elements (N, C, H, Pb, Cd) in *Agaricus bisporus* and the Air of the Al-Razzaza.

Code	Air	C%	H%	N%	Cd ppm	Pb ppm	E: Longitude	N: Latitude
3*	AbA	11.2	11.18	1.17	0.92	0.7	42.24387	32.03154
3	AbA	12	11.91	1.13	0.84	0.11	42.25235	32.03287
3	AbA	16.02	11.81	1.07	0.73	0.15	42.25437	32.02597
4**	A	34.68	16.08	1.95	2.19	1.9	42.24248	32.0265
4	A	30.06	15.67	2.93	3.53	1.9	42.24617	32.02126
4	A	45.99	16.2	2.96	2.59	1.6	42.25493	32.02145

1* Mean concentration between the concentration of elements in *Agaricus bisporus* and with surrounding air

2** Mean concentration in Air only, AbS: *Agaricus bisporus* with Air, A : Air only

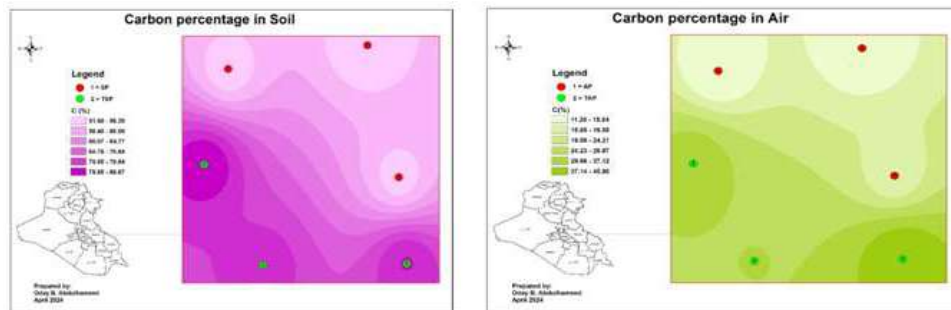


Fig. 1: Approach 1 and 2 spatial analysis of carbon concentration in soil and air.

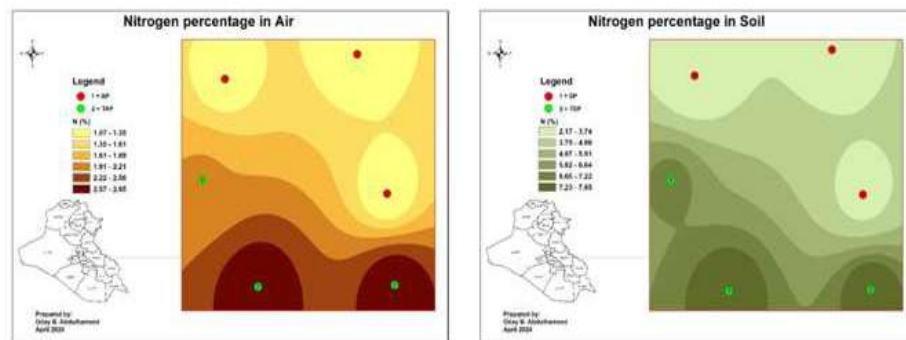


Fig. 2: Approaches 1 and 2, spatial analysis nitrogen in soil and air.

environmental indicators of the first level of pollution appear to include soil and air followed by biological levels in *Agaricus bisporus*.

Based on the longitude and latitude lines taken by GPS/ Geko 201, a graphical map was determined for the spatial analysis of all variables of the study areas on the basis of the

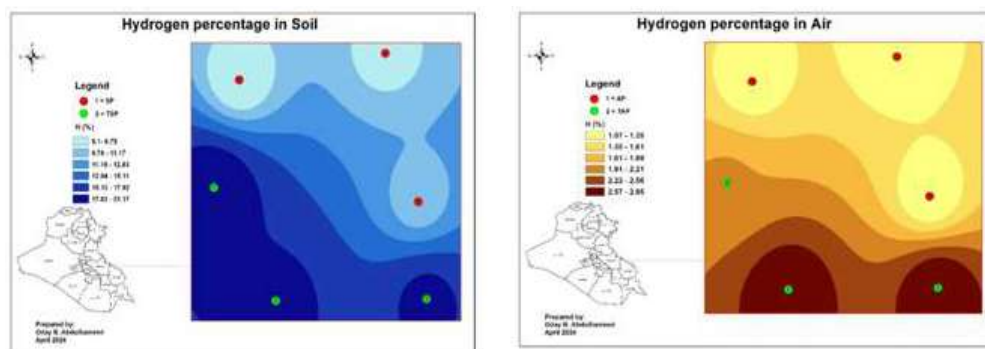


Fig. 3: Approaches 1 and 2, spatial analysis of hydrogen in soil and air.

affected natural environmental and biological factors. The graphical images obtained considered the input variables as indicators, indicating small points distributed in the data so that the first level environmental indicators of pollution are shown to include soil and air, followed by biological levels in *Agaricus bisporus*. The general maps for the spatial analysis are shown below in Figs. 1,2, and 3 approaches 1 and 2 Spatial Analysis of carbon, hydrogen, and nitrogen concentration in soil and air.

The resulting contour maps of the investigated metal levels and standard deviation maps are shown in Figs. 4

and 5. Approaches 1 and 2 of spatial analysis examined lead and cadmium in soil and air. Depending on the type of element, transportability and absorption influence the accumulation in plant tissues. Metals have accumulated in growing plants in the soil, which has led to a decrease in their concentrations in the air and soil of developing areas. These areas exhibit relatively high concentrations compared to those not surrounded by plants.

Agaricus bisporus, as a biomonitoring device, is directly proportional to the average surrounding bioavailability of nitrogen (N), hydrogen (H), and carbon (C). These elements

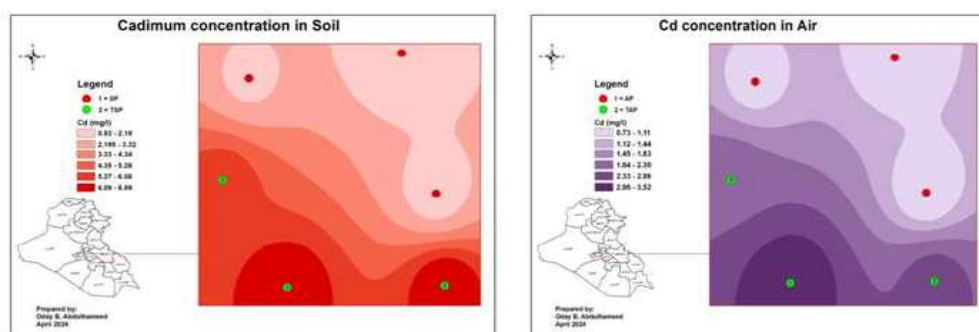


Fig. 4: Approach 1 and 2 spatial analysis of cadmium in soil and air.

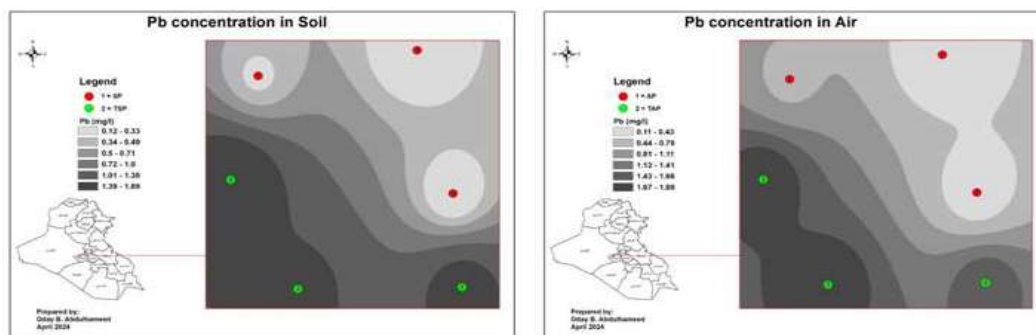


Fig. 5: Approaches 1 and 2, spatial analysis lead in soil and air.

are biologically available to living organisms due to changes in response to environmental conditions and geochemical processes that control their availability at each stage. Any impact on their accumulation leads to an imbalance in the concentrations of remaining major elements, such as lead and cadmium. This distinction is evident in the contour maps and the differences observed between the soil and air concentrations of each element.

A balanced amount of essential nutrients and minerals plays a key role in the metabolism of the food chain. These approaches or model equations in our results allowed the use of all available data as a biomarker *Agaricus bisporus* to predict and function the GIS variable and give coefficients with additional accuracy in the supplementary materials because they do not focus on differences in relative size according (Wolski & Kruk 2020) but rather focus on distance-weighted variables such as conditions, soil nature, and activities. Also, the characteristics of spatial data have changed significantly during the last ten years with the increase in population density and conflicts, as mentioned in (Wolski & Kruk 2020).

As a result, materials are at risk of forming significant accumulations during environmental assessment processes, and soil pollution is recognized as a significant threat, especially in open natural areas, as it can lead to serious health and environmental risks (Münzel et al. 2023). The next step involved creating hypotheses based on layers and neurons in artificial neural networks in order to identify and classify complex non-linear relationships between data sets related to *Agaricus bisporus*. This was chosen as the initial modeling method to address scientific problems and make predictions using complex non-linear relationships within the obtained dataset.

The study found that increasing the depth of the neural network and the number of hidden neurons could help in dealing with various sources of pollution. It was also determined that the dependent and independent variables play a crucial role in establishing the optimal model for biological neural networks in analyzing plant element concentrations and estimating air and soil pollution (Gholami et al. 2023). While there are significant amounts of certain elements in the soil, they play a crucial role in supporting plant growth and health, including (N, C, H), as well as heavy metals, which can have harmful effects on the soil. Nevertheless, an elevated level of these substances in the soil could exceed a specific concentration, leading to harmful effects that are toxic to plants in the future, as stated by Alengebawy et al. (2021) and Rashid et al. (2023).

An adequate balance of essential nutrients and minerals is crucial for the food chain's metabolism. The approaches or

model equations used in our study allowed for the utilization of all available data to predict and analyze the GIS variable as a biomarker for *Agaricus bisporus*. The coefficients provided in the supplementary materials offer improved accuracy, as they do not emphasize differences in relative size (Wolski & Kruk 2020) but rather focus on distance-weighted variables such as conditions, soil nature, and activities. Also, the characteristics of spatial data have changed significantly during the last ten years with the increase in population density and conflicts, as mentioned in Jones & Turner (2024).

As a result, materials can easily accumulate in environmental assessment processes, leading to soil pollution, which is considered a significant risk, especially in undeveloped areas, as it can pose serious threats to both health and the environment (Münzel et al. 2023). The next step involved creating hypotheses using layers and neurons in artificial neural networks to identify and categorize complex non-linear relationships between datasets related to *Agaricus bisporus*. This was chosen as the initial method for modeling, problem identification, and prediction building due to its ability to model the complex relationship between input and output. By increasing the depth of the neural network and the number of hidden neurons, enlightenment can be achieved in dealing with various sources of pollution, as concluded in the study by Gholami et al. (2023).

The study also determined that the dependent and independent variables are crucial in determining the optimal model for biological neural networks in estimating the concentration of plant elements and evaluating air and soil pollution. Even though there are significant amounts of elements present, they play a crucial role in the health of plants, including (N, C, H) and heavy metals, which can have harmful effects on the soil. Nonetheless, if there is an excessive amount of them in the soil, it may reach a harmful level that could be toxic to plants in the future, according to Alengebawy et al. (2021) and Rashid et al. (2023).

Using deep learning artificial neural networks to predict element levels could serve as a valuable alternative for determining the concentrations of soil and air elements. The current method for making predictions relies on authentic data and results, which are then compared with statistical methods. The current approach's validity was confirmed by splitting the data into three parts and using one of them to verify it. As a result, verification is achieved by analyzing real-world quantitative test data, followed by the use of artificial neural networks to forecast the levels of crucial heavy metals. This is done by establishing a connection between the fluctuations of these metals and the presence or absence of plants in wild areas, whether agricultural or desert and considering their potential impact on the

environment. Additionally, the relationship between these factors and climate changes during the growing season of *Agaricus bisporus* is consistent with the findings of Jones & Turner (2022).

The neural network's structure comprises the input layer with three separate spike inputs from the air and soil, denoted as (N, C, H). The concealed layer connects three neurons to the output layer, and the pair (Pb, Cd) signifies distinct states. Before commencing the analysis, the input and output data underwent normalization. The data was then categorized into three groups (*Agaricus bisporus*, soil, air) for durability testing. These separate the data into three categories for different trace elements and keep track of rejected data to regulate the accuracy of predictions (Ryoo et al. 2024).

The hyperbolic tangent function is used in the hidden layer, while the sigmoid logistic function serves as the activation function for the output layer. In mathematical biology, sigmoid functions are commonly employed in

prediction models. The error function is typically the sum of squared errors, and the maximum impact time on elements such as soil and air is determined by the number of consecutive steps without a decrease in error. This allows the function to represent the relative error value in successive steps. In the soil and *Agaricus bisporus* R. To determine the comparison of some variables in soil and *Agaricus bisporus* R, a programming language and software environment for statistical computing and graphics, is used to determine the comparison of some variables in soil and *Agaricus bisporus* R. A paired t-test was conducted to depict if there are significant differences in the mean average of soil and air variables and absence data *Agaricus bisporus*. Figs. 6 and 7 present the outcome of such an analysis. Conventional architecture focuses on elements of the network diagram in *Agaricus bisporus* with soil and air.

According to Al-Zuhairi et al. (2018) and Münzel et al. (2024), the paired t-test hypotheses show that all p-values

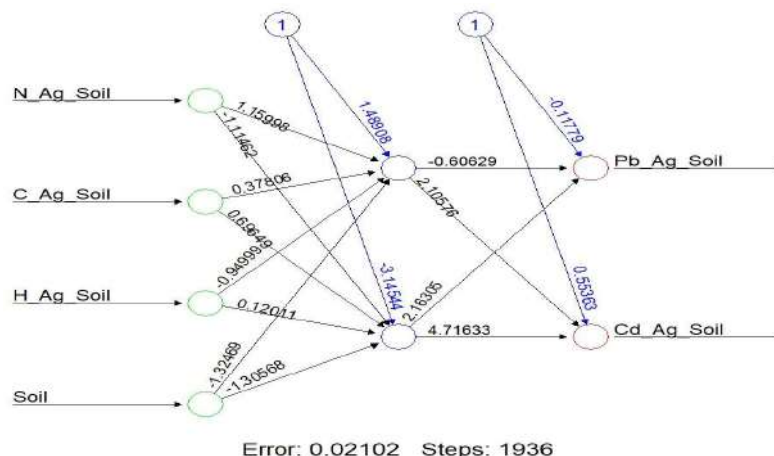


Fig. 6: The typical architectural concentration components of the network diagram in *Agaricus bisporus* alongside the soil.

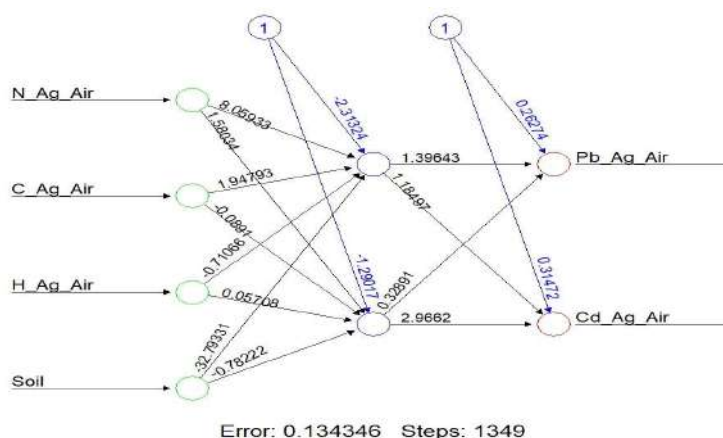


Fig. 7: The typical architectural concentration components of the network diagram in *Agaricus bisporus* alongside air.

in the soil are <0.05 , indicating major differences in the mean averages of soil variables before and after planting *Agaricus bisporus*. The correlation coefficient assesses the strength of the linear relationship between two variables, with a perfect positive linear relationship represented by $R^2 = 0 - 1$. Here, 0 indicates no relationship, while 1 indicates a strong relationship. The null hypothesis is $H_0: x_1 = x_2$ (not major), and the alternative hypothesis is $H_1: x_1 \neq x_2$ (major).

Linear regression techniques were employed to develop empirical predictive models based on field data. Statistical significance was determined with p-values <0.05 , while p-values >0.05 were deemed not major. Regarding air and *Agaricus bisporus*, R, a programming language widely used in statistical analysis, data analytics, and scientific research, was used to compare and analyze soil variable data. Specifically, a paired t-test was conducted to determine if there were major differences in the mean averages of soil and air variables before and after the planting of *Agaricus bisporus*. Since all p-values in air are <0.05 , it indicates major differences in the mean averages of air variables before and after the planting of *Agaricus bisporus*. The correlation coefficient, again, measures the strength of the linear relationship between two variables, with a perfect positive relationship signified by $R^2 = 0-1$, where 0 means no relationship and 1 indicates a strong relationship.

Linear regression methods were used to build empirical predictive models from field data. Statistical p-values <0.05 were regarded as major, while p-values >0.05 were considered not major. The figure displays predicted Pb values plotted against actual Pb values for the entire dataset, with results aligning closely within an acceptable range around the ideal prediction line. This suggests that the figure remains reasonable when viewed alongside the actual values. Tables 3 and 4 present quantitative results revealing relative errors in the respective test data. Given that the actual values are substantially greater than zero, the relative errors provide plenty of insight into the effectiveness of the current technology. What's more, bear in mind that the dataset is major, as evidenced by the calculated sum of square error (Ryoo et al. 2024).

The R-squared value, which ranges from 0 to 1, represents a perfect positive linear relationship. A correlation value of 0 denotes no relationship, while a value of 1 indicates a strong positive linear relationship in the soil. This is illustrated through tables (3 and 4), which show that the significance of the correlation among the independent variables can be identified by the increase from 0 (indicating no relationship) to 1 (indicating a strong relationship). The expected values, actual values, and residual values of the elements exhibit nearly perfect alignment, with the remaining values showing

a perfectly satisfactory agreement with the expected versus actual values (Wolski & Kruk 2024).

CONCLUSIONS

Data-driven mathematical models are important for making classifications or predictions, especially when using tools such as dependent and independent variables or artificial neural networks, without addressing causal relationships. Deep learning neural networks can effectively predict soil element levels and their transfer to plants, providing a cost-effective and time-efficient alternative. These predictions are based on real, validated data, which is divided into three segments for validation purposes. Artificial neural networks forecast the prevalence of key heavy metals by correlating them with plants' presence and their environmental impacts. The incorporation of technology, AI, machine learning, and GIS enhances environmental management by allowing for the monitoring and forecasting of contamination levels. By using an interdisciplinary strategy, this approach integrates physical geography, environmental sciences, and social sciences, using GIS to accomplish effective environmental assessment and sustainability goals.

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Isolation and Characterization of Hexavalent Chromium Reducing Bacteria for Application in Microbial Fuel Cells

Vijay Samuel G.^{1†} , R. Anitha¹, Govindarajan R.¹, D. Sangeetha², Nibedita Dey³, Premkumar Thangavelu⁴ and M. Anthony Raj⁵

¹Department of Biotechnology & Chemical Engineering, Hindustan Institute of Technology & Science, Chennai-603103, Tamil Nadu, India

²Department of Mechanical Engineering, Institute of Energy Studies, CEG Campus, Anna University, Chennai-600025, Tamil Nadu, India

³Department of Medical Biotechnology, Institute of Biotechnology, Saveetha School of Engineering, Saveetha University, Saveetha Institute of Medical and Engineering Sciences, Thandalam, Chennai-602105, Tamil Nadu, India

⁴Department of Infectious Diseases, Christian Medical College and Hospital, Vellore-632004, Tamil Nadu, India

⁵Department of Chemistry, Rajalakshmi Institute of Technology, Chennai-600124, Tamil Nadu, India

†Corresponding author: Vijay Samuel G.; vijaysamjuly@gmail.com

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ABSTRACT

Wastewater from several businesses that produce paints, steel, tannery products, dyes, and chrome-plated items contains hexavalent chromium [Cr(VI)]. The main contributing factor to pollution concentrations in water bodies is the wastewater that tannery businesses dump, which includes organic pollutants and heavy metals, particularly Cr(VI). The harmful consequences of Cr(VI) on humans include eczema, allergies, ulceration, respiratory tract problems, lung cancer, as well as genotoxic and mutagenic effects. Using bacteria to biotransform hexavalent chromium to trivalent chromium [(Cr(III))] is a practical strategy with proven viability in bioremediation. Following their isolation from the tannery industry's raw effluent, bacterial strains were investigated biochemically and molecularly. Based on this study, it was concluded that the microorganisms resistant to Cr(VI) were *Bacillus albus* and *Bacillus australimaris*. In a two-chambered microbial fuel cell reactor, the isolated Cr(VI) resistant bacteria will be employed as electrogenic bacteria with SPEEK (Sulphonated polyether ether ketone) as PEM (proton exchange membrane), which can synergistically aid in the reduction of hexavalent chromium and green energy generation.

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INTRODUCTION

Cr(VI) is widely used in a number of industrial processes, such as tanning, wood preservation, and electroplating. Cr(VI) is available commercially as potassium chromate (K_2CrO_4) and potassium dichromate ($K_2Cr_2O_7$). Hexavalent chromium is found in a lot of the solid and liquid waste that is produced by the chromium manufacturing sector. Before releasing these wastes into the environment, they must be treated (Alam et al. 2020). Compounds containing Cr(VI) are very soluble in water, poisonous, and carcinogenic to mammals. Trivalent chromium, on the other hand, is thought to be harmless as it forms insoluble oxides and hydroxides in soil and water systems at pH values greater than 5.5. One of the exciting new technologies that is anticipated to be crucial to the clean-up of waste sites is bioremediation. The goal of the biotreatment or bioremediation technique is to immobilize Cr(VI) in the soil matrix by detoxifying it in the wastewater and reducing it to Cr(III).

Enzymatic redox reactions are a common metabolic activity in microorganisms. Reactions that take place on the surfaces of bacteria can also decrease Cr(VI) non-metabolically (Kapahi & Sachdeva 2019). Kapahi & Sachdeva (2019) have

proposed this as the main reduction mechanism in natural geologic environments. Intracellular precipitation is a third method of Cr reduction (Rahman & Thomas 2020, Tang et al. 2021). The first process, in which Chromium is metabolically reduced in the presence of abundant electron donors, has been the subject of the majority of research (Lin et al. 2020). Chemical-reducing agents that necessitate biological interactions encompass the utilization of lactic acid, molasses, exclusive formulations like cheese whey, and the Hydrogen Release Compound (HRC). These substances give off carbon dioxide into the atmosphere, but in an anaerobic environment, they need to undergo biological changes in order to produce hydrogen. Both aerobic and anaerobic routes can be used by bacteria to enzymatically decrease Cr(VI) (Kalsoom et al. 2021). The biological mechanisms compete with other nonbiological routes for Cr reduction (Niu et al. 2024, Gu et al. 2024). Since biological reduction is sluggish in anaerobic environments, abiotic reduction by iron or hydrogen sulfide is anticipated to predominate. Only in aerobic settings can microbial decrease take on kinetic significance. The prime factors affecting the reduction rate are the quantities of oxygen in the system, pH, and geochemical conditions. The environment is always full of microorganisms. Research is still needed to identify their role in Cr reduction.

Pollution in the environment poses a major threat to the world. The magnitude of this problem seems to increase every day. The major cause of this issue is urbanization and industrialization. This alters the flow of substances in nature and instills chemicals that constitute the water bodies and their surrounding environments, like soil, flora, and fauna. Water pollution tops all pollution in the present modern world (Solanki et al. 2022). Wastewaters from industries heavily contaminate plants, and human and animal lives with toxic heavy metals. Bioaccumulation in aquatic bodies and subsequently reaching the human tissues through the

ecosystem has been reported by many scientists in recent years (Garg et al. 2012). Chromium pollution is mainly caused by tanning industries. More than 80% of the 2500 tanneries in India deal with chromium, which gets released into the surrounding environmental bodies eventually (Shukla et al. 2009). Chrome from high exhaust of tanneries can contaminate water bodies with up to 500-1000 mg Cr(VI) (Aravindhana et al. 2004). Hence disposal issues of the same are of great concern to the Environmental Protection Agency (EPA) and the World Health Organization (WHO).

The presence of chromium in the habitat affects the optimal functioning of the ecosystem and imposes an economic burden. 0.1 mg.L^{-1} is the permissible limit for chromium allowed by the pollution control board. Industries dealing with hide processing release around 250 kg of solid wastes and 100 kg of wastewater. Among the various tanning processes shaving of chrome leather and splitting of chrome takes 40% of the production process. This generates a lot of waste in the form of chromium. The trivalent form of chromium is found to be less toxic when compared to its hexavalent counterpart. The tanning industry uses approximately 6000 tonnes of chromium, of which 40% is discharged into the surroundings as waste. When chromium is used to tan hides, the end product seems to have better mechanical resistance, good suitability for dyeing, and exceptional hydrothermal resistance (Shaibur 2023). Workers exposed to chromium along with respiratory illness are also prone to dermatological complications like 'chrome holes'. Severe cases lead to morbidity by 40%, of which 16% is due to respiratory illness (Kasim et al. 2014). Dry cough is attributed to 5.6%, irritation in the throat by 3.6%, and congestion in the lungs by 3% (Tremper 2005).

The microbial consortia were isolated from the raw effluent of the tannery industry situated in the North Tamil Nadu district and were used to reduce the hexavalent chromium

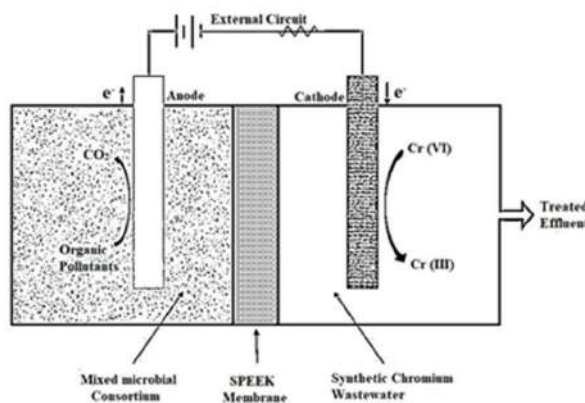


Fig. 1: Microbial Fuel Cell.

levels in the chrome tanning effluent. The effectiveness of the consortia was analyzed in this work, and the same will be used as an inoculant for microbial fuel cell experiments wherein SPEEK (sulfonated polyether ether ketone) will be used as PEM (proton exchange membrane) (Fig. 1).

The present study utilizes a group of microorganisms isolated from the raw effluent of the tannery industry situated in the North Tamil Nadu district and is used to reduce hexavalent chromium into trivalent. The previously reported studies exploited a variety of other methods, such as using a reductant like Epigallocatechin gallate (EGCG) and other chemicals like (Polystyrylmethyl) trimethylammonium borohydride, sodium metabisulphite, catalytic membrane reactors containing palladium, and using bacteria like *Halomonas maridiana* EA1.

MATERIALS AND METHODS

Sample Collection

The sterile nutrient broth (NB) was used to serially dilute the effluent sample (1 mL) from 10^{-1} to 10^{-5} dilution before plating it on nutrient agar (NA) (HiMedia). The plates were incubated at 37°C for 24 h. The well-isolated colonies were picked up using a sterile inoculation loop and further examined for growth on effluent-containing plates. Tannery effluent-based agar medium was prepared, in which tannery effluent was the sole carbon source (Sanjay et al. 2020). The tannery effluent was (1L) filtered, and 15 g of agar powder (HiMedia, Mumbai) was added for solidification. Then, the media was sterilized and cooled, and the agar plates were prepared, which were later inoculated with the isolated bacteria. After 24 h, the bacterial colonies were characterized for their shape, size, color, margin, and gram staining. Glycerol stocks of the culture were maintained in NA slants and stored at 4°C .

Biochemical Characterization of Bacteria

The physical and biochemical features of the isolated bacterium were carried out as per Bergey's Manual of Determinative Bacteriology was used to characterize the chosen bacterial isolate. Morphological traits were seen for each bacterial colony after a 24-hour incubation period at 37°C . After Gram staining, the colony morphology was investigated under a microscope, and biochemical analysis like Kovacs- indole test, methyl red test, Voges-Proskauer, endospore, catalase, and starch hydrolysis tests were done on the bacterial isolate.

DNA Isolation

Samples that were modified using the normal salting-out

process were used to isolate DNA. 10% of the 10L SDS (Sigma Aldrich) and 500 L of Solution 1 were added to the sample tube. Make use of a sterile homogenizer to blend the bacterial sample. Additionally, Proteinase K of 5 liters ($20 \text{ mg} \cdot \text{mL}^{-1}$) was added. For easier digestion, the mixture was maintained at 55°C for two hours in a water bath with sporadic mixing and rapid vortexing. The sample was placed on ice for ten min following full digestion. 250 μL of Solution 2 was added to this, and it was mixed by repeatedly inverting it. The samples were then refrigerated for five min on ice. The samples were subjected to centrifugation (Eppendorf 5804R) for 15 min at 8000 rpm. Following that, roughly 500 liters of clear supernatant were gathered and placed into fresh, labeled 1.5 mL tubes. To precipitate the DNA, twice as much one hundred percent molecular biology grade ethanol (Sigma Aldrich) was added. After that, the samples were centrifuged for 15 min at 11,000 rpm. Following that, 500 L of 70% ethanol was added for washing, and the supernatant was disposed away. The sample was spun for five min at 11000 rpm. Remove the supernatant with care, then let it air dry at room temperature. DNA that had partially dried was reconstituted in 100L of elution and kept at -20°C .

Molecular Region

In order to confirm the bacterial species using molecular means, the standard 16s rRNA gene was chosen. Using universal primers, PCR was used for 16S rRNA gene amplification.

Primers

Universal 16S rRNA gene primers Forward 27F 5'-AGAGTTTGATCMTGGCTCAG-3' Reverse 1492R 5'-GGTTACCTTGTTACGACTT-3' were used for the amplification.

Polymerase Chain Reaction Condition

The polymerase chain reactions were conducted using a thermal cycler (Thermo Fisher, Model. No. GeneAmp 9700). A molecular weight marker (a 100 bp ladder) was used to determine the molecular weight of the products amplified using 1.5% AGE (agarose gel electrophoresis).

Molecular Identification by 16S rRNA Gene Sequence

The samples that were altered from the conventional salting-out process were used to extract the genomic DNA of the bacterial isolates. For the bacterial species' molecular confirmation investigations, the standard 16s rRNA gene was chosen. Using universal primers, PCR was used for the 16S rRNA gene. Amplification. Primers for the whole 16S rRNA gene were chosen for amplification, encompassing all variable regions, based on prior research. Forward 27F

and 5'-AGAGTTTGATCMTGGCTCAG-3', Reverse 1492R and 5'-GGTTACCTTGTTACGACTT-3' are the primer name and sequences, respectively. A molecular weight marker (a 100 bp ladder) was used to determine the molecular weight of the amplified products using 1.5% AGE.

Sequencing the Amplified Product

Sequencing using BigDye Terminator v3.1: The sequencing process was conducted in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA).

Sequence analysis: The sequence quality was verified using Applied Biosystems' Sequence Scanner Software v1. MEGA 7 was used for sequence alignment and any necessary modification of the acquired sequences.

BLAST analysis: It was determined that the amplified sequences match the 16S rRNA by utilizing the similarity index created by the NCBI's BLAST program. The study's species were categorized according to their greater percentage of similarity to the reference species.

Effect of Chromium (VI) on Bacterial Growth

In 10 ml of nutrient-rich broth, the effects of several dosages

of Cr(VI) on the resistance of the bacterial growth were examined in triplicate at 37°C and 200 rpm in a water bath. The optical density (OD) (Shimadzu, UV-1900i model double-beam) of the culture at 600 nm was used to track the growth.

Chromium(VI) Assay

Bacterial growth and Cr(VI) reduction time courses were assessed in a shake flask culture using 100 mL of nutrient broth (HiMedia) at 37°C and 200 rpm, using 20 mg.L⁻¹ K₂Cr₂O₇. 2.0 ml of samples were taken out every 0.5 or 2 h. The OD at 600 nm was used to track the increase. The samples were centrifuged for 5 min at 10,000 rpm, and the supernatants were tested for the presence of leftover Cr(VI).

Determination of Bacterial Resistance or Tolerance to Cr

Using a broth dilution approach, a number of bacterial isolates that showed a significant capacity to detoxify or decrease Cr were investigated to ascertain their tolerance or resistance to the element. By using this method, bacterial cells with a density of 10⁶ cells.mL⁻¹ were cultured in brain heart infusion broth supplemented with varying amounts of K₂Cr₂O₇ for 24 h at 37°C and agitation at 100 rpm. With regard to the bacterial

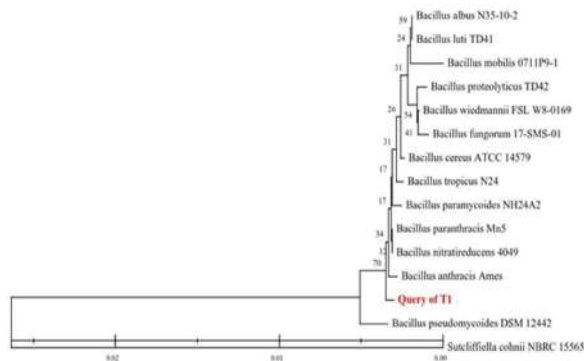


Fig. 2: Evolutionary relationships of taxa (T1).

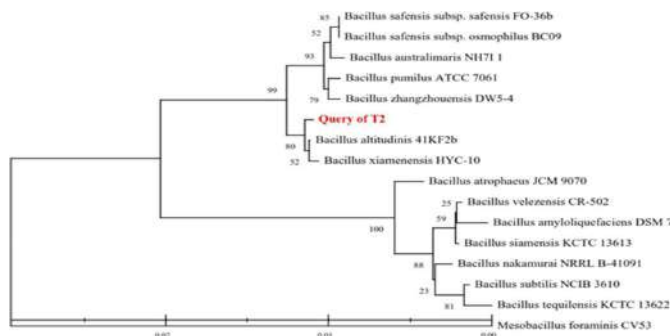


Fig. 3: Evolutionary relationships of taxa (T2).

Table 1: Identification of chromium (VI)-reducing bacteria by 16S rRNA gene sequence analysis.

Strain name	Name of Bacteria	Percentage Identity
T1	Bacillus albus	59
T2	Bacillus australimaris	93

growth, the turbidity of the broth was evaluated at 600 nm using a UV-visible spectrophotometer. By examining the isolates that demonstrated a significant concentration of Cr shows, good to moderate growth was observed.

RESULTS AND DISCUSSION

From NB plates containing Cr(VI), five distinct colonies were chosen and introduced with effluent obtained from a common leather tannery effluent treatment plant in the North Tamil Nadu district. To test their tolerance to Cr(VI) ranging from 500 to 2,000 mg.L⁻¹, the samples have been further inoculated in NB broth treated with various doses of Cr(VI). The highest possible level of tolerance was displayed by isolates T1 and T2, which grew well in the presence of 2,000 mg.L⁻¹ Cr (VI) (Fig. 2), while some isolates did not accelerate their growth rate over 1,000 mg.L⁻¹ Cr (VI) and hence the isolates T1 and T2 were chosen for further study. Hussain and Al-Saadi (2022) isolated certain bacteria that can withstand or reduce Cr(VI) at values of 1,500–2,500 mg.L⁻¹ and are considered chromium-resistant. Both biochemical and 16S rRNA gene sequence analyses were carried out to identify the isolates T1 and T2 (Table 1). The isolate

T1 exhibited 59% identity with *Bacillus albus* (Fig. 1), and isolate T2 showed 93% identity with *Bacillus australimaris* (Fig. 3) (Escamilla-Rodríguez et al. 2021, Kookhaee et al. 2022). Sanjay et al. (2020) reported *Bacillus* sp. Play a major role in reducing hexavalent chromium isolated from tannery effluent samples and chromite mine soils, respectively.

Isolation and Characterization of Bacteria

Identification of the isolated bacteria: The bacterial isolates that were chromium tolerant were identified using Bergey's Manual of Determinative Bacteriology (Sharma et al. 2023). The selected isolates shared common morphological and biochemical characteristics like size, shape, and arrangements, Gram-positive, negative indole, negative MR (methyl red), positive catalase, positive citrate, and positive starch hydrolysis (Table 2). The isolates were chosen for further identification after being identified as *Bacillus* sp. N35-10-2 and NH711 are based on their morphological features.

Given samples were serially diluted from 10¹ to 10⁹ and spread-plated on LB agar medium. After 16 h of incubation at 37°C, plates were subjected to observation. Two different morphologies were dominantly present in all dilution plates. Those 2 colonies were selected and subjected to molecular identification (Figs. 4 and 5).

Determination of DNA Quality

The amount of DNA that was separated was measured



Fig. 4: Plate 1 – T1 (Marked in red color).



Fig. 5: Plate 2 – T2 (Marked in yellow color).

Table 2: Morphological and biochemical characteristics of isolated Cr-reducing bacteria.

Characteristics	Observations	
	T1	T2
Morphology and staining		
Gram's staining	Positive	Positive
Acid-fast staining	Negative	Negative
Shape	Short rod	Short rod
Arrangement	Single	Single
Biochemical tests		
Indole	Negative	Negative
Motility	Positive	Positive
MR-VP	Negative	Negative
Catalase	Positive	Positive
Citrate	Positive	Positive
Starch hydrolysis	Positive	Positive
Oxidase	Negative	Negative

using Nanodrop 2000 (Thermo, USA). By calculating the absorbance ratio of 260/280 nm, the quality was examined. A score of 1.7 to 1.8 denotes high-quality DNA free of protein or RNA contamination.

DNA quality determination: On 0.8% agarose gels, the DNA quality was examined (Figs. 6 and 7). On 1.5%, the amplified products were examined. Using a molecular weight marker (a 100 bp ladder), the molecular weight was determined via agarose gel electrophoresis.

Chromium Reduction Potential

Redox processes can be catalyzed by microorganisms through a variety of methods, such as intracellular reduction and precipitation, nonmetabolic reduction by bacterial surfaces, and enzymatic extracellular reduction. Microorganisms capable of reducing Cr(VI) to Cr(III) include bacteria (Desulfomamaculum, Achromobacter, Aeromonas, Bacillus, Enterobacter, Escherichia, Pseudomonas, Micrococcus),

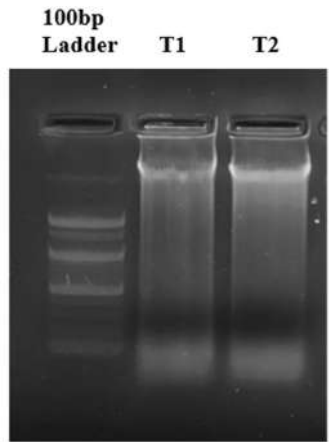


Fig. 6: Genomic DNA.

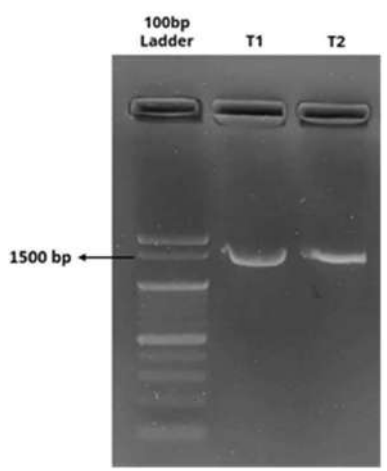


Fig. 7: PCR products of 16s rRNA gene.

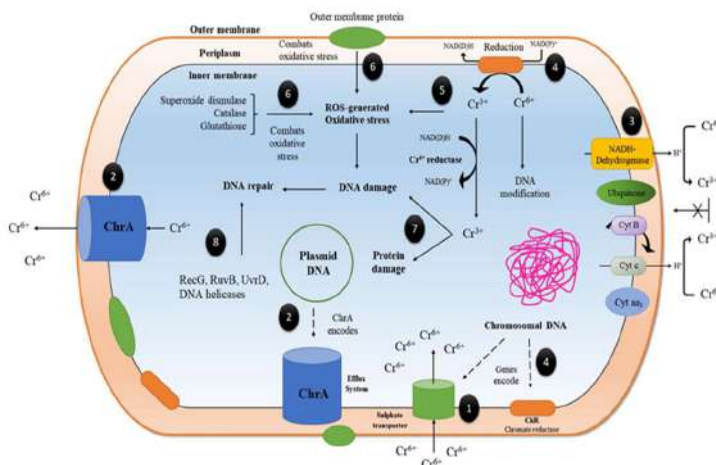


Fig. 8: Intracellular Cr (VI) reduction mechanism.

algae, yeasts, and fungi. (Fig. 8) (Rahman & Thomas 2021, Wang et al. 2017).

One frequent mechanism for reducing Cr(VI) intracellularly is the action of NADH, flavin protein, and other heme proteins on the reduction of Cr(VI). Among these, related reductases function as intermediates and electron donors, respectively, as NADH and NADPH. The activity of chromate reductase in the cell-free extract of *Halomonas* sp. TA-04 can be significantly increased by NADH. For bacteria to reduce Cr(VI) intracellularly, they must first absorb Cr(VI) through the appropriate functional groups, then move Cr(VI) into the cell via sulfate or phosphate channels, reduce Cr(VI) intracellularly using certain pertinent substances, and finally immobilize and accumulate the reduced products of Cr(III). The plasmid can transfer Cr(III) products out of the cells, leaving a small amount of these compounds within the cell. Nonetheless, there have also been reports of the phenomena when decreased Cr(III) products precipitated and accumulated within the cell. When Cr(VI) is reduced, free radicals and reactive oxygen species (ROS) are produced, which can harm DNA under aerobic conditions. Furthermore, there is a harmful effect of decreasing Cr(III) on cells. An extracellular decrease is, therefore, preferable (Tahri Joutey et al. 2016).

According to earlier studies carried out under microaerobic circumstances, chromium was precipitated from Cr(VI)-containing waters by two diverse mechanisms, such as sulfate reduction and precipitation as sulfide. Sulfate reduction required fatty acids as organic substrates, which at first led to minimal sulfide accumulation. Based on unique morphological and biochemical traits, the chromium-resistant bacterial strain KK15—which was isolated from chromium-contaminated soil—was identified as *Pseudomonas olearans*. The strain's hexavalent chromium

resistance demonstrated its ability to withstand extremely high concentrations of K_2CrO_4 in nutrient agar medium, and an assay involving crude cell-free extracts, resting cells, and permeabilized cells showed that the hexavalent chromium reduction is primarily linked to the soluble fraction of the cell. The strain holds great potential for the bioremediation of waste containing Cr(VI) (Smock et al. 2020).

When treating industrial effluents in bulk using chemical procedures, the level is frequently not reduced enough to comply with environmental laws. Bioremediation is seen to be a superior option for pipe treatment at the end of the pipeline. There have been reports of heavy metal contamination at Kolkata's main sewage treatment plant, East Calcutta Wetlands, including chromium (VI). Thus, it's possible that the bacterial population in this area can withstand chromium (VI) and could be helpful for chromium (VI) bioremediation. A *Bacillus subtilis* strain that was obtained from this area was cultivated with chromium (VI) at concentrations ranging from $2.5 \mu g.L^{-1}$ to $7.5 \mu g.L^{-1}$. After 24 h, the residual chromium concentration in growth media decreased by 97% and 90%, respectively, from its starting values of $2.5 g.L^{-1}$ and $5 g.L^{-1}$. The greatest removal was noted at $30^\circ C$. Using MATLAB® 7.4, non-linear regression analysis revealed that the *Bacillus* strain's growth in the presence of chromium (VI) provided the best fit for the Tessier model. The *Bacillus* strain may be able to remove chromium (VI) at the end of the pipe treatment (Upadhyay et al. 2017). This suggests the potential statistical significance of chromium reduction using microbial strains.

Bravibacterium sp. CrT-11 to CrT-14 were among the bacterial strains that were isolated from tannery effluents. All of these strains were able to withstand very high concentrations of K_2CrO_4 , up to $40 mg.mL^{-1}$ on nutrient agar and $25 mg.mL^{-1}$ in nutrient broth. They also showed

resistance to several metals (Pb, Co, Cu, Mn, Zn, Ni, etc) and antibiotics like streptomycin, ampicillin, tetracycline, kanamycin, and chloramphenicol. Every strain exhibited the ability to aerobically convert Cr (VI) to Cr (III). Compared to the other strains, *Bravibacterium* sp. CrT-13 collects and reduces more Cr (VI) at all applied concentrations. Additionally, these bacterial strains absorb and degrade Cr (VI) found in industrial effluents; the addition of various metallic salts had no discernible effect on their ability to decrease Cr (VI) (Marzan et al. 2017).

Elahi et al. (2019) identified and characterized a multiple heavy-metal resistant hexavalent chromium-reducing *Microbacterium testaceum* strain B-HS2. The isolated strain is a promising option to restore Cr(VI) contaminated environments because of its strong capability for Cr(VI) reduction and multi-heavy metal tolerance. Yuliani et al. (2019) identified and studied a chromium-lead-resistant bacterium from the leather tanning sector in Malang, Indonesia (Yuliani et al. 2019). The isolated strain was identified as *Bacillus subtilis* based on its morphological and biochemical features (Elahi et al. 2019).

Microbial Fuel Cell Performance

The viability of using the sMFC for soil bioremediation has been demonstrated by numerous investigations. According to these reports, the sMFC may have an impact on the remediation of soil heavy metal (loid) by (1) modifying the soil chemistry considerably, (2) changing the amount of organic matter in the soil, and (3) changing the composition of the microbial community. The sMFC-induced alterations in the physical, chemical, and biological characteristics of soil can have a significant impact on the transportation and destiny of certain redox-sensitive heavy metal (loid) pollutants. The behavior of heavy metal (loid) s co-precipitated with mineral oxides is utilized as an illustration of the influence of the sMFC. Mineral oxides are used as a final electron acceptor by the extracellular respiring electrogenic bacteria in anaerobic environments when the anode is not present. As a result, the metal(loid) contaminants that co-precipitated with the mineral oxides dissolve and are released.

Microbial fuel cells (MFCs) have the ability to treat oxidized pollutants reductively through the use of their biocathode. In this work, we found putative Cr(VI) reducing microorganisms and showed a reduction of biological Cr(VI) in an MFC cathode. The MFC's ability to generate power and reduce Cr(VI) was continuously observed. The only external carbon source introduced to the cathode compartment was bicarbonate, while the anode compartment received acetate as substrate. Microbial activity in the cathode, which uses

electrons and protons created by the oxidation of acetate in the anode compartment, facilitated most of the Cr(VI) reduction. The minimal effect of abiotic processes and biomass degradation on Cr(VI) reduction served as evidence for this. The oxidation of acetate in the anode compartment produces protons and electrons that are used by microbial activity in the cathode to aid in Cr(VI) reduction. Abiotic processes and biomass decomposition have very little effect on the decrease of Cr(VI). The contribution of biomass decay and abiotic processes to Cr(VI) reduction was negligible. At starting Cr(VI) values less than 80 mg.L⁻¹, a relatively quick Cr(VI) decrease was seen.

Nevertheless, Cr(VI) reduction was incredibly sluggish at 80 mg.L⁻¹. The highest Cr(VI) reduction rate of 0.46 mg Cr(VI)/g VSS· h was attained, leading to a corresponding increase in current (123.4 mA.m⁻¹²) and power density (55.5 mW.m⁻²), respectively. The catholyte's supernatant contained no trace of reduced chromium, indicating total chromium elimination as Cr(OH)₃ precipitate. The cathode biomass was primarily dominated by phylotypes closely related to *Pseudomonas aeruginosa* and *Trichococcus pasteurii*, the potential Cr(VI) reducers, according to the analysis of the 16S rRNA gene-based clone library (Yuliani et al. 2019).

Hexavalent chromium can be easily reduced in the lab using a dual-chamber MFC. Anolyte is generally the raw waste effluent and the bacteria acts like the substrate. In the present study, *Bacillus australimaris* and *Bacillus albus* are used as substrates. Electrons are conducted in the anode, which travels through the external circuit and enters the cathodic chamber. SPEEK is like a proton membrane and reduces chromium, which gets deposited on the cathode. Table 3 depicts the performance of different MFCs in chromium reduction and energy generation studied in the previous years by many eminent scientists.

Present studies have reported that an MFC with 500 ml working volume was analyzed for chromium with Nifion 117 and SPEEK-treated cathode. Fig. 1 depicts the proposed mechanism of chromium reduction. When the circuit was completed, chromium concentration was reduced by generating electrons. This reduction has been depicted in Fig. 9. The initial starting concentration was kept at 81 ± 1.3 mg.L⁻¹. Nafion 117 treated MFC showed a gradual decrease in chromium concentration by 14.4% with a final value of 69 ± 2.5 mg/L post a fortnight. SPEEK-treated MFC exhibited a reduction of 51% in chromium content with a final value of 39 ± 2 mg.L⁻¹ for a similar time period. A synergistic relationship between the microbial strains and SPEEK might be the reason for better chromium reduction in the second setup. SPEEK acts as a functionalized membrane for proton

Table 3: Comparative data on previously studied MFCs that reduce chromium using microbes

Chromium initial concentration [mg.L ⁻¹]	Source	Removal efficiency (chromium) [%]	Performance [mW.m ⁻²]	Reference
100	Lysogenic broth with <i>E.coli</i>	100	1540	(Gupta et al. 2017)
120	Lab inoculum	100	1221	(Li et al., 2018)
204	Anaerobic sludge	99.5	1600	(Li et al. 2008)
200	Domestic waste	100	150	(Wang et al. 2008)
80	Mixed Anaerobic sludge	-	55.5	(Tandukar et al., 2009)
-	Algal biomass	98	207	(Pang et al. 2013)
250	Anaerobic sludge	75	970	(Zhang et al., 2012)
500	Anaerobic effluent	76	986	(Zhang et al., 2012)
10	Sludge with <i>Hansenula anomala</i>	99.5	45	(Shi et al., 2017)
30	Sludge with <i>Hansenula anomala</i>	99	45	(Shi et al., 2017)
40	Sludge with glucose culture medium	100	164	(Song et al., 2016)
5	Sewage sludge	22, 100	47	(Kim et al., 2017)
10	Sewage sludge	19, 100	47	(Kim et al., 2017)
30	Sewage sludge	16, 63	150	(Kim et al., 2017)
50	Sewage sludge	23, 53	150	(Kim et al., 2017)
20	<i>Shewanella oneidensis</i> MR-1	100	229	(Pang et al., 2013)
5	Anaerobic sludge	Below detectable limits	92	(Sophia & Sai, 2016)
10	Anaerobic sludge	Below detectable limits	75	(Sophia & Sai, 2016)
5	Cow dung with lime	100	396	(Sindhuja et al., 2018)
26	Cow dung with lime	100	397	(Sindhuja et al., 2018)
10	<i>Shewanella oneidensis</i> MR-1	100	21	(Xafenias et al. 2015)
100	Biofilm from anode	100	10	(Gangadharan, Nambi and Senthilnathan, 2015)
100	Anaerobic sludge	100	700	(Gangadharan, and Nambi, 2017)
20	Activated sludge with glucose medium	80	132	(Fei et al., 2017)
81 ± 1.3	Tannery raw effluent with Nafion 117 as PEM	14.4	-	Present study
81 ± 1.3	Tannery raw effluent with SPEEK as PEM	51	-	Present study

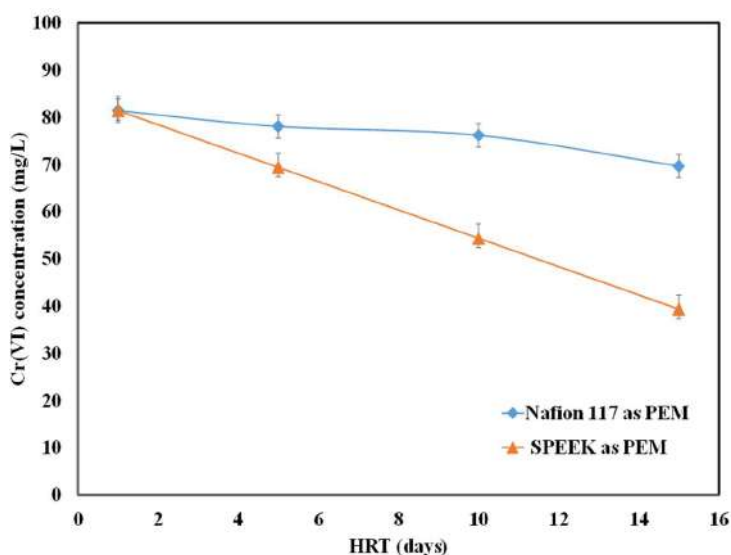


Fig. 9: Biochemical reduction of Cr (VI) in both reactors for HRT of 15 days at pH 2.

exchange and supports simple protonation at the cathode. The presence of a proton attracts the negatively charged HCrO_4^- by electrostatic attraction. Thus leading to a reduction of Cr(VI) to Cr(III). Although the Cr(VI) reduction is relatively lesser in the present study when compared to the previous studies (Table 3), the usage of low-cost SPEEK as PEM, the easy availability of microbial consortia as inoculum, and raw effluent as anolyte substrate, carbon paper as cheaper electrode make this a viable process for Cr(VI) reduction. However, the optimization studies to achieve complete Cr(VI) reduction and increased electricity production will be our priority for our forthcoming experimental studies.

LIMITATIONS

Currently, experimental and optimization studies are being carried out in the laboratory using a 10 L dual-chambered MFC in a semi-batch mode. pH plays a major role in determining the activity of microbes, reaction rates, degradation of substrate, and reduction of chromium at the respective compartments in the MFC. At lower pH, the reduction seems to be better, and as the pH increases the performance is found to decrease. The use of microbes in the anode chamber requires a neutral pH to enhance electron generation. Microbial growth and proliferation are majorly dependent on temperature. Depending on the species of microbes used, temperature requirements vary. The optimal temperature required for microbial growth is around 25–35°C. Extreme conditions could harm the microbial components of MFC. In an ideal MFC setup, energy generation seems to be double at 40°C. But a further rise of 10°C has reported a decrease in energy production by 4 times.

CONCLUSIONS

In the present study, bacteria were isolated and identified from leather tannery CETP, the isolated bacteria were identified to be *Bacillus australimaris* and *Bacillus albus* based on 16s rRNA sequencing. Nafion 117-treated MFC showed a gradual decrease in chromium concentration by 14.4%, whereas SPEEK-treated MFC exhibited a reduction of 51% for similar time periods. A synergistic relationship between the microbial strains and SPEEK might be the reason for better chromium reduction in the second setup. Chromium-contaminated soils, tannery sludge, and effluents have yielded a variety of Cr-resistant bacteria, including *Bacillus species*, *Lactobacillus strains*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, and *Bacillus methylophilicus* strains. The future study will focus on culturing of the isolated strains *Bacillus albus* and *Bacillus australimaris*. Upon obtaining successful results, the same will be scaled up to a 1000-liter pilot-scale reactor, and field studies will be conducted in

the leather tannery industries for further collaboration and commercialization.

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Waste in Flux: Temporal and Seasonal Shifts in Mysuru and Tiruchirappalli

Kavya P. Siddeshwar^{1†}, Rajeev Joseph D.², Prashanthi Devi M.¹ and Shivaraju H. P.³

¹Department of Environmental Science and Management, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

²Biomining Westart Communications India Pvt. Lt., Ekkattuthangal, Chennai, Tamil Nadu, India

³Department of Environmental Sciences, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India

†Corresponding author: Kavya P. Siddeshwar: spkavya1@gmail.com

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ABSTRACT

Efficient management of municipal solid waste is essential to fostering sustainable urban growth, especially in rapidly urbanizing countries such as India. This study conducts a comparative analysis of waste collection patterns in Mysuru and Tiruchirappalli, two major cities in India. The study specifically examines temporal shifts and seasonal variations. A study of daily waste collection data over six years indicates apparent differences among the cities. Mysuru has a greater average daily waste collection of 481.82 tons compared to Tiruchirappalli's 445.68 tons. A two-sample t-test assuming unequal variances indicates that this difference is statistically significant with a p-value of 0.0423. However, a more thorough analysis of seasonal patterns uncovers significant deviations. Mysuru exhibits elevated waste generation during the dry summer and wet winter seasons, whereas Tiruchirappalli encounters a surge in waste generation during the wet summer and wet winter periods. The seasonal variations highlight the impact of climate-related factors and consumption habits on waste generation. This study offers valuable insights into the intricacies of urban waste management in India, highlighting the importance of customized strategies that consider both temporal and seasonal fluctuations to improve the sustainability and resilience of waste management systems in rapidly developing urban areas.

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INTRODUCTION

Effective waste management is a critical concern with substantial implications for public health and environmental sustainability. Inadequate methods, such as uncontrolled waste burning and improper landfill management, can contaminate air, water, and soil, posing significant health risks, especially in urban areas where waste accumulation is more pronounced (Mangoro & Kubanza 2023, Oluwagbayide et al. 2024, de Titto & Savino 2024). The decomposition of waste also emits greenhouse gases that contribute to climate change, worsening global health and environmental issues (Somani 2023). In developing nations, these challenges are intensified by insufficient resources, infrastructure, and public awareness, resulting in unsustainable practices like unregulated disposal and burning (Mahajan 2023).

Current trends in municipal waste management reflect increasing complexity driven by urbanization and environmental concerns. There is a noticeable shift toward sustainable practices like recycling, composting, and waste-to-energy conversion. In India, rapid urbanization and population growth have led to innovative strategies such as decentralized waste management systems focusing on energy conversion, composting, and recycling. Initiatives like the "Swachh Bharat Abhiyan" have raised public awareness and improved sanitation infrastructure, promoting recycling and circular economy practices (Maalouf & Agamuthu 2023, Gupta et al. 2024).



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The COVID-19 pandemic has underscored the need for flexible waste management systems. Increased use of personal protective equipment (PPE) and changing consumer habits have significantly raised household waste levels. Medical waste generation surged dramatically during the pandemic, with increases ranging from 18% to 425%. For instance, Wuhan experienced a fivefold increase at its peak. Globally, daily medical waste escalated from 200 tons in February 2020 to over 29,000 tons by September 2020, straining existing storage and disposal systems (Richter et al. 2021). Waste composition shifted significantly during this period, with more packaging and food waste becoming prevalent. Many countries introduced new treatment policies for medical and municipal solid waste (MSW). For example, Poland saw a 6.7 percentage point rise in selective waste collection along with a 0.7-million-ton increase in recycled waste in 2020. However, the pandemic also posed challenges for policies aimed at reducing plastic use and enhancing recycling efforts (Chen et al. 2021, Urbańska et al. 2023).

Seasonal fluctuations further impact waste generation in India. Research indicates that yard and agricultural waste generation peaks during harvest seasons in February and March (Agrawal et al. 2013). A study on MSW in Guwahati revealed significant seasonal variation in organic waste and plastics, organic waste constituted 42.2% and plastics 25.2% of MSW, with variations ranging from 22% to 49% across different seasons (Singhal et al. 2022). Tourism exacerbates waste management issues in regions where infrastructure is strained by increased visitor numbers. In heritage sites like the Old City of Semarang in Indonesia, mismanaged waste detracts from historical value (Al Muqsit et al. 2024). The hotel industry faces challenges in adapting operations to minimize waste, with staff training identified as crucial (Diaz-Farina et al. 2023). Food waste from tourism activities presents substantial challenges that can be addressed through circular bio-economy principles (Mejjad et al. 2023). Regions like Chamundi Hills in Karnataka struggle with difficult terrain and overwhelming tourist numbers during peak seasons (Debnath et al. 2020). Deposit refund schemes could encourage responsible waste disposal among tourists (Shella et al. 2024).

Active community participation is vital for the sustainability of waste management systems. In Batam City, Indonesia, effective management relies on dynamic governance and community empowerment through stakeholder collaboration (Salsabila et al. 2024). In Bangladesh, youth-led initiatives demonstrate the potential for engaging younger community members while addressing gender-related challenges (Ahmed et al. 2024). Decentralized community-led solutions enable effective handling of waste even in space-limited areas (Ambastha & Aich 2019).

However, challenges persist due to insufficient knowledge among households about segregation and recycling practices, particularly in India (Minhas 2017, Singh & Ghosh 2023, Arunkumar et al. 2024).

In developed countries, the focus of waste management is primarily on incorporating circular economy principles to minimize resource depletion while fostering economic benefits like innovation and job creation. Waste-to-energy methods such as incineration are frequently utilized to manage waste sustainably (Alao et al. 2022). However, developing nations face challenges in implementing these technologies due to inadequate logistics and financial resources. Learning from successful practices in wealthier countries could enable quicker adoption of sustainable methods (Mochammad 2024). The Swachh Bharat Mission (SBM) has spurred technological advancements aimed at enhancing urban waste management in India by promoting sustainable practices and community involvement (Anand & Devi 2023). Smart segregation bins and mobile applications have been introduced, yet high costs and the need for public awareness remain barriers (Lonare 2023, Sajeew Krishna et al. 2023).

The success of SBM relies on ongoing innovation, efficient policy execution, and engaged community participation. Mysuru and Tiruchirappalli (Trichy) serve as significant cities in Southern India, each with unique strategies for handling municipal solid waste, influenced by their historical, cultural, and socio-economic backgrounds. Both cities have received recognition for their endeavors in upholding cleanliness and sustainability, but they have distinct challenges that are influenced by factors such as rising populations, urbanization, and seasonal fluctuations. The objective of this study is to provide a thorough comparative analysis of waste collection trends in the cities of Mysuru and Tiruchirappalli. The study aims to comprehend the underlying causes that influence the patterns in waste collection in both cities by analyzing the variances throughout different seasons. The results will provide a more comprehensive comprehension of the shift of waste collection dynamics over time and the possible influence of seasonal variations on urban waste management practices in Mysuru and Tiruchirappalli.

Study Area

Mysore, popularly referred to as Mysuru, is the second most densely populated city in Karnataka. It is situated at a latitude of 12°18'26" N and a longitude of 76°38'59" E. The city covers a total area of 156 km², including both the city itself and the surrounding census-designated places. It now has a population of roughly 1.26 million (Fig. 1). Mysuru

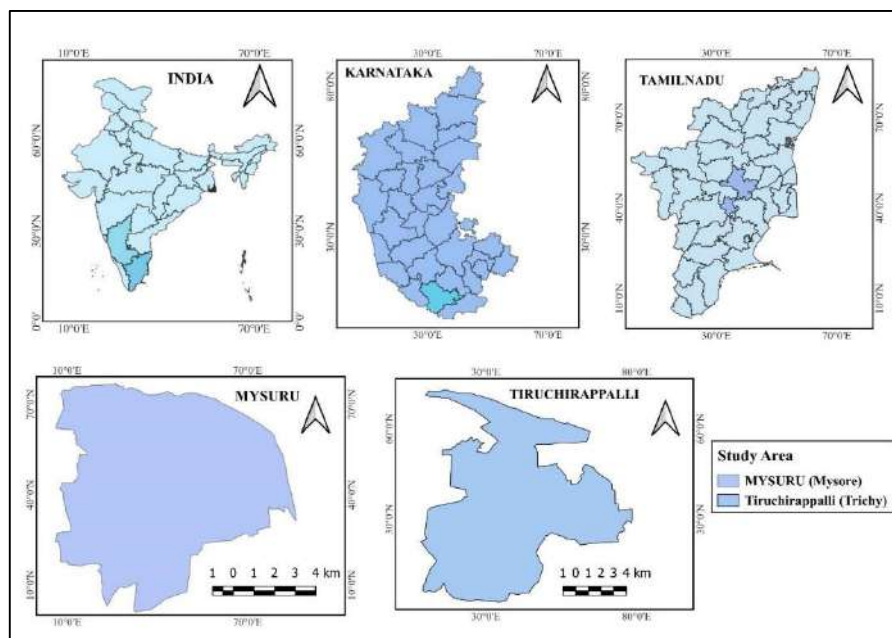


Fig. 1: Study area location map of Mysuru and Trichy cities.

is renowned for its abundant cultural heritage, earning it the titles of “City of Palaces” and “Cultural Capital of Karnataka.” As part of the Swachh Survekshan movement, this city in India is known for its exceptional cleanliness and dedicated efforts in maintaining sanitation. Mysuru, situated at the base of Chamundi Hills, is a popular destination for travelers, particularly during the annual Dasara celebration. The city serves as a nexus for exploring nearby tourist attractions and has been a focal point of urban growth within the state. Mysuru is acknowledged for its proactive waste management strategies, encompassing explicit zero-waste objectives, robust municipal governance, and engaged citizen involvement in waste segregation initiatives. The decentralized waste management system comprises various material recovery facilities (Dry Waste Collection Centres operating in numerous wards), composting units, and a door-to-door collection system, all of which facilitate effective waste processing and advance the city’s goal of achieving a zero-waste future.

Tiruchirappalli, often known as Trichy, is a significant II-tier city in Tamil Nadu and functions as the administrative center of Tiruchirappalli district. The city is located at a latitude of $10^{\circ}47'25''$ N and a longitude of $78^{\circ}42'17''$ E. It covers a total area of 167.23 square kilometers. Trichy, with a present population of over 1.2 million, ranks as the fourth-largest city and urban agglomeration in the state. Renowned for being the cleanest city in Tamil Nadu, it has also been ranked among the most liveable cities in India. Trichy is renowned for its rich historical background and cultural

importance. It is host to numerous notable structures, one of which is the famed Rockfort Temple. The city functions as a vital center for regional trade and education, asserting its importance as a prominent hub in Tamil Nadu. The city’s governance strategy has predominantly emphasized infrastructure development, including the biomining of old dumpsites. Nevertheless, the execution of waste management policies, especially in waste segregation, has been less consistent compared to Mysuru. With only one major landfill, Trichy faces challenges related to centralized disposal methods, leading to inefficiencies in waste collection, especially during peak waste generation periods.

Mysuru and Trichy generate waste from multiple sources, including residential, business, tourist activities, and industrial areas. Mysuru sees seasonal waste increases due to tourism, especially during the summer season and the Dasara festival. In contrast, Trichy generates significant amounts of household, commercial, and industrial waste, largely due to its substantial industrial activities and educational institutions. The varied sources of waste in both urban areas offer a comprehensive dataset for examining waste management strategies under differing city pressures. The selection of Mysuru and Trichy for this study is motivated by their contrasting urban characteristics. The tourism-driven waste generation in Mysuru contrasts with the industrial and educational waste profile of Trichy, facilitating comparative analysis of how diverse sources influence waste management requirements. Moreover, the Swachh Bharat Mission rankings for both cities have shown

a downward trend, emphasizing the importance of this study in assessing current waste management methods and the demand for modernization. The need for a temporal and seasonal waste analysis, which is central to this study, is further emphasized by the seasonal fluctuations in Mysuru's tourism and Trichy's industrial activities.

MATERIALS AND METHODS

The present study utilized waste collection data gathered from the Mysuru City Corporation (MCC) and the Tiruchirappalli City Corporation (TCC) to examine waste management practices in Mysuru and Trichy from 2018 to 2023. Data was collected monthly, specifically showing the average amount of waste collected every day in each month. The monthly averages served as a reliable indicator of waste collection patterns. These figures were then utilized to compute annual averages for each city, facilitating a comparison of waste collection trends across time. To comprehend the overall variations between the two cities, the year averages of waste collection were analyzed using descriptive statistical analysis and a two-sample t-test, assuming unequal variances. This approach was utilized to examine whether there were statistically significant variations in waste collection between Mysuru and Trichy.

Furthermore, a waste collection difference analysis was conducted to look further into the discrepancies between the cities throughout the six years. The collection of waste data was analyzed seasonally by dividing it into four distinct categories: dry summer (March, April, May), wet summer (June, July, August), wet winter (September, October, November), and dry winter (December, January, February). By segregating the data, it was possible to conduct

Table 1: Descriptive statistics for daily average waste collection in tons in Mysuru and Tiruchirappalli (2018-2023).

	Mysuru	Tiruchirappalli
Valid	6	6
Median	479.71	443.00
Mean	481.82	445.68
Std. Deviation	34.71	30.57
Coefficient of variation	0.072	0.069
Variance	1205.04	934.88
Range	89.30	81.50
Minimum	436.28	410.06
Maximum	525.58	491.56

a thorough analysis of how seasonal changes affect waste collection patterns in both cities. This analysis offers valuable insights into how climatic circumstances can impact waste management operations. To go beyond historical patterns, a predictive model was generated using waste collection data from 2018 to 2023. The model provides a seven-year forecast for waste collection in both cities, covering the years 2024 to 2030. The findings section presents the forecasting results, including expected waste collection figures, with a forecasting table.

RESULTS AND DISCUSSION

The descriptive statistics (Table 1) show that the average waste collection in Mysuru and Trichy is comparable, with Mysuru consistently collecting more waste than Trichy, with an average daily collection of Mysuru's Mean of 481.828 tons compared to Trichy's mean of 445.69 tons. However, Mysuru exhibits a considerably higher level of variation in its

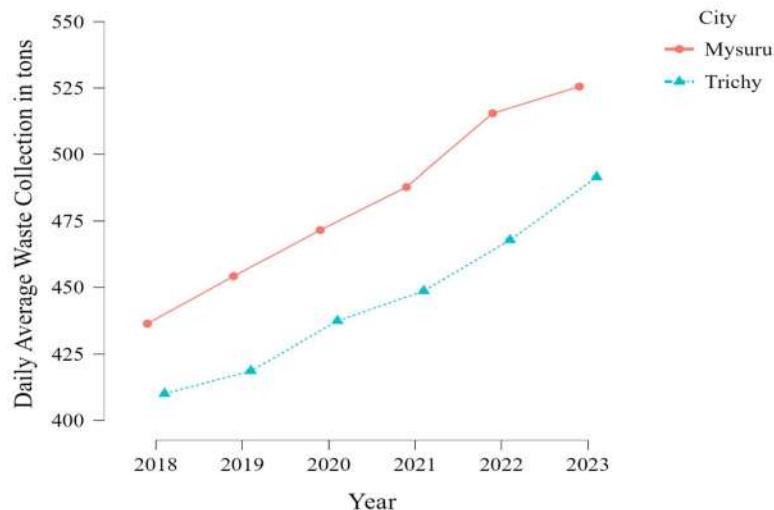


Fig. 2: Temporal Comparison of Average Daily Waste Collection in Mysuru and Trichy (2018-2023).

daily collection of waste, as evidenced by a larger standard deviation (34.71) and coefficient of variation (7.2%) in comparison to Trichy (Std. Dev = 30.57, CV = 6.9%). This indicates that Mysuru is more susceptible to variations in waste generation. In general, these statistics indicate that Mysuru faces challenges with higher and more variable waste collection, while Trichy's waste collection shows a constant performance.

The daily average waste collection trends for Mysuru and Trichy from 2018 to 2023, as depicted in Fig. 2, reveal noteworthy patterns. While both cities generally experienced an upward trend in waste generation over the six years, the COVID-19 pandemic led to a temporary dip in waste collection in 2020. This decline likely reflects the reduced economic activity and altered consumption patterns during the lockdowns. However, as societal activities resumed, waste generation rebounded, with both cities recording notable increases in daily waste collection from 2022 onwards. Mysuru's daily average collection rose from 436.28 tons in 2018 to 525.58 tons in 2023, while Trichy's increased from 410.06 tons to 491.56 tons during the same period. These trends underscore the dynamic nature of waste generation and the need for adaptable and resilient waste management strategies to effectively address long-term growth and unforeseen disruptions.

Although Mysuru and Trichy share many urban characteristics, a two-sample t-test assuming unequal variances was conducted to explore a potential difference in average waste collection (Mysuru: $M = 481.83$, Trichy: $M = 445.68$) (Table 2). A one-tailed test, based on the premise that even minor differences in city size and development

Table 2: Comparison of average daily waste collection: Mysuru vs. Trichy.

	Mysuru	Trichy
Mean	481.82	445.68
Variance	1205.07	934.78
Observations	6	6
Df	10	
t Stat	1.913	
P(T<=t) one-tail	0.0423	
P(T<=t) two-tail	0.0846	

stage might impact waste generation, revealed a statistically significant difference, $t = 1.914$, $p = .042$. This suggests that despite their similarities, Mysuru's waste management system handles a significantly larger volume. However, it is essential to acknowledge that a two-tailed test would not have reached the conventional significance level ($p = .085$). These findings highlight the complex interplay of factors influencing municipal waste generation, suggesting that city size alone may not fully explain variations in waste collection even among seemingly comparable urban environments.

The percentage difference in waste collection between Mysuru and Trichy from 2018 to 2023 reveals a dynamic trend shaped by factors beyond population size alone (Fig. 3). Mysuru consistently demonstrated higher waste collection rates throughout the observed period, maintaining a relatively stable difference between 6.39% and 8.74% in the pre-pandemic years. However, the onset of the COVID-19 pandemic (2020-2021) led to a narrowing of this gap, likely reflecting the impact of pandemic-related restrictions on economic activity and waste collection

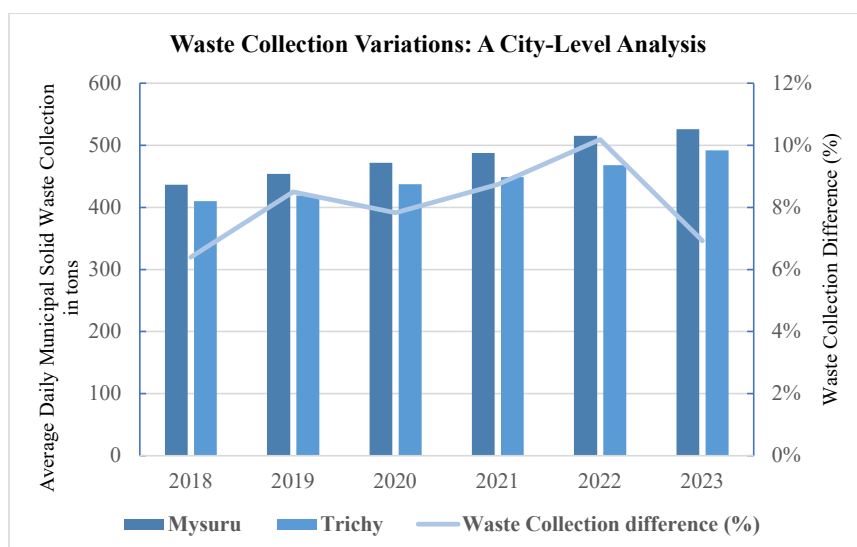


Fig. 3: Trends in Waste Collection: Mysuru vs. Trichy (2018 - 2023).

practices in both cities. Despite this temporary convergence, the difference widened again, peaking at 10.18% in 2022. This post-pandemic divergence, exceeding pre-pandemic levels, suggests a more rapid rebound or shift in the factors driving waste collection in Mysuru compared to Trichy. The observed fluctuations underscore the complex interplay of economic, social, and behavioral factors that influence municipal waste management, particularly during periods of significant disruption.

The 2023 Swachh Survekshan report outlines noteworthy variations between Mysuru and Tiruchirappalli's waste management policies. Mysuru achieved a national ranking of 23rd due to its impressive performance in several waste management aspects. It achieved a door-to-door collection rate of 97%, a source segregation rate of 70%, and a waste processing rate of 94%. Mysuru's Zero Waste Management concept has been highly effective, underpinned by effective governance and community involvement, which focuses on raising awareness and enforcing segregation norms among residents. However, Tiruchirappalli, holding the top position in Tamil Nadu, ranks 112th nationwide. It exhibits a better level of segregation at 89% but falls behind

in processing at 76%. The findings indicate that Mysuru's resource recovery approach is robust, but its efforts in segregating waste need enhancement. On the other hand, Tiruchirappalli's emphasis on waste segregation is hindered by inadequate processing infrastructure. Even with a higher segregation rate, the city's reliance on centralized waste disposal methods and insufficient processing facilities leads to inefficiencies in handling segregated waste. Both cities can benefit from exchanging best practices with each other to achieve a more equitable waste management system.

Both Mysuru and Trichy exhibit cyclical waste generation patterns as shown in (Fig. 4). While waste generation patterns likely fluctuate, analysis of waste collection in Mysuru and Trichy reveals cyclical trends, influenced by seasonal factors, local events, and external factors such as severe weather events, policy changes, and even global events like the recent pandemic. Seasonal variations significantly impacted waste collection in both Mysuru and Trichy, with higher waste generation typically observed during the dry summer (March, April & May) and wet winter (September, October & November) months. Both cities experienced lower waste

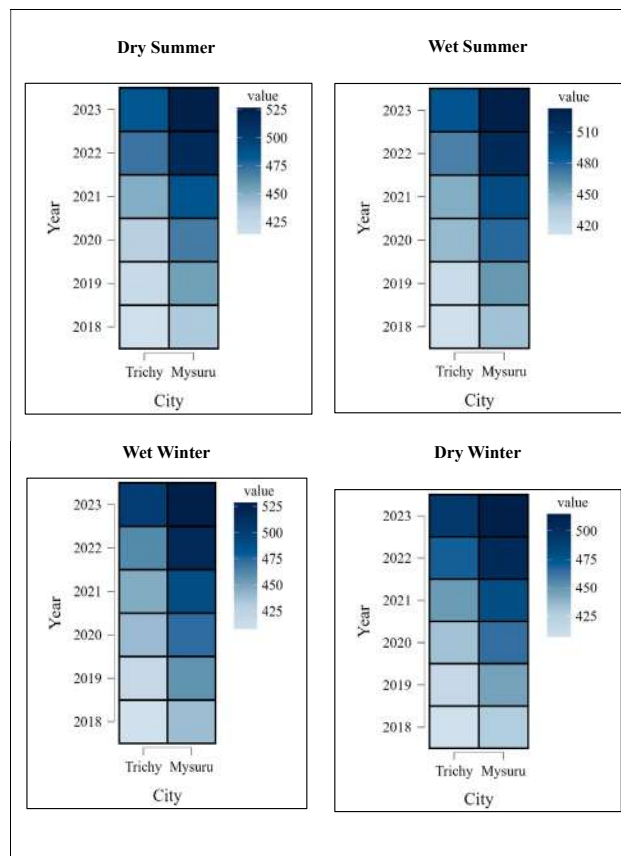


Fig. 4: Comparing Waste Seasonality: A Heatmap Analysis of Mysuru and Trichy (2018-2023).

collection during the dry winter (December to February), reflecting reduced consumption patterns.

- **Dry Summer (March, April, May):** Mysuru consistently shows higher waste collection during the Dry Summer months, with a significant rise from 437.77 tons in 2018 to 526.60 tons in 2023. Trichy follows a similar pattern but with lower volumes, starting at 413.93 tons in 2018 and increasing to 484.12 tons in 2023. This increase can be attributed to higher consumption during the summer holidays and more outdoor activities, leading to more waste generation.
- **Wet Summer (June, July, August):** In the Wet Summer, Mysuru sees a steady increase in waste collection, from 440.77 tons in 2018 to 532.58 tons in 2023. Trichy also experiences growth, with waste collection rising from 411.60 tons in 2018 to 488.82 tons in 2023. Despite both cities experiencing rainfall, the waste collection during this season is relatively high, possibly due to less severe monsoon impacts and continued urban activity.
- **Wet Winter (September, October, November):** During the Wet Winter, Trichy's waste collection is notably influenced by the northeast monsoon, which brings heavy rainfall. This season sees a substantial increase in waste collection, starting at 407.94 tons in 2018 and reaching 500.43 tons in 2023. Mysuru experiences a significant increase in waste generation during the Dasara festival, driven by heightened tourist activity and large-scale events. However, the city manages to efficiently collect this additional waste through highly effective strategies. These include increased collection frequency, deployment of dedicated waste management teams, and targeted public awareness campaigns promoting responsible waste disposal, ensuring that waste collection keeps pace with the surge in waste generation during this period. While the volume of organic waste likely remains substantial, this period sees a higher proportion of recyclable materials, such as plastic bottles, food packaging, and paper products, being used and discarded during the festival. This shift suggests a greater awareness and emphasis on recycling practices during such large-scale events. This concerted effort likely contributes to a more manageable waste stream despite the increased activity.
- **Dry Winter (December, January, February):** The Dry Winter season shows a similar trend in both cities, with waste collection steadily rising. Mysuru's collection increased from 427.33 tons in 2018 to 514.70 tons in 2023, while Trichy's collection grew from 406.77 tons in 2018 to 492.87 tons in 2023. This season typically involves year-end celebrations and New Year festivities, contributing to the higher waste collection during these months.
- The COVID-19 pandemic, particularly during 2020 and 2021, brought about a unique set of circumstances. While the generation of certain waste types, particularly single-use items, likely increased due to heightened hygiene concerns, the pandemic significantly disrupted waste collection services. This disruption is evident in the noticeably lower waste collection rates during those years. The Dry Summer months, coinciding with peak COVID-19 restrictions and lockdowns, witnessed a pronounced decline in waste collection. Additionally, the Dry Winter months also saw significantly reduced waste collection, reflecting the sustained impact of restricted urban activity and limitations on gatherings and tourism. This period reflected a combination of reduced economic activity, limitations on movement, and potential disruptions in waste management services, ultimately leading to lower overall waste collection compared to pre-pandemic and post-pandemic years. The pandemic years caused a slight dip in both cities, which quickly reversed as activities normalized in 2022, with waste collection exceeding pre-pandemic levels by 2023, highlighting the resumption of urban life.

Table 3: Projected Municipal Solid Waste Collection in Mysuru (2024-2030).

Future Trends in Mysuru's Waste Collection (2024-2030)					
Year	Daily Average Waste Collection in tons [t]	80% CI		95% CI	
		Lower	Upper	Lower	Upper
2024	543.44	535.36	551.51	531.08	555.79
2025	561.30	549.87	572.72	543.83	578.76
2026	579.16	565.17	593.14	557.76	600.55
2027	597.02	580.86	613.17	572.31	621.72
2028	614.88	596.82	632.93	587.26	642.49
2029	632.74	612.95	652.52	602.48	662.99
2030	650.60	629.23	671.96	617.92	683.27

A steady rise in Mysuru’s daily average waste collection, increasing from approximately 543 tons in 2024 to 651 tons in 2030, a substantial 20% increase. The projections, presented with both 80% and 95% confidence intervals, demonstrate a high degree of certainty in this upward trend. For example, in 2030, there is a 95% probability that the daily average waste collection will fall between 618 and 683 tons. This projected surge in waste generation presents a significant challenge, particularly with the ongoing transition demand to Greater Mysuru City Corporation, which will incorporate semi-urban and rural areas in the near future, further straining existing waste management systems. These (Table 3) forecasts show an increase in Mysuru’s daily average waste collection, demanding a proactive approach to accommodate the anticipated increase and ensure equitable and inclusive

waste management services across the expanded jurisdiction of Greater Mysuru City Corporation.

A forecast of Trichy’s daily average waste collection (Table 4) projects a consistent upward trend from approximately 508 tons in 2024 to 606 tons in 2030, representing a notable increase of about 19%. The projections are provided with both 80% and 95% confidence intervals, indicating a high level of certainty in this growth trajectory. For instance, in 2030, there is a 95% probability that the daily average waste collection will fall between 573 and 638 tons. This projected growth in Trichy’s daily waste collection underscores the escalating demands on the city’s waste management system. The consistent upward trend, reaching an anticipated 606 tons per day by 2030, necessitates

Table 4: Projected Municipal Solid Waste Collection in Trichy (2024-2030).

Future Trends in Trichy’s Waste Collection (2024-2030)					
Year	Daily Average Waste Collection in tons	80% CI		95% CI	
		Lower	Upper	Lower	Upper
2024	507.86	499.90	515.81	495.69	520.02
2025	524.16	512.90	535.41	506.95	541.36
2026	540.46	526.67	554.24	519.38	561.53
2027	556.76	540.84	572.67	532.42	581.09
2028	573.06	555.26	590.85	545.85	600.27
2029	589.36	569.87	608.85	559.55	619.16
2030	605.66	584.60	626.71	573.46	637.85

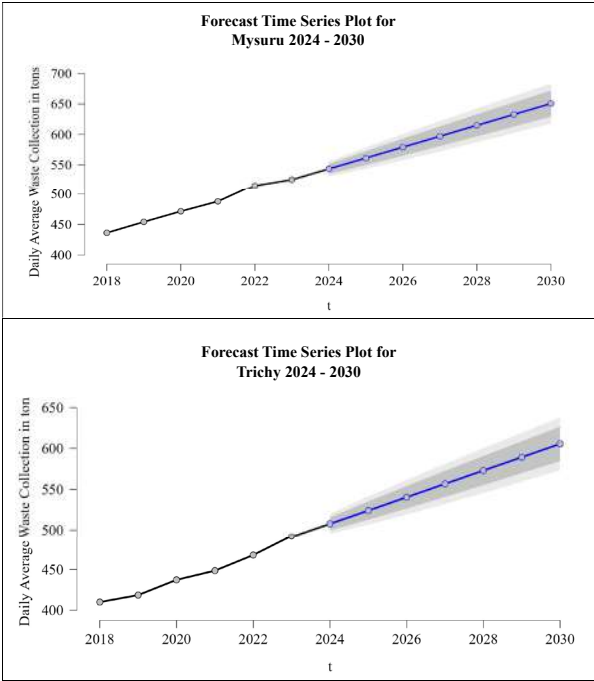


Fig. 5: Projecting Municipal Solid Waste Collection: A Comparative Forecast for Mysuru and Trichy (2024-2030).

a forward-looking approach to infrastructure development and waste management strategies to effectively handle this projected increase and avert potential challenges soon.

Autoregressive Integrated Moving Average models were employed to analyze historical daily waste collection patterns and forecast future trends in Mysuru and Trichy, as shown in Fig. 5. In both cities, ARIMA models effectively captured the observed upward trends. In Mysuru, the model revealed a statistically significant ($p = 0.002$) positive drift coefficient of 17.86 tons per year (95% CI: 10.87-24.86 tons), suggesting an average annual increase of 17.86 tons. Similarly, the model for Trichy identified a statistically significant ($p = 0.003$) positive drift coefficient of 16.30 tons per year (95% CI: 9.41-23.19 tons), indicating an average annual increase of approximately 16.30 tons in daily waste collection. Forecasts from both models, extending to 2030, project a continuation of these upward trends, underscoring the need for proactive waste management strategies in both cities. This study faced a challenge due to the absence of official government population statistics following the 2011 census. Nevertheless, to maintain accuracy in forecasting waste collection patterns for Mysuru and Trichy, the study relied on up-to-date population projections from credible sources like macro trends and World Population Review. These updated population data established a robust basis for employing the ARIMA model to predict future waste collection trends in both urban areas. Although the ARIMA model effectively captures historical waste collection patterns, it mainly relies on past data and fails to consider potential external influences that could impact future waste collection practices. In the absence of official government population projections, the study employed reliable independent estimations to provide supported predictions. The results indicate an increasing need for effective waste collection systems as populations expand, especially considering the transition from Mysuru City Corporation to the Greater Mysuru City Corporation and the rising urbanization in Trichy.

Forecasts for the average daily waste collection in Mysuru and Tiruchirappalli between 2024 to 2030 indicate substantial growth, highlighting the need for flexible waste management approaches to meet increasing requirements. Mysuru's decision-makers should explore the adoption of cutting-edge waste classification technologies, including optical sorting mechanisms and artificial intelligence-based waste recognition systems, to enhance source segregation and minimize contamination in recyclables. Furthermore, establishing anaerobic digestion plants could transform organic waste into biogas for energy production, thereby lessening the reliance on landfills. While Tiruchirappalli's focus on waste segregation is praiseworthy, the city could

enhance its waste management capabilities by increasing its processing capacity. This could be achieved through the implementation of decentralized waste processing units at the community level. Such systems would reduce transportation expenses and improve local waste management effectiveness. Additionally, the city could benefit from the installation of smart waste containers equipped with sensors. These advanced bins would enable real-time monitoring of waste levels, allowing for the optimization of collection routes and schedules, thereby boosting operational efficiency. Furthermore, both cities should launch educational programs to raise awareness among residents about the importance of sorting waste and using proper disposal techniques. These municipalities can foster a collective sense of responsibility and encourage community participation in waste management efforts by collaborating with local organizations, including community groups, NGOs, and educational institutions. Additionally, conducting routine policy reviews and data-driven analyses will ensure adaptability in responding to changing waste trends, allowing Mysuru and Tiruchirappalli to effectively address their waste-related challenges.

CONCLUSIONS

This study provides a comprehensive analysis of waste collection patterns in Mysuru and Tiruchirappalli between 2018 and 2023, focusing on the disparities in their waste management systems and the challenges faced by both cities. Although the cities share commonalities in urbanization and socio-economic aspects, they have implemented different strategies to handle the increasing amount of waste, influenced by the differing infrastructure and operational efficiencies of each city. Although Mysuru effectively manages its daily waste collection with its current infrastructure, the variations in waste collection metrics suggest the necessity for more scalable and flexible waste management solutions. Trichy demonstrated consistency in waste management, although operating with relatively fewer resources. This indicates that the effectiveness of waste management is not entirely determined by the size of the infrastructure, but rather by how efficiently available resources are utilized and managed.

An important factor to consider is the decrease in the national rankings of both Mysuru and Trichy in the SBM. Despite this decline, both cities have upheld their leading position within their respective states, highlighting their unwavering dedication to cleanliness. Nevertheless, this decrease emphasizes the necessity for ongoing innovation and enhancement in their waste management systems. Waste management is a dynamic process that requires cities to adapt to technological advancements. Adhering to obsolete methods will restrict the capacity to fulfill

growing demands and environmental standards. A crucial insight from this study is that just increasing the capacity of landfills is not a viable answer to the issue of managing urban waste sustainably. Instead, communities should prioritize the optimization of the longevity of current infrastructure by implementing new methods that reclaim materials and reduce the amount of waste disposed of. Mysuru can consider adopting modern waste processing systems to decrease its reliance on landfills and recover valuable materials from waste. These approaches decrease the amount of waste while simultaneously adhering to global sustainability requirements by reducing environmental degradation.

On the other hand, strategies that promote waste reduction at the source, such as waste segregation, recycling, and composting, have been effectively implemented in Mysuru and offer pathways that can be adapted in other urban centers like Trichy. By focusing on reducing waste generation at the source, cities can complement their operational strategies and ensure long-term sustainability in waste management. The success of waste management systems is significantly influenced by political will and governance. The efforts to process and reclaim waste resources in Mysuru have encountered obstacles such as political intervention, delays in decision-making, and administrative challenges. The scalability of waste management systems in Trichy might be hindered by governance concerns. To effectively adopt advanced waste reduction and resource recovery approaches, it is crucial to have consistent governmental and public backing. Collaborative governance, policy reforms, and community participation are essential for maintaining long-term resilience in managing the continuously growing urban waste generation. Both Mysuru and Trichy, by refining and adapting their waste management approaches, can serve as examples of how urban areas can navigate these complex challenges with a forward-thinking and sustainable approach. This study's findings underscore the temporal and seasonal fluctuations in waste collection, demonstrating their impact on waste management in Mysuru and Tiruchirappalli. Moreover, these findings can be utilized in other rapidly urbanizing cities in India and similar developing countries confronting waste management issues. By knowing the local contexts of waste generation and collection, policymakers and urban planners can develop sustainable strategies that successfully meet regional requirements and improve overall waste management efficiency.

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The Impact of Oil Pollution on the Growth of Local Plants as Bioindicators for Assessing Environmental Pollution Effects

Rana Fadhil Abbas¹, Omer Abdul Kareem Aswad², Huda Farooq Zaki¹, Estabraq Mohammed Ati¹,
Reyam Naji Ajmi^{1†} and Maryam Jasim Hasan²

¹Department of Biology Science, Mustansiriyah University, Baghdad, Iraq

²College of Science, Mustansiriyah University, Baghdad, Iraq

†Corresponding author: Reyam Naji Ajmi; isamtoffec@gmail.com; reyam80a@yahoo.com

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ABSTRACT

The study investigates the environmental impact of oil pollution in the vicinity of the Dora Refinery, with a particular focus on its effects on local flora. The research utilizes selected plant species such as *Salvia rosmarinus*, *Eucalyptus globulus*, *Ficus nitida*, *Conocarpus lancifolius*, *Nerium*, *Eucalyptus camaldulensis* and *Dodonaea viscosa* as biological indicators to assess the extent of contamination and the plants' adaptive responses to polluted conditions. Oil pollution is identified as a significant environmental issue in the Dora Refinery area, contributing to severe soil degradation and posing challenges to plant survival. The primary objective of this study is to evaluate the physiological and biochemical responses of these plant species to oil-contaminated soils, including growth performance, stress response mechanisms, and their potential role in environmental monitoring and land reclamation. The methodology involved selecting both polluted and non-polluted sites in proximity to the refinery. Soil samples were collected from these locations and used to cultivate the target plant species under controlled conditions. Growth rates were recorded, and several biomarkers associated with oil pollution exposure were analyzed. These included measurements of soil hydrocarbon content, chlorophyll concentration, antioxidant enzyme activities, and the accumulation of heavy metals and hydrocarbon pollutants in plant tissues. Results revealed that plants grown in polluted soils exhibited significantly reduced growth rates compared to those in uncontaminated environments. Notably, *Salvia rosmarinus* and *Dodonaea viscosa* demonstrated substantial accumulation of hydrocarbon pollutants, indicating their high sensitivity to contamination. In contrast, *Eucalyptus globulus* and *Conocarpus lancifolius* showed greater resilience, maintaining relatively stable growth and physiological parameters under stress. Polluted plants also exhibited clear signs of environmental stress, including decreased chlorophyll content and elevated antioxidant enzyme activity, reflecting their biochemical response to oxidative stress induced by pollutants. These findings suggest that the studied species vary in their tolerance to oil pollution, making them valuable bioindicators for environmental assessment. In conclusion, the physiological traits of these plant species significantly influence their susceptibility to oil pollutants. Their differential responses provide critical insights for environmental monitoring programs and offer promising avenues for the development of phytoremediation and land management strategies in oil-contaminated regions.

INTRODUCTION

Oil pollution is among the most dangerous contaminants found in industrial areas, such as those where refineries, like the Dora refinery area in Baghdad, are located. Oil spills and waste resulting from accidents in oil-related processes lead to the contamination of soil with harmful substances, resulting in a wide-scale negative effect on the local flora and disturbing the ecological balance. Plants that are present in the polluted soil face multiple issues, mainly the impact of these pollutants on their growth, as well as their susceptibility to environmental toxicity as well and stress caused by these pollutants. As mentioned by Zeki et al. (2019), previous studies have proved that an increased oil pollutant concentration significantly downgrades the plant growth process. A study conducted by Afolabi



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et al. (2022) reported that plants grown in oil-polluted soil usually experience stunted growth due to the fact that the amassed chemicals reduce the ability of the roots to absorb vital elements required for plant growth. Another study by Alkindi et al. (2023) has also pointed out that oil pollution causes physiological changes in plants by affecting main processes such as photosynthesis and cellular respiration, which will lead to a decrease in growth and adaptation to their surrounding area.

Plants succeeding in oil-polluted conditions are exposed to toxic agents at higher levels, including polycyclic aromatic hydrocarbons and heavy metals. The latter exerts toxic effects on the plant cells by way of interference with important cellular functions, resulting in the decline of the tissues in a plant.

In this regard, the research by Hassan et al. (2021) shows that with an increased accumulation of heavy metals such as lead and cadmium in the body of oil-polluted plants, the toxicity also increases and directly influences root and leaf growth; this effect can reduce the ability of the plants to adapt to the polluted environmental condition.

Aside from the toxicity, oil-polluted plants face severe environmental stressors as a result of the changes in the structure of the soil, such as pH and oxygen content. Studies by Khan et al. (2021) on the effects of environmental stress caused by oil pollution showed that plants demonstrate increased production of antioxidant enzymes as a protection mechanism, evidencing increased oxidative stress. This was coupled with the decline of leaf chlorophyll concentration, a factor that impairs photosynthesis and, consequently, growth. The oil pollution impacts the individual plants and causes a severe shift in the biodiversity of the entire ecosystem. In the case of long-term oil pollution, as that around the Dora Refinery, different plant species are affected based on their various sensitivities to the pollutants. A study by Murphy et al. (2023) confirmed that oil pollution reduces plant diversity; those species that cannot adapt to pollution vanish gradually, while only the strongest species remain. This consequently causes ecological imbalance and affects all levels of the food chain in the polluted region.

According to findings by Al-Ibady et al. (2025), the problem of oil pollution is one of the most important environmental hazards in Iraq, especially in areas that are adjacent to oil refineries, such as the Dora Refinery. There is limited research on the impact of oil pollution on plants locally; however, a few works have considered the topic of interest and the environmental impact caused by the operation of the oil industry.

Assessment of the impact of oil pollution on soil and flora of the Dora area (Smith & Wood 2022) was carried

out at the University of Baghdad, focusing on the impact of oil pollution on the soil and plants in the Dora Refinery vicinity area. These results were confirmed when soil and plants around the polluted area were tested and were found to have higher levels of petroleum hydrocarbons and heavy metals, which greatly reduced plant growth. The findings also suggested that the eucalyptus tree was relatively resistant to this form of pollution compared to many other species. The Iraqi Ministry of Environment carried out research, "Compare research on oil refineries' effects on the environment in Baghdad's ecosystem (Ibrahim et al. 2025) to assess the impact of oil operations on the environment in Baghdad, including the Dora refinery. The findings were that oil activities contributed negatively to soil and water quality, being the major influencing factors on vegetation degradation around the refinery. The study recommended stressing the use of resilient native plants as a means of environmental monitoring.

Therefore, the present study focused on the impact of oil pollution on native plant species growth in the region of Dora refinery, with its use as biological indicators in order to assess the repercussions of pollution. Moreover, it was going to be of importance in determining how well these species adapt to unfavorable conditions, which develops strategies for the restoration and management of areas that have been affected by contamination.

MATERIALS AND METHODS

To ensure a comprehensive study of the impact of oil pollution on the growth of native plants in the Dora Refinery area, a precise scientific methodology will be followed that includes several main steps covering soil analysis, testing plants in polluted and unpolluted conditions, and analyzing biological and chemical data to implement the experiment and obtain results that help in evaluating the impact of oil pollution include:

Selection of study sites: Contaminated sites near the Dora Refinery were selected as pilot sites, in addition to other uncontaminated sites used as a reference for comparison (control sites), based on their proximity to the refinery and direct exposure to oil spills or air and environmental pollution. The type of soil and its physical and chemical properties were taken into account at each site to ensure that the results were based primarily on the impact of oil pollution (Adeel et al. 2022).

Collection of soil samples: Samples were collected at a depth of 0-30 cm from the soil surface with three replicates for each selected site. Samples were stored in sterile containers for transport to the laboratory for analysis (Baig & Hameed 2023).

Soil analysis: A comprehensive analysis of soil chemical elements such as Total Petroleum Hydrocarbons (TPH) was conducted to determine the extent of oil contamination with organic compounds to assess toxicity, along with measuring pH and Cation Exchange Capacity (CEC) to determine soil fertility and heavy metals (Pb, Cd, Hg) (Fadhel et al. 2019).

Selection of Plant Species: Native plants found in the area selected by abundance and would have the possibility to stand pollution because it is the most common, and include the following: *Salvia rosmarinus*, *Eucalyptus globules*, *Ficus nitida*, *Conocarpus lancifolius*, *Nerium*, *Eucalyptus camaldulensis*, and *Dodonaea viscosa*.

Physiological growth measurements: In order to gauge the photosynthetic capacity of the plants, chlorophyll content was measured using a chlorophyll meter; meanwhile, drying of plants was performed to measure the dry weight of each plant as an overall growth rate indicator (Ati et al. 2022).

Environmental stress and toxicity analysis: Environmental stress and toxicity were assessed by quantifying the activities of key antioxidant enzymes, namely catalase and superoxide dismutase, to determine the extent of oxidative damage potentially induced by oil pollution. Furthermore, inter-species comparisons were conducted to evaluate plant tolerance, using the studied species as a biological indicator for biodiversity shifts by observing differential resistance levels and growth patterns (Khishamuddin et al. 2022).

Data analysis and statistics: Application of ANOVA tests to test for the differences between plants in the contaminated and uncontaminated soil. Determination of levels of significance: $P < 0.05$, Standard deviation calculation, and the percent relative change in growth, environmental stress, and toxicity between the two groups according to Rajpar et al. (2023).

RESULTS

The mean TPH in the contaminated soil surrounding the Daura refinery was around 8000 mg.kg^{-1} , while the TPH concentration in the uncontaminated soil was about 50 mg.kg^{-1} , with fluctuation between samples, where the standard deviation was about 1200 mg.kg^{-1} , indicating heavy oil pollution in the studied area. In addition, the concentrations of VOC and BTEX in the contaminated samples were higher, reaching up to 300 mg.L^{-1} and with about 50 mg.L^{-1} of standard deviation. The pH range value was between 5.8 and 6.2 for the contaminated soil, to be considered slightly acidic, while for the uncontaminated, the pH range was between 7.0 and 7.2, approximately neutral, with a standard deviation for the contaminated samples of 0.15. The CEC in the contaminated soil was 15 mEq.100g^{-1} , and in the uncontaminated soil,

25 mEq.100g^{-1} , with a standard deviation of $1.8 \text{ mEq.100g}^{-1}$. The contents of toxic heavy metals in the contaminated soil were in high proportion, with the average lead Pb-250 mg.kg^{-1} , cadmium Cd-8 mg.kg^{-1} , and mercury Hg-1.5 mg.kg^{-1} , in comparison with the uncontaminated soil: Pb-15 mg.kg^{-1} , Cd-0.5 mg.kg^{-1} , Hg-0.1 mg.kg^{-1} , while standard deviation of the contaminated samples was about: Pb-20 mg.kg^{-1} , Cd-0.9 mg.kg^{-1} , Hg-0.2 mg.kg^{-1} . In the polluted soil, the organic matter content is about 1.2%, while the organic matter of the unpolluted soil is approximately 2.5%, with a standard deviation of 0.3%. Table 1 shows the soil parameters in the Dora refinery area.

It is seen from the results in Table 2 that oil pollution had an effect on the development of different plant species in contaminated soil compared to the uncontaminated one. It is observed that the mean height of the plants was reduced in contaminated soil, where in contaminated soil, the height of *Salvia rosmarinus* reached about $30 \pm 2.5 \text{ cm}$ as compared to $55 \pm 3.5 \text{ cm}$ in uncontaminated soil. About *Eucalyptus globulus*, for example, it attained $32 \pm 3.0 \text{ cm}$ in the contaminated soil against $60 \pm 4.0 \text{ cm}$ in the uncontaminated soil. Also, the area of the leaf diminished since *Ficus nitida* reached $18 \pm 1.8 \text{ cm}^2$ in the contaminated soil as opposed to $30 \pm 2.0 \text{ cm}^2$ in the uncontaminated one. The number of branches was also few. The mean number of branches of *Conocarpus lancifolius* was 6 ± 0.5 branches on contaminated soil compared to 11 ± 0.8 branches on uncontaminated soil.

According to Table 3, results indicated that oil-contaminated soils decreased chlorophyll and ascorbic acid contents in the studied plant species. The chlorophyll content of plants grown on contaminated soils was generally highly reduced compared with those grown on uncontaminated soils. As an example, the chlorophyll content of *Salvia rosmarinus* dropped from 36 SPAD units on uncontaminated soils to 21 SPAD units on contaminated soils. There was also a reduction in the chlorophyll content of *Eucalyptus globulus* from 42 SPAD units in uncontaminated soils to 23 SPAD units in contaminated ones, while that of *Nerium* decreased from 32 to 18 SPAD units. However, on the level of ascorbic acid, a great reduction was evidenced in the plants grown in contaminated soils; for example, the level of ascorbic acid in *Eucalyptus globulus* reduced from 13.0 mg.g^{-1} grown on uncontaminated soil to 7.0 mg.g^{-1} on contaminated soil. Similarly, *Ficus nitida* recorded a reduction in ascorbic acid level from 11.0 mg.g^{-1} on uncontaminated soil to 5.8 mg.g^{-1} grown on contaminated soil.

The results indicated that the fresh and dry weights of the plants in Table 4 were highly affected by oil contamination, reflecting deterioration in growth and the productive

Table 1: The oil-polluted soil parameters in the Dora refinery area.

Parameter	Average in Polluted Soil	Average in Unpolluted Soil	Standard Deviation
Total Petroleum Hydrocarbons (TPH)	8000 mg.kg ⁻¹	50 mg.kg ⁻¹	1200 mg.kg ⁻¹
Volatile Organic Compounds (BTEX)	300 mg.L ⁻¹	-	50 mg.L ⁻¹
pH Level	5.8-6.2	7.0-7.2	0.15
Cation Exchange Capacity (CEC)	15 meq.100g ⁻¹	25 meq.100g ⁻¹	1.8 meq.100g ⁻¹
Lead (Pb)	250 mg.kg ⁻¹	15 mg.kg ⁻¹	20 mg.kg ⁻¹
Cadmium (Cd)	8 mg.kg ⁻¹	0.5 mg.kg ⁻¹	0.9 mg.kg ⁻¹
Mercury (Hg)	1.5 mg.kg ⁻¹	0.1 mg.kg ⁻¹	0.2 mg.kg ⁻¹
Organic Matter Content	1.2%	2.5%	0.3%

Table 2: The analyzed parameters in all the plant species in the polluted and unpolluted soils.

Plants Species	Height [cm]	Leaf Area [cm ²]	Number of Branches
<i>Salvia rosmarinus</i>	30 ± 2.5 (Polluted)	20 ± 2.0 (Polluted)	6 ± 0.5 (Polluted)
	55 ± 3.5 (Unpolluted)	35 ± 2.5 (Unpolluted)	12 ± 0.8 (Unpolluted)
<i>Eucalyptus globulus</i>	32 ± 3.0 (Polluted)	22 ± 2.3 (Polluted)	7 ± 0.6 (Polluted)
	60 ± 4.0 (Unpolluted)	40 ± 3.0 (Unpolluted)	15 ± 1.0 (Unpolluted)
<i>Ficus nitida</i>	28 ± 2.8 (Polluted)	18 ± 1.8 (Polluted)	5 ± 0.7 (Polluted)
	52 ± 3.2 (Unpolluted)	30 ± 2.0 (Unpolluted)	10 ± 0.9 (Unpolluted)
<i>Conocarpus lancifolius</i>	29 ± 3.1 (Polluted)	19 ± 2.1 (Polluted)	6 ± 0.5 (Polluted)
	58 ± 3.8 (Unpolluted)	34 ± 2.7 (Unpolluted)	11 ± 0.8 (Unpolluted)
<i>Nerium oleander</i>	27 ± 2.4 (Polluted)	16 ± 1.9 (Polluted)	4 ± 0.4 (Polluted)
	50 ± 3.0 (Unpolluted)	29 ± 2.4 (Unpolluted)	9 ± 0.7 (Unpolluted)
<i>Eucalyptus camaldulensis camaldulensis</i>	31 ± 2.7 (Polluted)	21 ± 2.2 (Polluted)	7 ± 0.6 (Polluted)
	54 ± 3.6 (Unpolluted)	36 ± 2.8 (Unpolluted)	13 ± 0.9 (Unpolluted)
<i>Dodonaea viscosa</i>	26 ± 2.0 (Polluted)	15 ± 1.5 (Polluted)	5 ± 0.5 (Polluted)
	48 ± 3.2 (Unpolluted)	28 ± 2.3 (Unpolluted)	8 ± 0.6 (Unpolluted)

Table 3: Chlorophyll and ascorbic acid content measurement results.

Plant Species	Chlorophyll Content (SPAD Units)	Ascorbic Acid Content [mg.g ⁻¹]
<i>Salvia Rosmarinus</i>	21 ± 1.5 (Polluted)	6.5 ± 0.5 (Polluted)
	36 ± 2.0 (Unpolluted)	12.5 ± 0.7 (Unpolluted)
<i>Eucalyptus globulus</i>	23 ± 2.0 (Polluted)	7.0 ± 0.4 (Polluted)
	42 ± 1.8 (Unpolluted)	13.0 ± 0.6 (Unpolluted)
<i>Ficus nitida</i>	19 ± 1.2 (Polluted)	5.8 ± 0.3 (Polluted)
	31 ± 1.5 (Unpolluted)	11.0 ± 0.5 (Unpolluted)
<i>Conocarpus lancifolius</i>	20 ± 1.8 (Polluted)	6.0 ± 0.5 (Polluted)
	38 ± 2.1 (Unpolluted)	11.5 ± 0.4 (Unpolluted)
<i>Nerium oleander</i>	18 ± 1.4 (Polluted)	5.5 ± 0.2 (Polluted)
	32 ± 1.9 (Unpolluted)	10.0 ± 0.3 (Unpolluted)
<i>Eucalyptus camaldulensis</i>	22 ± 1.7 (Polluted)	6.8 ± 0.5 (Polluted)
	40 ± 2.0 (Unpolluted)	12.0 ± 0.6 (Unpolluted)
<i>Dodonaea viscosa</i>	20 ± 1.5 (Polluted)	6.2 ± 0.4 (Polluted)
	34 ± 2.3 (Unpolluted)	11.0 ± 0.5 (Unpolluted)

capacity of plants upon exposure to contamination. For instance, the fresh weight of *Salvia rosmarinus* plants was approximately 70 g in soil with no contamination, but was reduced to 40 g when tested against contaminated soil. *Eucalyptus globulus* also suffered a similar depression trend in fresh weight, from 80 g for the uncontaminated soil to 45 g in the case of contaminated soil. A similar depressive effect is reported by other species, such as *Nerium* and *Dodonaea viscosa*, indicating that contaminated soil harms all the studied species. This was also reflected in the drastic decline in the dry weights of plants grown on contaminated soil, with *Eucalyptus camaldulensis* falling from 26 g in uncontaminated soil to 14 g in contaminated soil. The respective dry weights of *Dodonaea viscosa* were recorded from 21 g in uncontaminated soil to 10 g in contaminated soil.

Table 5 results showed significant activities of antioxidant enzymes and petroleum hydrocarbon accumulation in the polluted plants. Values ranged between 5.0 to 10.0 units.g⁻¹ dry weight in the polluted plants. This increase in catalase activity is used as an indication of the plant's response to oxidative stress brought about by pollution. In the case of superoxide

dismutase activity, the range of this enzyme was between 3.0 and 8.0 units.g⁻¹ dry weight in contaminated plants, indicating high levels of oxidative stress in plants when compared to uncontaminated ones. The value of TPHAC in the case of total petroleum hydrocarbon accumulation reached 500 to 1500 mg.kg⁻¹ dry weight in contaminated plants, reflecting the intensity of the pollution effect.

Table 6 is an effective analytical tool with which one can understand the effects of pollution on various plant species by measuring some biomarkers. In *Salvia rosmarinus* and *Nerium*, chlorophyll contents showed high values (in mg.g⁻¹), which means both species contain high chlorophyll content, indicating good photosynthetic activity and their ability to tolerate higher levels of pollution. On the other hand, *Ficus nitida* presented a significant decrease in chlorophyll content, proving it is of poor photosynthetic capacity and highly affected by pollution, while other plants underwent a decrease in chlorophyll level to a medium range, reflecting variable pollution effects. Catalase enzyme activities (U.g⁻¹ dry weight) in *Salvia rosmarinus* and *Nerium* also presented high activities, proving their response to pollution-

Table 4: Dry and fresh weight measurement results in all plant species.

Plant Species	Fresh Weight [g]	Dry Weight [g]
<i>Salvia rosmarinus</i>	40 ± 3.0 (Polluted)	12 ± 1.0 (Polluted)
	70 ± 4.5 (Unpolluted)	25 ± 2.0 (Unpolluted)
<i>Eucalyptus globulus</i>	45 ± 4.2 (Polluted)	13 ± 1.2 (Polluted)
	80 ± 5.0 (Unpolluted)	30 ± 2.5 (Unpolluted)
<i>Ficus nitida</i>	35 ± 3.5 (Polluted)	10 ± 0.9 (Polluted)
	65 ± 4.0 (Unpolluted)	20 ± 1.5 (Unpolluted)
<i>Conocarpus lancifolius</i>	40 ± 4.0 (Polluted)	11 ± 1.1 (Polluted)
	75 ± 4.5 (Unpolluted)	22 ± 1.8 (Unpolluted)
<i>Nerium oleander</i>	30 ± 3.2 (Polluted)	9 ± 0.8 (Polluted)
	60 ± 3.8 (Unpolluted)	18 ± 1.4 (Unpolluted)
<i>Eucalyptus camaldulensis</i>	42 ± 3.5 (Polluted)	14 ± 1.0 (Polluted)
	78 ± 4.0 (Unpolluted)	26 ± 1.9 (Unpolluted)
<i>Dodonaea viscosa</i>	32 ± 2.8 (Polluted)	10 ± 0.9 (Polluted)
	62 ± 3.5 (Unpolluted)	21 ± 1.6 (Unpolluted)

Table 5: The environmental stress and toxicity analysis.

Item	Value	Interpretation
Catalase Activity [U.g ⁻¹ dry weight]	5.0 - 10.0 (in contaminated plants)	Increased activity due to oxidative stress
Superoxide Dismutase Activity [U.g ⁻¹ dry weight]	3.0 - 8.0 (in contaminated plants)	Another response to oxidative stress
Total Petroleum Hydrocarbons Accumulation [mg.kg ⁻¹ dry weight]	500 - 1500 (in contaminated plants)	High accumulation indicates the effect of pollution on plants
Statistical Analysis (p-value)	Less than 0.05	Statistical significance of differences between groups

induced effective stress levels. The high activities in *Salvia rosmarinus* and *Nerium* showed a good ability to deal with free radicals resulting from pollution. Low activities were detected in *Ficus nitida*, reflecting weak resistance against environmental stress.

The total petroleum hydrocarbons in mg.kg^{-1} , high values were indicated by *Ficus nitida*. This must mean that this is more affected by pollution compared to other species. *Salvia rosmarinus* and *Nerium* had low values, indicating their resistance to pollution. While *Salvia rosmarinus* demonstrated a significant increase in growth rate by +20%, indicating its responsiveness under negative environmental conditions, *Ficus nitida* showed a drastic decline by -50%, indicating the intensity of the impact of pollution on it. Other species reflected moderate changes in growth, indicating variable effects of pollution, and these species could act as efficient bioindicators in the monitoring of pollution due to their capacity for supporting the high availability of chlorophyll and enzyme activity. On the other hand, the model represented by *Ficus nitida* indicated the negative impacts of pollution by reducing the availability and growth. All these results contribute to a better understanding of the importance of the appropriate selection of plant species for pollution monitoring and environmental impact studies.

DISCUSSION

Results indicated that oil pollution in the soil of the surrounding area of the Daura refinery causes an increased buildup of petroleum hydrocarbons that inhibit plant growth,

hence increased accumulation of toxic organic compounds in the soil. It agreed with a study result by Ajmi et al. (2018) that the accumulation of heavy organic compounds has a similar effect to have on the surrounding environment. The increase of heavy organic compounds such as aromatic hydrocarbons and benzene increases the levels of toxicity in soil; this places extra stress on plants and results in reduced vital activities, which is also supported by those who explained how these compounds can impede root activity and limit plant growth. Moreover, the decrease in pH of the contaminated soil indicates changes in soil properties under oil pollution, which interfere negatively with the plant's nutrient uptake processes. According to Yousaf & Umer (2022), such changes in soil can lead to physiological disorders that may be harmful to the health and development of the plants. The decrease in cation exchange capacity in polluted soils indicates the loss of fertility, as the soil's capacity to retain the necessary nutrients will be limited. This again confirms that oil pollution reduces the effectiveness of soil in supporting plant growth and ecological balance, according to Saeed et al. (2024).

The results obtained indicate that oil pollution negatively affects plant growth, limiting their development and hindering their vital functions in polluted soil. These effects are supported by previous research, where Bhatia et al. (2021) indicated that oil pollution leads to changes in the biochemical composition of native plants, affecting their ability to adapt and grow and reducing their efficiency in restoring the ecosystem. Also, the study conducted by Ati et al. (2023) showed that soil pollution with oil pollutants

Table 6: The bioindicators for assessing the impact of oil pollution on different plant species.

Bioindicator	<i>Salvia rosmarinus</i>	<i>Eucalyptus globulus</i>	<i>Ficus nitida</i>	<i>Conocarpus lancifolius</i>	<i>Nerium</i>	<i>Eucalyptus camaldulensis</i>	<i>Dodonaea viscosa</i>
Chlorophyll Content [mg.g^{-1}]	High (4.5-5.5)	Moderate (3.0-4.0)	Low (1.0-1.5)	Moderate (2.5-3.5)	High (4.0-5.0)	Moderate (2.0-3.0)	Moderate (2.5-3.5)
Catalase Activity [U.g^{-1} dry Weight]	High (10.0-15.0)	Moderate (5.0-10.0)	Low (1.0-3.0)	Moderate (4.0-6.0)	High (8.0-12.0)	Moderate (5.0-8.0)	Moderate (5.0-7.0)
Superoxide Dismutase Activity [U.g^{-1} dry weight]	High (5.0-8.0)	Moderate (3.0-5.0)	Low (1.0-2.0)	Moderate (3.5-5.5)	High (4.5-7.0)	Moderate (3.0-4.5)	Moderate (4.0-5.0)
Total Petroleum Hydrocarbons [mg.kg^{-1}]	Low (200-500)	Moderate (300-700)	High (800-1200)	Moderate (400-600)	Low (250-500)	Moderate (350-650)	Moderate (400-700)
Growth Rate [% change]	+20%	+10%	- 50%	+5%	+15%	+10%	+5%

and heavy metals affects the environmental properties of leaves and surrounding soil, leading to a deterioration in the natural growth of plants and increasing environmental stress. Heavy organic compounds such as aromatic hydrocarbons negatively affect plants, increasing soil toxicity and impairing their ability to retain nutrients. This effect is demonstrated by the decrease in leaf area and number of branches in plants grown in contaminated soil, as shown by the decrease in the number of branches of *Nerium oleander* to 4 ± 0.4 branches in contaminated soil compared to 9 ± 0.7 branches in uncontaminated soil. Thus, these results highlight the need to take measures to rehabilitate contaminated areas, especially since some plants such as *Eucalyptus globulus* and *Conocarpus lancifolius* have shown a degree of tolerance to pollution, making them good candidates for environmental rehabilitation in areas affected by oil pollution, therefore, show that oil contamination of soil exerts adverse effects on the photosynthetic performances of plants. Two views are brought forward by Hasan et al. (2024) and Ati et al. (2023), respectively, on how oil contamination can interfere with photosynthesis and limit the plant's capability to produce chlorophyll, which is important for health. Ascorbic acid is an important antioxidant that plays a major role in protecting plants against various environmental stresses. Its reduced level in plants that grow in polluted soil indicates that these plants are under some sort of environmental stress due to their impaired ability to synthesize antioxidant compounds. This was in agreement with the work of Rahmatullah and Ajmi et al. (2022) that pollution reduces the plant's potential for responding against the oxidative stress mediated by the pollutant. The lessened content of chlorophyll and ascorbic acid expresses that pollution has a negative effect on physiological performance and the ability of the plant to respond to adverse environments, supporting the hypothesis of Ali and Iqbal (2023) that soil pollution with oil negatively affects the physiological performance of the plants in terms of growth and response to the adverse environments.

The ability of plants to absorb water and nutrients necessary for their growth, as none of the plant species tested showed resistance to these levels of pollution. This is corroborated by the findings of Aswad et al. (2025) and Resmi et al. (2023), who made an inference on the negative effect of oil pollution on soil and plants' structural properties and hindering plants from developing dry matter that was necessary for plant development. This has caused deleterious effects, leading to less productive growth of the plants grown in the polluted soil. In comparison with those grown in the polluted one, a significant increase in fresh and dry weights was recorded for the plants grown in the unpolluted soil.

Also, according to Yousaf & Umer (2022), oil pollution helps by decreasing the plants' potential for environmental

stress tolerance, thus rapidly reducing overall plant growth and failing to achieve good productivity in the soil. It also seemed that some species, such as *Ficus nitida* and *Nerium*, might be more sensitive to pollution than others, which may indicate a true difference in the sensitivity of plants to pollution. This agrees with the observation by Zeki et al. (2019) concerning the variations of plant remediation mechanisms among different species while dealing with various pollutants like mercury. The agreement here is that variation exists in the ability of plants to tolerate and deal with pollution in many different ways.

While polluted plants have a highly active catalase enzyme, it tries to combat the oxidative stress the plant faces due to pollution, since catalase works on the decomposition of hydrogen peroxide that has built up due to the oil pollutants. Farooq & Murtaza (2022) affirm that soil microbial communities provide a probability for improvement in the plant repair mechanism in petroleum hydrocarbon-contaminated soil and point toward the environmental interactions regarding the mitigation of pollution impacts. The high activity of the superoxide dismutase enzyme in the polluted plants also suggests that these plants are under immense oxidative stress—as proved by Hassan et al. (2021) on the role of ascorbic acid in mitigating oil-pollution-induced negative effects on plant growth.

Such high levels of accumulation of total petroleum hydrocarbons in polluted plants reflect the capability of plants to absorb such toxic elements from the general environment. On the other hand, such big aggregations may cause serious obstacles for them to grow and develop due to toxic substances' accumulation in their tissue and influencing their physiological functions, as explained by Hasan et al. (2024) in their work regarding bio-pollution indicators in the Qayyarah refinery area in Iraq, where oil pollution has negative effects on the general health and growth of plants, this indicates that *Salvia rosmarinus* and *Nerium* exhibit a clear tolerance to pollution by maintaining high levels of chlorophyll, catalase, and superoxide dismutase activity. This is in agreement with previous studies indicating the abilities of such species to adapt to polluted environments. Farooq & Murtaza (2022) have also supported the role of soil microbial communities in enhancing the environmental restoration process and thereby enhancing the survivability and growth potential of these plants under polluted conditions.

For comparison, some previous studies on the impact of oil pollution on soil and plants are presented, such as TPH Concentration shows that oil pollution results in the accumulation of hydrocarbons in the soil in a similar manner to Arora et al. (2022). This study reported high TPH concentrations in polluted areas around fuel stations and refineries and their adverse effects on plant growth.

Volatile Organic Compounds (VOC) and BTEX: Some studies have evidence that oil contamination is one of the main causes of the increase in volatile organic compounds, including BTEX, in soil (Mouazen et al. 2021). Significant increases in BTEX soil concentrations were observed as a result of oil pollution in areas next to refineries.

Oil pollution has been linked to a decrease in soil pH, as previously observed in studies where contaminated soils exhibited lower pH levels compared to adjacent, unpolluted areas (Cai et al., 2020). The present findings support this, highlighting that the reduced pH results from the slow decomposition of hydrocarbons and a decline in the soil's cation exchange capacity (CEC). This reduction in CEC impairs the soil's ability to retain and absorb essential nutrients. Supporting this, Zhang et al. (2023) reported that oil pollution significantly diminishes the soil's ion absorption capability.

Multiple studies have confirmed that oil pollution leads to the buildup of heavy metals such as lead (Pb), cadmium (Cd), and mercury (Hg) in the soil. For example, Liu et al. (2022) found a significant increase in the concentration of these metals in oil-contaminated soils. This contamination also affects plant health. Pundir et al. (2020) reported reduced plant growth and visible deterioration in leaf quality due to oil pollution. Similarly, Sharma et al. (2021) observed a decline in chlorophyll content and a rise in antioxidant activity in plants exposed to polluted soil. These findings collectively suggest that oil pollution negatively impacts both soil quality and plant physiological responses.

The effect of oil pollution on fresh and dry weight is similar to what was mentioned in the study by Bajpai et al. (2022). This study confirmed that oil pollution leads to a reduction in fresh and dry weight of plants due to negative effects on growth and an increase in the activity of antioxidant enzymes in polluted plants. Zhao et al. (2023) showed that there was increased enzyme activity due to oxidative stress in the plants growing in oil-polluted soil.

On the contrary, *Ficus nitida* recorded a severe negative response to pollution, thus confirming what was highlighted in studies on the effect of oil pollution on photosynthesis and growth in plants. In addition, Dias & Taveres (2023) confirmed environmental factors like ascorbic acid are essential in mitigating the effects of pollution. These further support the fact that less tolerant plants, like *Ficus nitida*, may act as models to define the adverse effects of pollution and hence give importance to the appropriate selection of species in environmental studies and monitoring of pollution. It was evident when Hasan et al. (2024) analyzed the pollution indicators around polluted areas such as the Qayyarah refinery in Iraq.

CONCLUSIONS

1. TPH concentration in the contaminated soil of the Daura refinery was relatively high, about 8000 mg.kg⁻¹, showing high oil contamination.
2. While the uncontaminated soil showed relatively low levels of contamination at about 50 mg.kg⁻¹, on the other hand, the contaminated one showed high levels of volatile organic compounds and high levels of heavy non-volatile compounds, such as BTEX, that elevated the toxicity levels while inhibiting plant growth.
3. The pH value in the contaminated soil was slightly acidic, hence delaying the uptake of nutrients by plants since the pH ranged from 5.8 to 6.2, while CEC was highly reduced in the contaminated soil when compared to the uncontaminated one, which is reflective of decreased soil fertility.
4. The main contaminants in soil are heavy metals Pb, Cd, and Hg, which show very dangerous levels in polluted soil against plant health and the surrounding environment. The organic matter content of the contaminated soil decreased, indicating a decline in biological activity and soil fertility.
5. Oil pollution resulted in a reduction in plant height, leaf area, and the number of branches. This means that pollution reduces both the growth and development of the plants. In the plants that grew on contaminated soil, there was a considerable decline in the content of chlorophyll and ascorbic acid, reflecting an interference with the plant's photosynthesis process and with some plant metabolic processes to resist the environment.
6. Pollution affected weight gain; it significantly reduced the fresh and dry weights of plants, tending to disallow the growth and yield of plants.

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Eco-efficient Bricks for Sustainable Urban Plastic Waste Management

Yacouba Zoungranan^{1†} , M'Bra Ignace Christian², Soro Sirata Ibrahima Francis², Ekou Lynda² and Ekou Tchirioua²

¹Department of Mathematics, Physics and Chemistry, University Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire, Ivory Coast

²Department of Chemistry, University Nangui Abrogoua, Abidjan, Côte d'Ivoire, Ivory Coast

†Corresponding author: Yacouba Zoungranan: zoungranan@gmail.com

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ABSTRACT

In developing countries, plastic packaging waste and the proliferation of cement plants have become real problems in terms of hygiene and public health. Common plastic packaging is produced by the petrochemical industry. It is (Ivory Coast) non-biodegradable and releases numerous toxic substances when heated or incinerated. In this study, building blocks were produced by incorporating waste plastic packaging (low-density polyethylene) as reinforcement in fired clay bricks. The incorporation into the raw clay matrix was carried out in proportions of 0%, 1%, 2%, 3%, 4%, and 5% of plastic, corresponding respectively to brick types Bcp0, Bcp1, Bcp2, Bcp3, Bcp4 and Bcp5. The Bcp4 bricks showed optimal physical properties (water absorption rate, apparent porosity, density, and compressive strength). The introduction of 4% plastic waste into the clay increased the compressive strength, decreased the water absorption rate, and significantly reduced the apparent porosity. The influence of firing temperature (T_f), firing time (t_f), and amount of mixing water (m_{water}) was investigated on Bcp0 and Bcp4 bricks. The better plastic incorporation for the operating parameters $T_f = 200^\circ\text{C}$, $m_{\text{water}} = 20$ g, and $t_f = 6$ h. The study shows that it is possible to have eco-efficient brick production with low energy consumption.

INTRODUCTION

In some African cities, such as Abidjan, the economic capital of Côte d'Ivoire, the various domestic and industrial activities that take place daily led to a proliferation of waste on the streets (Jambeck et al. 2018), especially plastic waste. This capital alone produces more than 288 tons of plastic waste every day, of which only 5% is recycled (Koumi et al. 2021). This situation is due to the country's lack of advanced technology facilities and appropriate rules and regulations on the production, use, and management of plastic waste. Due to their high stability and durability, most of these plastic wastes have limited natural degradation. They take between 100 and 1,000 years to decompose (Pilapitiya & Ratnayake 2024). This chemical stability poses pollution problems in receiving environments, impacting human and animal health (Emadian et al. 2017, Kanellopoulos et al. 2021). In addition, strong urban demographic growth has also led to a high demand for housing and induced the development of several cement works that supply cement for making bricks for housing. Unfortunately, the day-to-day operation of these cement plants results in the release of numerous toxic substances liable to harm the environment and human health (Subashi De Silva & Hansamali 2019, Venkatarama Reddy & Jagadish, 2003). Despite the presence of these cement plants, which are intended to facilitate access to housing, the housing crisis is a reality (N'goran et al. 2020). The bricks have a long history as a building material, from the use of clay to the incorporation of composite materials. Molding may be followed by a bricks firing stage, or they may remain unfired (Jiménez-García et al. 2023). Although raw clay bricks are environmentally friendly and recyclable, their physico-chemical

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properties may sometimes be insufficient for certain applications. In such cases, to improve the properties of the bricks, the manufacturing processes require them to be fired at very high temperatures or to use composite materials in combination with clay (Rauniyar et al. 2024). Several studies have been carried out on composite bricks made from clay and associated organic or inorganic materials. Among these studies, materials such as glass powder waste (Khan et al. 2022), rice straw (Akinyemi et al. 2022), incineration bottom ash (Adediran et al. 2024), tea waste (Hussien et al. 2024), and dolomite powder industrial waste (Kiziničević et al. 2023) have been used recently. Most of these results showed that the use of inorganic materials improved certain mechanical properties but reduced compressive strength (Ebadi-Jamkhaneh et al. 2021). This could be explained by a reduction in the vitreous phase content (Achik et al. 2020).

In the traditional process, the brick kiln is heated to 1000-1500°C, where alumina, silica, and iron are converted to molten glass, which acts as a binder to ensure adhesion. It then takes several days to slowly cool the kiln and recover the bricks (Murmur & Patel 2018). Côte d'Ivoire has several clay deposits (Bakary Soro et al. 2023, Kouakou et al. 2023) used in the manufacture of numerous ceramics (He et al. 2024) and in the construction of rural dwellings in general. The poor quality of traditional bricks has led people to turn to materials from local or imported cement works. However, these materials remain costly and even inaccessible to low-income populations. It is, therefore, necessary to improve the quality of traditional bricks to produce sustainable materials that are both inexpensive and environmentally friendly. Several studies have been carried out to improve the quality of fired clay bricks by adding various reinforcements, such as non-biodegradable plastic waste, to help solve plastic waste management problems (Dalkılıç & Nabikoğlu 2017, Haque & Islam 2021, Ikechukwu & Shabangu, 2021, Kulkarni et al. 2022, Gounden et al. 2024). The present study aims to contribute to a solution for recycling plastic bags from the city of Abidjan by incorporating them as reinforcement alongside clays in the manufacture of ecological bricks. The

aim is to design composite bricks with various proportions of incorporated plastics and to assess their physical and mechanical characteristics. In this way, local clay raw materials could be put to good use in the production of widely used building materials.

MATERIALS AND METHODS

Dry Material Preparation

Raw clay was collected from a clay pottery site (8°8'32.34" N and 5°6'21.38" W) in Katiola, a town located in central Côte d'Ivoire, 393.5 km from the economic capital Abidjan. After drying, the clay was ground, and the resulting powder was sieved on a sieve (Saulas, Paris, France) with an opening of 800 µm. The sieved material was stored in a jar and used as the base matrix for the ecological bricks. The plastic waste used as reinforcement is black low-density polyethylene packaging (Fig. 1). This type of plastic is a popular household packaging material, easy to obtain due to its low cost and high capacity to hold a variety of household products. It is, therefore, one of the most abundant types of plastic waste to be found on the streets of Abidjan. Polyethylene bag waste was collected in residential areas across the streets. The collected waste was thoroughly washed under running water and then dried for 48 h at room temperature. Multiple crumbling was performed using a blender shredder to obtain a fine plastic straw. Sieving on a sieve (Saulas, Paris, France) with an opening of 800 µm produced a homogeneous straw, as variation in dry matter particle size can affect brick properties and the repeatability of results (Kulkarni et al. 2019).

Brick Production

Each type of brick (Bcp0, Bcp1, Bcp2, Bcp3, Bcp4, Bcp5) was produced using a mass $M = 75$ g of dry materials ($m_{\text{clay}} + m_{\text{plastic}}$) and a mass m_{water} of distilled water (wet material). For each brick of a given type, the mixing of dry materials is carried out by determining the corresponding mass of plastics for a proportion (0%, 1%,

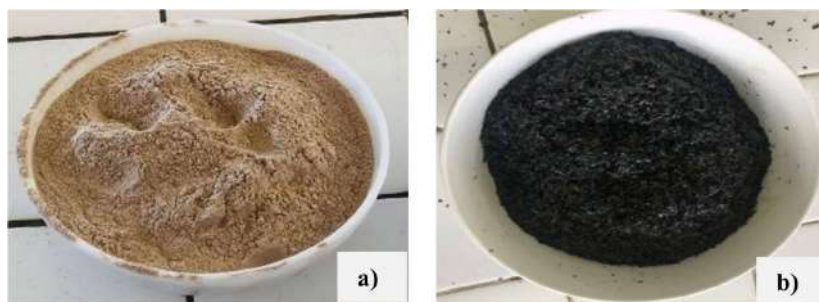


Fig. 1: Dry materials: a) clay matrix, b) plastic waste powder.

Table 1: Composition of the dry mix.

Sample	Plastic waste powder [%]	Clay [g]	Plastic waste powder [g]
Bcp0	0	75	0
Bcp1	1	74.25	0.75
Bcp2	2	73.5	1.5
Bcp3	3	72.75	2.25
Bcp4	4	72	3
Bcp5	5	71.25	3.75

2%, 3%, 4%, 5%) to always have a total mass of 75 g of dry materials.

For each sample, the dry mixture is prepared by means of a glass rod and then mixed with the required amount of water and kneaded. The resulting paste is poured into an 80 mm × 50 mm × 50 mm metal mold (Fig. 2) fitted with a removable compression plate.

The bricks were formed using a manual mechanical press, then demolded and dried at room temperature for 48 h. After drying, the bricks were fired in a muffle kiln (Nabertherm GmbH, Bremen, Germany) at 200°C.

In the first stage of the study, ten (10) bricks of each type (Bcp0, Bcp1, Bcp2, Bcp3, and Bcp4, Bcp5) were produced by setting the mass of mixing water at $m_{\text{water}} = 15$ g, the firing temperature at $T_f = 200$ °C and the firing time at $t_f = 4$ h. This stage made it possible to select the type of dry mass mixing ratio required to obtain the type of brick with

optimum characteristics (Bcp_{opt}). In the second stage of the study, the influence of the parameters firing temperature (T_f), firing time (t_f), and wet mass (m_{water}) on the physicochemical properties of Bcp_{opt} bricks was studied in comparison with Bcp0 bricks.

Operating Parameters and Their Influence

The water quantity (m_{water}): For these brick series Bcp_{opt} and Bcp0, mixing was carried out with different masses $m_{\text{water}} = (15 \text{ g}, 20 \text{ g}, 25 \text{ g}, \text{ and } 30 \text{ g})$. The firing temperature was set at 500 °C for 4 h.

The firing temperature: Bcp_{opt} and Bcp0 brick series are always considered, with $m_{\text{water}} = 20$ g and firing temperatures successively set at 200°C, 300°C, 400 °C and 500°C for a duration of 4 h.

The firing time: The effect of time was carried out with Bcp_{opt} and Bcp0 brick series for $m_{\text{water}} = 15$ g. The firing time for each trial was determined by the firing temperature. The firing times for each trial were 4h, 5h, and 6h. The firing temperature remained fixed at 200°C.

Brick Physicochemical Characterisation

To determine the water absorption rate, the brick is dried to a constant mass (m_1) and then immersed in distilled water for 24 h. The sample is then removed, wiped with a damp cloth, and weighed (m_2). Water absorption is given by equation 1:

$$\text{Absorption (\%)} = \frac{m_2 - m_1}{m_1} \times 100 \quad \dots(1)$$



Fig. 2: Moulding device.



Fig. 3: Compressive strength tests with the UTEST (UTCM-6431, Ankara, Turkey) device.

Density is calculated after firing and cooling a mass m of brick. The dimensions of the brick are then measured with a caliper so that the volume V is known. The density is then calculated using the following formula:

$$\text{Density} (t / m^3) = \frac{m}{V} \quad \dots(2)$$

To assess apparent porosity, the brick is dried to a constant mass (m_1) and then immersed in distilled water for 24 h. The sample is removed, wiped with a damp cloth, and weighed to obtain a mass (m_2). The volume V of the brick is given by measuring its dimensions with a caliper. Apparent porosity is calculated using the following formula:

$$\text{Apparent porosity} (\%) = \frac{m_2 - m_1}{V} \times 100 \quad \dots(3)$$

Compressive strength tests were carried out using an automatic compression machine (Fig. 3).

The breaking force causing the fracture is estimated by the machine, and together with the surface area, we calculate the compressive strength using the following formula:

$$\text{Compressive strength} (MPa) = \frac{\text{Maximum load at failure} (N)}{\text{Area under compression} (mm^2)} \quad \dots(4)$$

Bricks Composition EDS Analysis

EDS analyses of the clay samples were carried out using Bruker's QUANTAX EDS microprobe with an X Flash 6/30 detector. The structural specificities of the Bcp_{opt} and Bcp0 bricks were analyzed using a Hiro SH 400 M Scanning Electron Microscope (Limonest, France).

Data Processing

All calculations were performed with Microsoft Office Excel

Table 2: Elemental composition of raw clay.

Elements	Normal Mass [%]	Atomic Mass [%]
O	57.32	72.36
Al	13.43	10.05
Si	17.60	12.66
Ti	0.69	0.29
Fe	9.20	3.33
Na	0.75	0.66
Mg	0.43	0.36
K	0.58	0.30
Total	100.00	100.00

2016 Professional. Mean values and standard deviation were determined from three individual measurements.

RESULTS

Composition of the Raw Clay

Semi-quantitative EDS analysis of the clay sample enabled us to specify the composition of the clay in the study. The dispersion spectrum of the elements is shown in Fig. 4, and the elemental proportions are given in Table 2.

The results of the semi-quantitative EDS analysis of the raw clay sample are shown in Fig. 4, which presents the spectrum of the chemical elements contained in the study clay (O, Al, Si, Ti, Fe, Na, Mg, K). Table 1 shows the different mass percentages of these chemical elements. Silicon (Si) and aluminum (Al) are highly present, mainly due to the presence of chemical elements in oxide form, respectively SiO₂ (silica) and Al₂O₃ (alumina). Alumina is a determining factor in the identification of clays. A high alumina content indicates the presence of kaolinite (Qlihaa et al. 2016). However, it

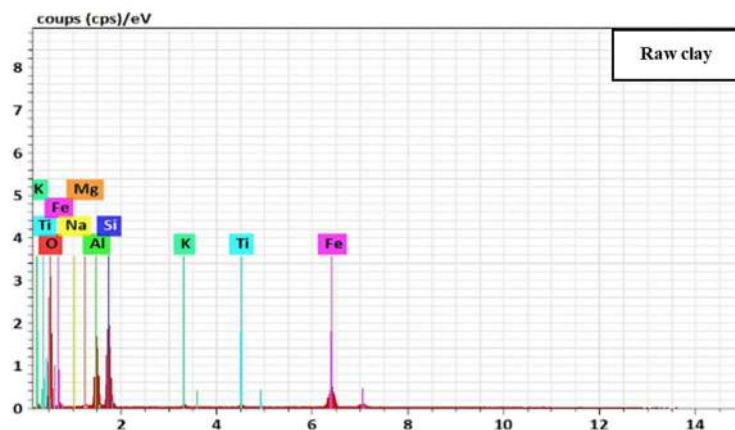


Fig. 4: Energy Dispersion Spectrum (EDS) of raw clay.

is important to highlight the low presence of the chemical elements Fe, Mg, and K. The presence of Ti would indicate the presence of titanium dioxide (TiO_2) in the raw clay. In addition, the clay in this study is predominantly a mixture of kaolinite and illite, as observed by Kpangni et al. (2008).

Optimal Dry Matter Composition

The bricks were shaped progressively, increasing the amount of plastic in the total dry matter from 1% to 10% initially. Baked at 200°C for 4 h, dislocation of the bricks was observed for proportions of 6% or more of plastic waste (Fig. 5). These bricks detach on cooling and can, therefore, not be characterized.

The specific characteristics of the bricks obtained in the first stage of the study are shown in Fig. 6.

Water absorption is an important characteristic for assessing the durability, thermal conductivity, and quality of a brick (Iftikhar et al. 2020). Fig. 6 shows that at a firing temperature of 200°C , the water absorption capacity of bricks decreases as the amount of plastic incorporated increases. Bcp4 bricks remain the least absorbent. The apparent porosity is positively correlated with the water absorption capacity. It decreases and reaches its lowest value also for

Bcp4 bricks. The moderate addition of plastic, therefore, makes the bricks less porous. The density of the bricks is an important parameter in defining their field of application. The gradual addition of plastic reduces the density of bricks. The lowest-density bricks are Bcp5 bricks. The incorporation of up to 4% plastic waste as dry reinforcement in the Bcp0 brick optimizes its compressive strength. Given the above, it appears that the composition of Bcp4 bricks, fired at only 200°C , provides a significant improvement in durability and compressive strength. Scanning electron microscopy (SEM) allowed us to observe the comparative texture (Fig. 7) of Bcp0 and Bcp4 bricks. The clay particles in Bcp0 are in the form of clusters of fine polygonal platelets (in the micrometer range), characteristic of a kaolinite (Bakary Soro et al. 2023) rather than an illite crystalline morphology. The addition of plastic waste makes the surface of Bcp4 much less glassy but more compact and, therefore, less susceptible to deformation. There is also a good distribution of plastic in the porous structure of the clay in Bcp4.

Effect of Operating Parameters

For this stage of the study, tests were carried out with Bcp0 and Bcp4 bricks.



Fig. 5: Aspects of some types of eco-friendly bricks.

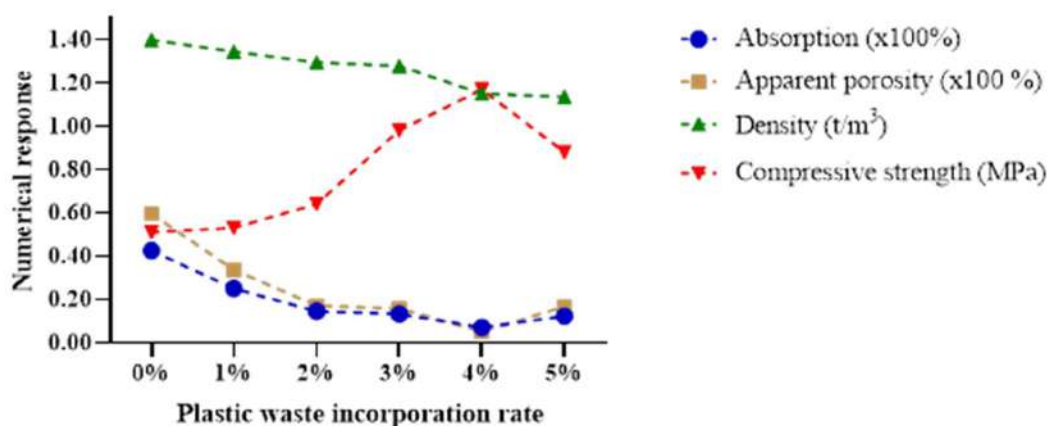


Fig. 6: Evolution of bricks characteristics, $T_f = 200^\circ\text{C}$, $m_{\text{water}} = 15 \text{ g}$, $t_f = 4 \text{ h}$.

Firing temperature: The effects of increasing the temperature to 400°C and 500°C were not compared due to the dislocation of the Bcp4 bricks at these temperatures. The results of the temperature effect are shown in Fig. 8.

As the temperature increases from 200°C to 300°C, there is an increase in water absorption capacity, porosity, and a

slight increase in density. On the other hand, a decrease in the compressive strength of the material is observed.

Effect of firing time: The results of firing at $T_f = 200^\circ\text{C}$ at various times are shown in Fig. 9.

Firing for 6 h improves compressive strength and density. The bricks become slightly denser and more resistant to compression.

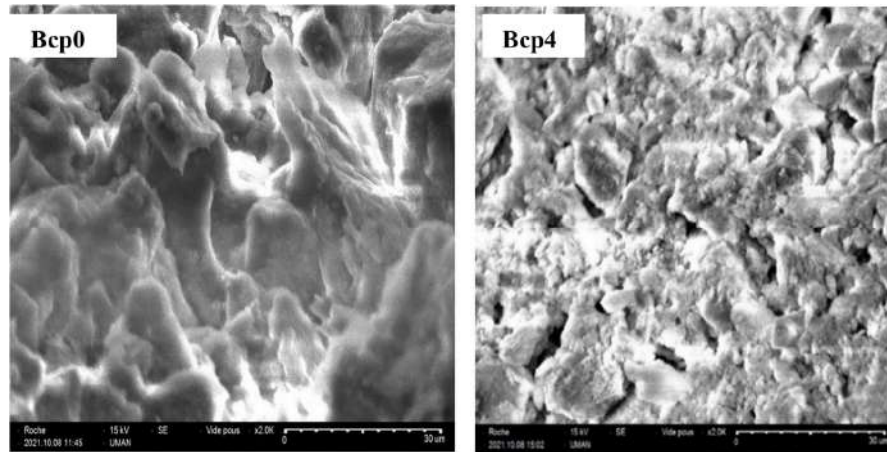


Fig. 7: SEM images comparing Bcp0 and Bcp4 bricks. $T_f = 200^\circ\text{C}$, $m_{\text{water}} = 15\text{ g}$, $t_f = 4\text{ h}$.

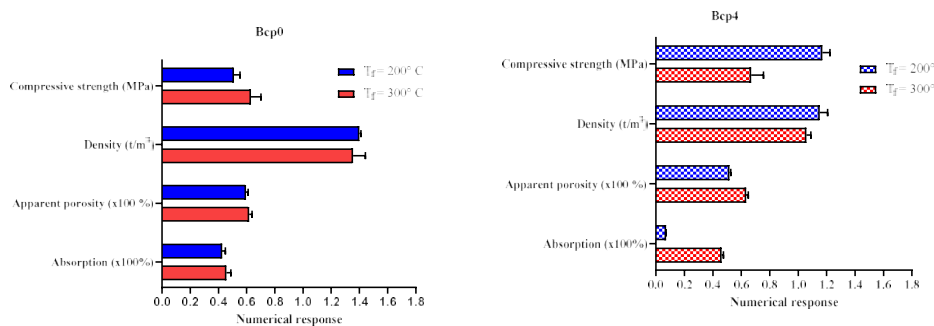


Fig. 8: Effect of firing temperature on the characteristics of Bcp0 and Bcp4 bricks, $m_{\text{water}} = 15\text{ g}$, $t_f = 4\text{ h}$.

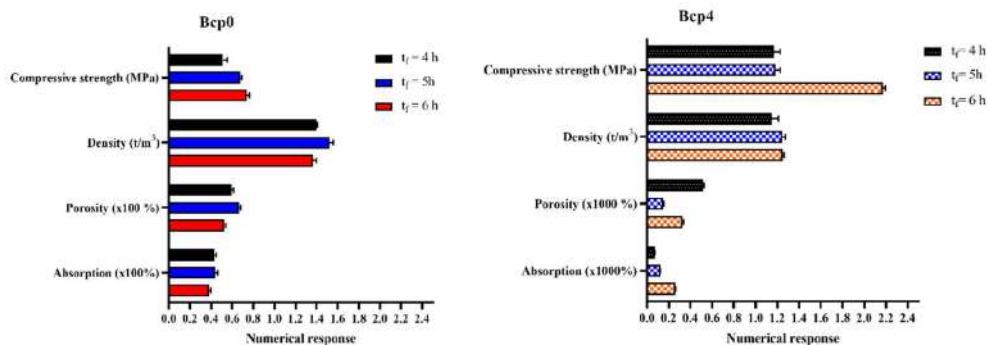


Fig. 9: Effect of firing time on the characteristics of Bcp0 and Bcp4 bricks, $m_{\text{water}} = 15\text{ g}$, $T_f = 200^\circ\text{C}$.



Fig. 10: Material loss during compaction of Bcp4 at $m_{\text{water}} = 30$ g.

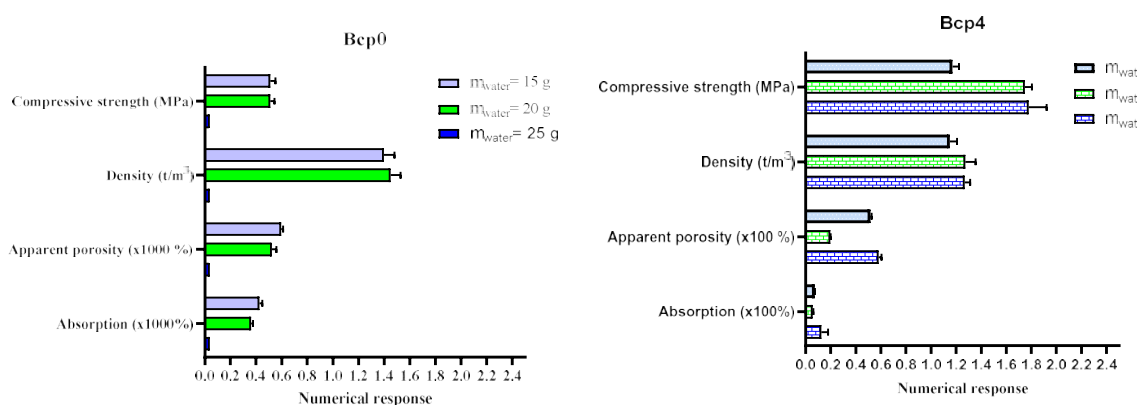


Fig. 11: Effect of wet mass on the characteristics of Bcp0 and Bcp4 bricks, $T_f = 200^\circ\text{C}$, $t_f = 4$.

Effect of wet mass: Above $m_{\text{water}} = 25$ g, wet matter acts as a limiting factor for brick shaping at the compaction stage. For example, the production of bricks Bcp4 at $m_{\text{water}} = 30$ g resulted in a deformed brick (Fig. 10) due to material leakage during compaction. The same molding difficulties were observed with Bcp0 from $m_{\text{water}} = 25$ g.

The amount of wet matter influences the characteristics of the bricks produced. For the Bcp4 brick type, as the amount of moisture increases, the compressive strength and porosity increase. This increase in porosity leads to an increase in water absorption capacity. This could be a limiting factor in the durability of the bricks. However, at $m_{\text{water}} = 20$ g, the compressive strength is improved. This amount of $m_{\text{water}} = 20$ g also improves the physicochemical and mechanical properties of Bcp0 bricks (Figs 10 & 11).

DISCUSSION

Composite bricks were developed by dispersing plastic (polyethylene) in clay. The results of the composite bricks obtained present much better brick characteristics compared to bricks elaborated with raw clay alone. A study of the influence of the dry matter mixing ratio revealed that moderate incorporation of polyethylene improved the quality

of composite bricks, especially their compressive strength. The study revealed that compressive strength was highest at 4% plastic. Moderate increases in plastic content (up to 5%) lower density and porosity, significantly reducing the water absorption capacity of composite bricks. The experimental study by Ebadi-Jamkhaneh et al. (2021) on the mechanical properties of fired clay bricks incorporated with plastic waste showed that the addition of 3% fine polyethylene plastic particles could include the fired clay brick as a load-bearing brick. Another study by Hussien et al. (2024) on the tea waste additive effects on the physical and mechanical properties of unfired clay bricks also showed that samples containing 2.5% and 5% of the additive gave better compressive strength results. The present study also showed that increasing the firing temperature to 300°C increased density and lowered compressive strength. There was also an increase in the water absorption and porosity of composite bricks. This result could be due to the behavior of polyethylene. Polyethylene melts at 85°C and degrades at 274°C (Wahane et al. 2023). Thus, at temperatures below 300°C and above its melting point, it remains in the liquid state, enabling it to adhere better to the clay and close the pores of the clay material. Above the degradation temperature of polyethylene, the liquid shrinks to form pellets, creating voids in the clay

material. This shrinkage phenomenon is likely to explain the increase in porosity and absorption, leading to a reduction in the strength of composites. As water absorption capacity is recognized as an indicator of durability and porosity (Vasić et al. 2020), a temperature of 200°C is more suitable for firing clay-polyethylene composite bricks. A low absorption capacity and high density could reduce brick permeability and resistance to certain climatic conditions (Vasić et al. 2020). The increase in porosity linked to the plastic additive improves thermal conductivity (Hussien et al. 2024) and makes it possible to obtain lightweight insulating bricks. As for the effect of firing time, it shows that it is after 6 h of firing that resistance and density are high. Finally, a study of the effect of wet mass shows that maximum compressive strength values are obtained at 20 g.

CONCLUSIONS

Conventional bricks made from high-temperature clay require a high consumption of energy and clay raw materials. In this study, clay-plastic composite bricks were developed using different proportions of polyethylene plastic waste. The Bcp4 bricks exhibited optimum physical and mechanical properties at only 200°C, with a 15 g water mixture and a cooking time of 4 h. This is equivalent to an incorporation rate of 4% of the mass of the brick. The effects of operating parameters such as baking temperature, amount of wet material, and baking time were investigated. The optimum baking time for bricks was found to be 6 h per 20 g of wet mass and a cooking temperature of 200°C. The results show that the incorporation of plastic waste into the bricks can lead to a significant improvement in the performance of the bricks. Bricks produced under the conditions revealed by the study could be an alternative means of reducing polyethylene plastic waste. This suggests positive prospects for solutions in the construction industry.

Overall, the results suggest that polyethylene plastic waste can be reused to produce environmentally friendly bricks, which could significantly reduce plastic waste in the environment. These composites are promising for use as durable bricks for walls, paving, and other construction applications. However, it would be necessary for future studies to expand the type of plastic waste and increase the mechanical characterization tests.

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Response Surface Modeling for COD Removal in Electroplating Effluent Using Sacrificial Electrodes by Electro Fenton Process: Optimization and Analysis

Nandhini V. , Dhanakumar S.†  and Durga M. 

Department of Environmental Science, PSG College of Arts and Science, Coimbatore 641014, Tamil Nadu, India

†Corresponding author: Dhanakumar S.: ecodhanan.phd@gmail.com

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ABSTRACT

The effluent produced by the electroplating industry contains hazardous and toxic chemicals that pose a threat to living organisms and ecosystems. Consequently, it is essential to employ advanced treatment technologies to remove the toxicants from the wastewater. Over the past two decades, the concept of Electro Fenton has been developed and demonstrated as an effective method for significantly alleviating pollutants in wastewater, making it a promising solution for treating wastewater. In the present investigation, the efficiency of the Electro Fenton (EF) process in removing Chemical oxygen demand (COD) from electroplating wastewater using stainless steel as the sacrificial electrode was examined. The influence of various operating parameters, including pH, hydrogen peroxide concentration, reaction time, and Fe^{2+} concentration, was investigated with the help of Box-Behnken design (BDD) in Response surface methodology (RSM). Notably, EF treatability studies demonstrated that optimal conditions of pH 2, Fe^{2+} concentration of 0.005M, H_2O_2 concentration of 0.5M, and RPM of 450 resulted in more than 75% COD removal. Hence, the sacrificial electrodes can be effective in removing COD from the wastewater.

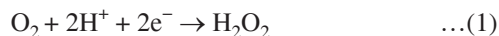
INTRODUCTION

The swift growth of industrialization and urbanization has resulted in the generation of a wide range of pollutants, which has raised concerns about the environment and human health (Xing et al. 2022). Currently, the world is grappling with significant environmental challenges, particularly water contamination, which is primarily driven by various industrial activities. The electroplating industry is one of the most impactful pollution-generating industries, producing a vast amount of wastewater that contains heavy metals and toxic substances, which are persistent (Guan et al. 2022). The electroplating process involves applying a thin layer of metal to an object's surface by immersing or suspending the object in an electrolytic solution. The cathode is used for the electrolytic deposition process, while the plate of the metal to be deposited acts as the anode. A substantial amount of freshwater is consumed during the process, with about 40% of the water used being discharged as electroplating effluent into the Groundwater (Prajapati et al. 2016). Further, the effluents consist of a variety of heavy metals, including copper, zinc, nickel, chromium, and lead, as well as calcium salts, organic compounds, and other toxic substances. The release of these heavy metals into the environment has detrimental effects on human health, the ecosystem, and other living organisms, due to their potential for bioaccumulation (Yong et al. 2021). As a result, effective measures are necessary to reduce the threats these toxins pose to ecosystems and humans. Preventative measures are necessary to mitigate the harmful consequences of water discharge. Although sedimentation techniques are often utilized in the

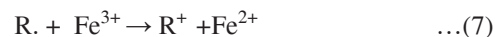
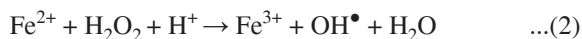
electroplating industry, they may result in increased sludge production, which leads to the transportation of sludge and the recovery of metals being unfeasible. Therefore, wastewater treatment methods such as coagulation (Wei et al. 2013), biosorption (Abdel-Shafy et al. 2019), adsorption (Boddu et al. 2022), reverse osmosis (Alharthi et al. 2022), ion exchange, electro dialysis (Zelinski et al. 2023), and electrodeposition (Klishchenko & Chebotarova, 2023) and chemical precipitation (Verma & Balomajumder et al. 2020) were used. They do not completely degrade the pollutants in the effluent, and it is expensive.

Advanced oxidation processes (AOPs) have been developed in recent decades to convert various refractory organic molecules with strong oxidants into water, CO₂, and inorganic salts (Mustafa & Aziz 2023). Among the AOPs, the Fenton process has demonstrated its effectiveness in reducing harmful contaminants in industrial effluents through electroplating, out of all the examined AOPs. On the other hand, hydroxyl radicals can regulate the rate of reaction and are highly reactive, non-selective, easily developed, powerful oxidants, short-lived, and harmless (Zhu et al. 2019). In the case of adsorption and Reverse Osmosis, both techniques are removal processes rather than degradation methods. Adsorption concentrates pollutants on the adsorbent surface (Boddu et al. 2022), while RO separates them, often resulting in brine or concentrate that requires further treatment (Alharthi et al. 2022). In contrast, EF actively degrades pollutants rather than simply removing them. The hydroxyl radicals generated in the EF process target the pollutants, breaking down their molecular structure, which leads to mineralization. This degradation reduces further waste management (Shokri et al. 2023).

As the reaction shows, continuous in-situ electrochemical production of H₂O₂ takes place in an acidic medium via oxygen reduction at the cathode (Brillas et al. 2009) produces OH which has a strong oxidation potential (2.8 V/SHE) which oxidizes any organic pollutant which leads to the complete mineralization of organics in the wastewater (Oturán et al. 2021).



The classical Fenton process involves the homogeneous treatment by adding Fenton's reagent, which consists of iron (II) and hydrogen peroxide, to the wastewater. On the other hand, the EF process is a heterogeneous treatment that uses an electrochemically assisted Fenton process. The process can continue because Fe²⁺ can be regenerated through chemical or electrochemical processes. (Eq. (2-7) (Asaithambi et al. 2022).



Electro-Fenton process is an increasingly viable option for sustainable industrial wastewater treatment, particularly for industries dealing with persistent, complex pollutants like those in electroplating effluent. It relies on the electrochemical generation of hydrogen peroxide (H₂O₂) and ferrous ions (Fe²⁺) in situ, which react to produce hydroxyl radicals capable of oxidizing various contaminants, leading to the generation of smaller chemical footprints that eventually decrease the reagent cost, making it as cost-effective (Shokri et al. 2023). In the EF process, the performance of the electrolytic system is heavily dependent on the electrode material, as it is the source of the OH radical, which is generated on the surface of the electrode when it oxidizes with water. Stainless steel electrodes are commonly used in the EF process due to their affordability, high catalytic conductivity, corrosion resistance, and simplicity. During the EF process, a significant amount of iron is sacrificed, which reacts with the existing Fenton reagents and results in the demineralization of pollutants (Radwan et al. 2018). In this study, stainless steel was employed as a sacrificial electrode and evaluated for its effectiveness in treating electroplating wastewater using the electro-Fenton (EF) process. Investigations were conducted on the impacts of several operating parameters that affect the EF process, including pH, Fe²⁺ concentration, H₂O₂ concentration, temperature, and time. In addition, reaction kinetics and response surface methodology (RSM) with Box-Behnken design (BBD) are studied in detail.

MATERIALS AND METHODS

Materials

Electroplating effluent was collected from the Zinc electroplating industry in Coimbatore, Tamil Nadu. The effluent samples were collected in pre-cleaned polyethylene bottles, sealed, and subsequently transported to the laboratory for analysis. The samples were stored at 4°C. All the chemicals utilized in the experiments were of the highest analytical grade. All solutions were prepared using double-distilled water. The physico-chemical parameters such as pH, electrical conductivity, acidity, chloride, nitrate, phosphate, sulphate, COD, Iron, and zinc, of the wastewater samples were assessed as per APHA 2017 guidelines. The concentration of heavy metals such as Iron (Fe) and Zinc (Zn) was determined using Atomic Absorption Spectroscopy (AAS). Sodium hydroxide (NaOH) and hydrochloric

acid (HCl) were utilized to adjust the pH. Sodium sulfate (Na_2SO_4) served as the electrolyte. Hydrogen peroxide (H_2O_2) and ferrous sulfate heptahydrate ($\text{Fe}_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$) were used as oxidants and catalysts, respectively.

Experimental Procedure

The main components of the EF process are electrodes, a reactor, and a power supply. Electrodes play a crucial role in the Electro-Fenton (EF) process, as they are directly involved in generating the essential reactants and facilitating pollutant degradation. Both the anode and cathode serve specific functions in the process, influencing the generation of hydrogen peroxide, the regeneration of ferrous ions, and, ultimately, the overall efficiency of pollutant removal (Zhou et al. 2024). The cathode in the Electro-Fenton process is primarily responsible for generating hydrogen peroxide (H_2O_2) and regenerating ferrous ions (Fe^{2+}), which are essential for producing hydroxyl radicals. At the cathode, dissolved oxygen (O_2) is reduced to form H_2O_2 , a key reactant for hydroxyl radical production. The pH range of 2 to 5 is crucial for the Electro-Fenton process because it ensures the optimal generation of hydroxyl radicals ($\cdot\text{OH}$) (Sirés & Brillas 2017). At lower pH values, the formation of ferric ions (Fe^{3+}) is favored, which are essential for the Fenton reaction (Xu et al. 2020). Additionally, a lower pH helps in maintaining the stability of hydrogen peroxide (H_2O_2). Studies have shown that the degradation efficiency of contaminants is highest within this pH range (Nidheesh et al. 2018). The concentration of ferrous ions (Fe^{2+}) is another critical parameter. A concentration range of 0.001 M to 0.005 M is optimal because it provides enough Fe^{2+}

to react with H_2O_2 and generate hydroxyl radicals without causing excessive iron precipitation (Xu et al. 2020). Higher concentrations can lead to the formation of insoluble iron hydroxides, which can reduce the efficiency of the process (Sirés & Brillas 2017). The concentration of hydrogen peroxide (H_2O_2) is also important for the generation of hydroxyl radicals. A concentration range of 0.1 M to 0.5 M ensures that there is sufficient H_2O_2 to react with Fe^{2+} ions, but not so much that it leads to the scavenging of hydroxyl radicals or excessive consumption of H_2O_2 (Nidheesh et al. 2018). This range balances the need for effective radical generation with the practical considerations of cost and reagent availability (Xu et al. 2020). These parameters are supported by various studies and reviews on the Electro-Fenton process, which highlight the importance of optimizing pH, Fe^{2+} concentration, and H_2O_2 concentration to achieve efficient degradation of contaminants (Sirés & Brillas 2017). The electro-Fenton setup is shown in Fig. 1. The electrodes should be cleaned before and after each run by washing with acetone, and after rinsing with aqua distillate. The next step was to add 0.5 L of effluent to a glass reactor. The pH of 2-4 (acidic) is ideal for the EF reaction (Zhao et al. 2020). Therefore, the pH of the wastewater is regulated using HCl. During the electro-catalytic reaction, the reaction mixture was kept uniform by employing a magnetic stirrer. Wastewater was stirred constantly at a speed of 450 rpm. The appropriate amount of Na_2SO_4 was added, which is necessary to increase the wastewater sample's conductivity and enable simultaneous electrical current passage through it. Electrodes are positioned inside the reactor, and the electrodes are wired to the DC supply. The anode-cathode electrode pairs used



Fig. 1: Electro Fenton setup with Sacrificial stainless steel electrodes.

in this experiment were separated by a 1 cm inter-electrode gap. A precision DC power supply provided the current for the trials. After a pre-determined time, the electricity was shut off, and samples of the reactor were removed for further analysis. Following the completion of the run, the wastewater sample generated was filtered, and 100 mL of the sample was then collected for COD analysis (APHA 2017). The removal efficiency of the COD was calculated using Equation (8)

$$COD_{Removal} = \left(1 - \frac{COD_i}{COD_t}\right) \times 100 \quad \dots(8)$$

Where COD_i is the initial COD and COD_t is the final COD of the electroplating effluent.

Optimization Analysis Using RSM

To determine the optimum number of parameters for the effective degradation of COD in electroplating wastewater, RSM was used in this study. RSM is a more systematic approach to experimentation that simultaneously predicts outcomes, which are called responses; it depends on the independent variables (Nu et al. 2021). The concentrations of Fe^{2+} , H_2O_2 , and the initial pH of the solution, were among the process variables that were optimized. The Box-Behnken Design (BBD) within Response Surface Methodology (RSM) provides distinct advantages by reducing the number of experimental trials needed to identify potential interactions between parameters and their effects on the electro-Fenton (EF) degradation of electroplating effluent (Rajoria et al. 2024). BBD was used to optimize the process variables, with three components set at levels -1 and 1, representing low and high values, respectively (Table 1). The high and low values for each factor varied from the factorial design to identify the optimal range for maximizing degradation efficiency.

Utilizing Design-Expert® software version 13.0 (Stat-Ease Inc., Minneapolis, Minnesota, USA), the experimental design and statistical analysis were completed. The software also generated the trial runs at random. At the 5% level of significance, the ANOVA was utilized to ascertain the model's significance as well as the main effects and higher-order interactions of the components. The experimental validation of the model was evaluated by comparing the experimental results with the predicted values obtained under optimal conditions, which were tested in replicates. A second-order polynomial equation (9) was used to fit the experimental data and identify the significant variables in the model.

$$COD_{Removal\ efficiency} = \beta_0 k + \sum \beta_i Z_i + \sum \beta_{ii} Z_i^2 + \sum \beta_{ij} Z_{ij} + e \quad \dots(9)$$

The variables, in this case, are k , the intercept is denoted by β_0 , the input factor Z_i 's linear effect is represented by β_i , the linear-by-linear interaction between Z_i and Z_j is represented by β_{ij} , the input factor Z_i 's quadratic effect is

Table 1: Independent input variables for Box-Behnken design.

Factor	Variable	Unit	Low value	High Value
A	Initial pH	-	2	5
B	Fe^{2+}	M	0.004	0.006
C	H_2O_2	M	0.1	0.5

represented by β_{ii} , and the statistical error is denoted by e . To investigate the link between the process components and the replies, ANOVA was employed. Second-degree polynomials were employed to characterize the data, and regression analysis, variance coefficient of regression (R^2), and p-value of the ANOVA were used to assess the acceptability of the model with the best fit (9).

RESULTS AND DISCUSSION

Characteristics of Wastewater

The physicochemical characteristics of the electroplating wastewater were analyzed and tabulated (Table 2):

Optimization Analysis

The BBD technique was applied to maximize COD degradation at the initial pH, Fe^{2+} , and H_2O_2 conditions. The removal efficiency was taken into consideration as the surface responses to the variables in the intended experiment, which comprised fifteen distinct runs. The outcomes of the trials carried out with BBD, along with the predicted and observed removal percentages, are mentioned in Table 3.

A second-order polynomial regression model was used to establish the relationship between the independent variables and the response variable (Rajoria et al. 2024).

$$COD\ Degradation\ (mg.L^{-1}) = +77.94 - 0.8988A - 0.0175B + 0.8738C + 1.40A^2 - 3.98B^2 - 0.8479C^2 - 0.6450AB + 0.1175AC - 0.5350BC$$

Table 2: Characteristics of Electroplating Effluent.

Parameter	Value
pH	4.8
Electrical conductivity [$mS.cm^{-1}$]	2.2
Total Dissolved Solids [$mg.L^{-1}$]	1538
Acidity (as $CaCO_3$) [$mg.L^{-1}$]	30
Chloride [$mg.L^{-1}$]	301.32
Nitrate [$mg.L^{-1}$]	2.12
Phosphate [$mg.L^{-1}$]	1.70
Sulphate [$mg.L^{-1}$]	13.23
Iron [$mg.L^{-1}$]	2.68
Zinc [$mg.L^{-1}$]	5.17
COD [$mg.L^{-1}$]	1080

Table 3: Experimental matrix and observed responses of Electroplating wastewater in BBD.

Std	Run	Factor 1	Factor 2	Factor 3	Response 1
		A: Initial pH	B:Fe	C:H ₂ O ₂	COD Degradation %
8	1	6	0.005	0.5	78.34
2	2	6	0.004	0.3	75.16
9	3	4	0.004	0.1	71.99
15	4	4	0.005	0.3	78.61
6	5	6	0.005	0.1	76.31
12	6	4	0.006	0.5	73.17
10	7	4	0.006	0.1	72.54
7	8	2	0.005	0.5	80.45
14	9	4	0.005	0.3	78.61
1	10	2	0.004	0.3	75.12
4	11	6	0.006	0.3	74.32
3	12	2	0.006	0.3	76.86
13	13	4	0.005	0.3	76.61
11	14	4	0.004	0.5	74.76
5	15	2	0.005	0.1	78.89

Where A is the Initial pH, B is the ferrous ion concentration, and C is the H₂O₂ concentration, respectively. The 2% nonconformity of the observed and the predicted values depicts that the model was fit to the data (Nu et al. 2021).

Statistical Analysis

The statistical ANOVA of COD degradation for the

EF treatment of electroplating wastewater is shown in Table 4. Fisher's distribution test (F-test) and the Regression coefficient (R^2) were used to assess the models' sufficiency and significance. Consequently, the computed F-values came out to be 12.83, which is significant because it is less than the $p\text{-value} < 0.005$. These results can be explained by a suitable correlation between the factors and degradation efficiency. A, C, A², and B² are important model terms in this instance. Additionally, the non-significant Lack of Fit mean squares indicates that there are no extra interactions affecting the performance of the second-degree model or its removal efficiency. The non-significance of AC means that H₂O₂ levels can be optimized independently. Similarly, AB and BC are significant, implying that Fe²⁺ and H₂O₂ can be optimized independently, which may lead to more efficient use of H₂O₂ and Fe²⁺ without compromising the process efficiency, which makes the process cost-effective. The Prob > F values, which are below 0.05 with a 95% confidence level, demonstrate the significance of the model parameters and the accuracy of the regression model. The low coefficient-of-variation (C.V.) value of 1.14 for COD degradation percentages reflects a high level of precision. The sequential F-test and other adequacy indicators support the use of a quadratic model. Analysis of the sequential model sum of squares and model summary statistics showed that P-values for A, C, A², and B² were all below 0.01, indicating a strong relationship between these terms and the response variable. According to the Response Surface Methodology (RSM), there was no significant interaction between the two factors (2FI) and the linear model. The quadratic model, as indicated by the model summary statistic (Table 4 & 5), provided the highest regression coefficients ($R^2 = 0.9585$

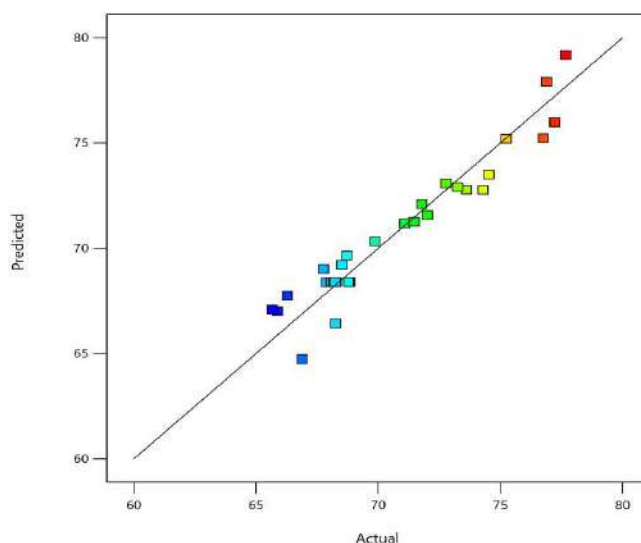


Fig. 2: Predicted vs Actual Plots for COD degradation.

for % COD degradation). The close agreement between the observed and predicted values is evidenced by the minimal difference of less than 0.2 between the adjusted R^2 value of 0.8838 and the expected R^2 value of 0.7428 (Fig. 2). The near alignment of the points to the straight line suggests that the model's predictions were largely consistent with the experimental results.

Adequate Precision (AP) is characterized by a signal-to-noise ratio, with a preferred value of at least 4. In other words, AP is defined as the range in the expected response related to its associated error. Since all of the AP values for COD degradation were greater than 4 (11.9342), it may be concluded that the system is performing effectively (Kacem et al. 2024).

Interaction of Process Parameters

Three-dimensional response surface graphs generated by RSM were analyzed to investigate the effects caused by factor interactions, including initial pH, Fe^{2+} , and H_2O_2 (Fig. 3). The interaction parameters were discussed individually as follows:

Effect of pH on COD Removal

To investigate the impact of pH on COD removal, the range of pH values from 2 to 5 was studied. The COD removal

efficiency of 80.45% is maximum, and it was observed at a pH of 2 after 30 min of treatment. It is evident that the effect of pH decreased with higher pH values from 2 to 5, and COD removal decreased, leveling off afterward. This occurrence can be attributed to the fact that higher pH, leads to the precipitation of dissolved iron ions as Fe^{2+} , leading to the formation of $Fe(OH)_n$, which can only decrease the concentration of dissolved Fe^{2+} , and these ions accumulated on the surfaces of the electrodes, preventing the further regeneration of Fe^{2+} . On the other hand, low pH levels lead to an increase in hydroxyl radical radiation, which oxidizes the compounds. A similar trend was observed in a study by Latha et al. (2024), where COD removal decreased from 85 to 45 % by increasing the pH from 3 to 6. This can be explained by the increased concentration of $\bullet OH$ radicals generation in the reactor over time. In acidic solutions, the presence of additional protons facilitates the conversion of dissolved oxygen into hydrogen peroxide. In conditions of higher pH, hydrogen evolution is enhanced, leading to a decrease in the availability of protons for the generation of hydrogen peroxide and its decomposition. Furthermore, at higher pH levels, the formation of various hydroxide species of iron ions occurs, along with increased scavenging effects of hydroxyl radical, which leads to auto-decomposition, resulting in a decrease in removal efficiency.

Table 4: ANOVA for Quadratic model.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	86.42	9	9.60	12.83	0.0059	significant
A-Initial pH	6.46	1	6.46	8.64	0.0323	
B-Fe	0.0024	1	0.0024	0.0033	0.9566	
C- H_2O_2	6.11	1	6.11	8.16	0.0355	
AB	1.66	1	1.66	2.22	0.1961	
AC	0.0552	1	0.0552	0.0738	0.7967	
BC	1.14	1	1.14	1.53	0.2710	
A ²	7.26	1	7.26	9.70	0.0264	
B ²	58.50	1	58.50	78.18	0.0003	
C ²	2.65	1	2.65	3.55	0.1183	
Residual	3.74	5	0.7482			
Lack of Fit	1.07	3	0.3582	0.2686	0.8461	not significant
Pure Error	2.67	2	1.33			
Cor Total	90.16	14				

Table 5: Fit Statistics.

Std. Dev.	0.8650	R ²	0.9585
Mean	76.12	Adjusted R ²	0.8838
C.V. %	1.14	Predicted R ²	0.7428
		Adeq Precision	11.9342

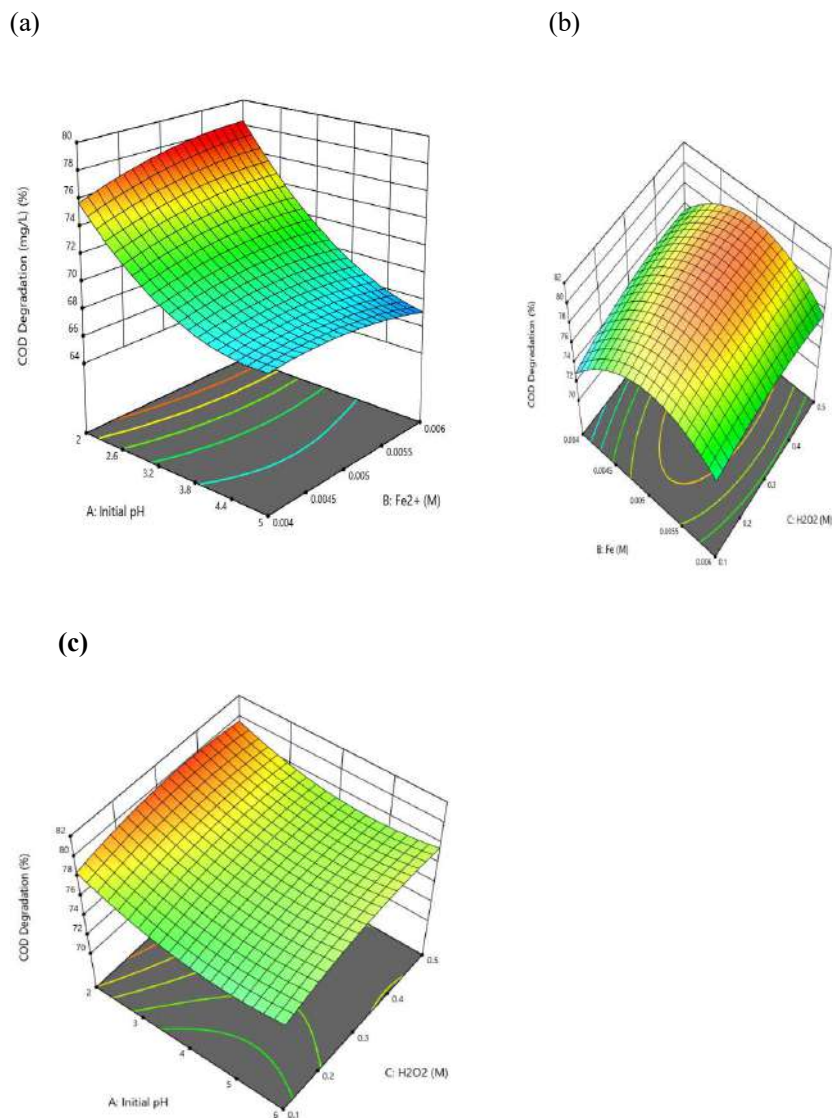


Fig. 3: 3-D surface graph for the Electroplating effluent (a-c) COD degradation vs Initial pH, Fe²⁺, H₂O₂.

Effect of Fe²⁺ on COD Removal

The concentration of Fe²⁺ plays a crucial role in regulating the rate of the Fenton reaction for the production of •OH. The highest COD removal efficiency of 80.45% was attained at a Fe²⁺ concentration of 0.005 M. The lower concentration of Fe²⁺ slower hydroxyl radical formation, resulting in a decrease in removal efficiency. As the Fe²⁺ concentration increased up to 0.005M, COD removal efficiency significantly improved; however, it decreased at higher concentrations and led to iron sludge formation. Nguyen et al. (2021) also reported the Fe²⁺ concentration was increased, resulting in higher removal efficiency for various pollutants. Although the increase

was negligible at higher Fe²⁺ concentrations, it was still significant.

Additionally, high Fe²⁺ concentrations may exceed the desired levels. Excessive application of Fe²⁺ ions can also increase the amount of sludge produced and associated sludge disposal costs. It can be inferred that increasing the relative concentration of Fe²⁺ in relation to the organic substrate enhances hydroxyl radical formation, thereby accelerating substrate degradation. However, this enhancement also requires more electrical charge due to the intensification of competing reactions during electrolysis. Fenton's reaction produces more •OH when the Fe²⁺ content rises; nevertheless,

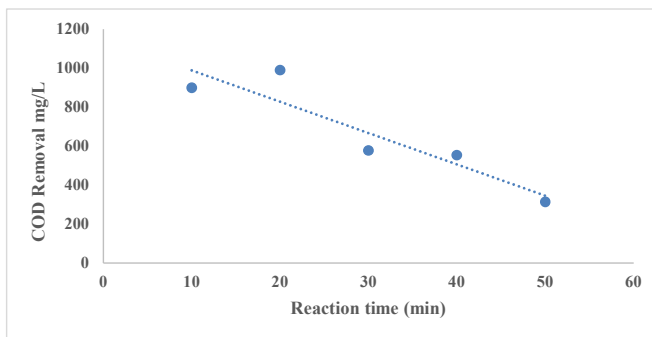
greater concentrations may even somewhat suppress the radical's generation, decreasing the process's potency. The increase in the rate of waste response explains the deleterious effects of this excess of Fe^{2+} ions (10), which consequently consumes $\cdot\text{OH}$.



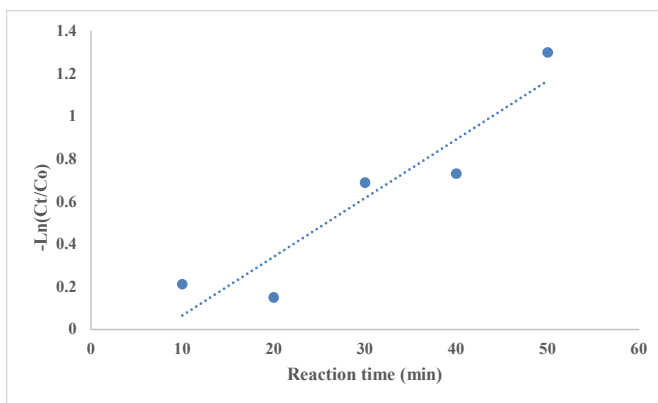
Effect of H_2O_2 on COD Removal

To investigate the influence of hydrogen peroxide (H_2O_2) concentration on COD and its removal from electroplating wastewater, A range of H_2O_2 values, from 0.1 to 0.5 M, was investigated. It is crucial to determine the optimal H_2O_2

(a)



(b)



(c)

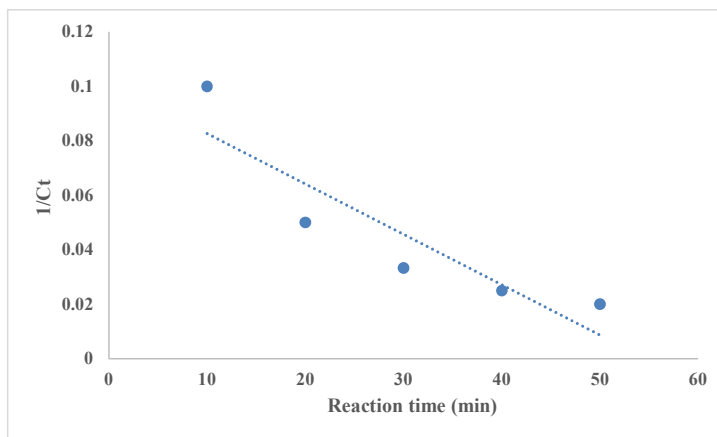


Fig. 4: Kinetics for COD removal in electroplating effluent (a) Zero order Kinetics, (b) First order Kinetics, (c) Second order Kinetics.

concentration in the EF process for both efficiency of COD removal and economic feasibility, particularly with respect to the cost of H_2O_2 . The results indicate that the highest COD removal efficiency was achieved with a 0.5 M H_2O_2 concentration after a 30-minute treatment. As the H_2O_2 concentration increased from 0.3 M to 0.5 M, the COD removal efficiency improved. This improvement is likely due to the increased generation of hydroxyl radicals in the system resulting from the higher H_2O_2 concentration. A study by Dokhani et al. (2024) on the Fenton process observed that COD removal improved as the H_2O_2 concentration increased from 20 to 170 $\mu\text{L.L}^{-1}$. This increase is attributed to the acceleration of the Fenton reaction and the generation of a higher amount of $\bullet\text{OH}$ radicals. However, further increasing the H_2O_2 concentration reduced removal efficiency due to the deactivation of OH radicals and greater depletion of oxidizing agents. Therefore, a 0.5 M H_2O_2 concentration, which achieved the highest COD removal efficiency, was deemed optimal for subsequent experiments.

Reaction Kinetics for COD Removal

The effectiveness of the EF process for eliminating COD was assessed at pH 2, a Fe^{2+} concentration of 0.005M, H_2O_2 concentration of 0.5M, and RPM of 450, which resulted in COD removal of over 80%. Furthermore, the COD removal kinetics model was investigated at optimum operating conditions (Fig. 4) using equations 11 and 12.

$$\ln(\text{Co}/\text{C}) = -k_1 t \quad \dots(11)$$

$$1/\text{C} = 1/\text{Co} + K_2 t \quad \dots(12)$$

Where k_1 (min^{-1}) and K_2 ($\text{mg.L}^{-1}.\text{min}^{-1}$) are the apparent first and second-order rate constants, respectively. Also, C (mg.L^{-1}), Co (mg.L^{-1}), and 't' are the COD dosages at the final and initial electrolysis time (t), respectively.

The relationship between $\ln(\text{Co}/\text{C})$ and time during the EF process exhibited a precise linear correlation, with an R^2 value of 0.9068. This finding is compared to the relationship between $1/\text{C} - 1/\text{Co}$ and time, which had an R^2 value of 0.8132, which is shown in Table 6. The kinetic model for COD removal followed pseudo-first-order kinetics. A study conducted by Jiad et al. (2024) also demonstrated first-order kinetics, indicating that the reaction proceeds rapidly, leading to a significant reduction in pollutant concentration over time. This enhanced reactivity may result from the generation of highly reactive hydroxyl radicals ($\bullet\text{OH}$) through the EF process. Therefore, high rate constants suggest that the rate-limiting step is favorable, resulting in efficient pollutant degradation.

A high R^2 value, or coefficient of determination, is crucial in practical applications as it indicates a strong fit

Table 6: Kinetics for COD removal in electroplating effluent.

Kinetics	Rate constants	Precision (R^2)
Zero order	$\text{K} = -16.08 [\text{mg.L}^{-1}]$	0.8508
First order	$k_1 = 0.027 [\text{min}^{-1}]$	0.9068
Second Order	$\text{K}_2 = -0.001 [\text{mg.L}^{-1}.\text{min}^{-1}]$	0.8132

between the kinetic model and experimental data, enhancing the predictability and control of the treatment process. The pseudo-first-order model accurately describes the degradation rate when the R^2 value approaches 1, enabling precise estimation of the time required for pollutant reduction (Eilertsen et al. 2024). A strong R^2 value enables the detection and correction of deviations from expected rates, ensuring efficiency and stability in real-time operations (Bhangare et al. 2022, Subash et al. 2022).

CONCLUSIONS

In the present study, the performance EF process for the removal of COD in the electroplating wastewater using stainless steel electrodes was analyzed, and the effects of pH, Fe^{2+} , and H_2O_2 were investigated. The cathode and anode, made up of stainless steel, were used. The observations of these investigations demonstrate the significance of selecting the ideal parameters to achieve high COD elimination efficiency, which is crucial for any practical applications. The operating variables of the EF process that impact the COD removal efficiency are assessed; at optimal conditions of pH 2, Fe^{2+} concentration of 0.005 M, H_2O_2 concentration of 0.5 M, and RPM of 450, the COD removal efficiency of 80.45%. The kinetics study obeys the pseudo-first-order kinetics with a precision of R^2 value of 0.9068. The results showed that the EF method can be applied successfully in COD removal. The developed treatment procedure with the optimal conditioning parameters was employed for the effective removal of COD from electroplating wastewater. There are certain drawbacks in the system, including the formation of iron sludge due to the Fenton reagents; therefore, the sludge is isolated and can be processed further without risk of contamination. Although the experiment was conducted on a lab scale, scaling up to the industrial level presents major challenges. Key areas needing further study include energy consumption, the durability and resistance of stainless steel and iron-based electrodes, and the handling and disposal of iron sludge, which can introduce secondary pollution concerns. Additionally, process optimization is required to address potential decreases in treatment efficiency due to sludge buildup, as well as to minimize operational costs associated with sludge management. Addressing these challenges is essential to ensure the process's feasibility and sustainability on a larger scale.

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Utilization of Jute Waste in Polybutylene Succinate-Based Biocomposites and Analysis of Mechanical Properties and Biodegradability

A. Sharma^{1,2}, S. Kulshreshtha^{1,5}✉, N. S. Rajput³ and A. Goyal⁴

¹Amity Institute of Biotechnology, Amity University, Jaipur-303002, Rajasthan, India

²Amity School of Applied Sciences, Amity University, Jaipur-303002, Rajasthan, India

³Amity School of Engineering and Technology, Amity University, Jaipur-303002, Rajasthan, India

⁴Department of Mechanical Engineering, Manipal University Jaipur, Jaipur-303007, Rajasthan, India

⁵Amity Centre for Water Studies and Research, Amity University Rajasthan, Jaipur-303007, Rajasthan, India

✉Corresponding author: Shweta Kulshreshtha, shweta_kulshreshtha@rediffmail.com

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ABSTRACT

A polybutylene succinate (PBS) composite reinforced with natural jute (*Corchorus olitorius*) fibers (50-80 µm) was investigated for its mechanical properties and biodegradability. This study aims to investigate the effects of fiber additions and size variations on composite performance and environmental sustainability. The PBS80/JF20 composite with 80 µm jute particles demonstrated the lowest MFI at 26 g.10 min⁻¹, significantly lower than that of pure PBS (p<0.05), indicating reduced flowability. Tensile strength decreased with the addition of jute fiber, reaching 21.4 MPa with 50 µm particles. Density was also reduced, with the lowest recorded at 1.27 g.cm⁻³ in PBS95/Jute5 (p<0.05). The composites with 80 µm fibers exhibited a slightly higher weight loss (9.5%) compared to those with 50 µm fibers (6.8%), likely due to insufficient interfacial adhesion in larger fibers, making them more susceptible to microbial degradation. Results indicate that adding natural jute fibers into the PBS matrix leads to significant decreases in melt flow index, tensile strength, and impact energy, while significantly enhancing density, water absorption, and biodegradability with respect to neat PBS. Further analysis of fiber sizes revealed that increasing fiber size (from 50 to 80 µm) results in a non-significant decrease in melt flow index, tensile strength, density, impact energy, water absorption, and biodegradation rates. These findings suggest that while the addition of natural fibers compromises mechanical properties, it significantly improves the environmental attributes of the composites, like water absorption and biodegradation (p<0.05). Fibers with a smaller diameter are preferable for maintaining mechanical integrity, while fibers with a larger diameter enhance biodegradability. The paper provides valuable insights into the development of a biocomposite material that balances mechanical performance with environmental sustainability.

INTRODUCTION

Jute caddies, known as jute processing waste, produce 40,000 tonnes annually in India (Nayak et al. 2011). It consists of non-spinnable fibers, batching oil, machine oil, grease, and bark from the jute plant. This waste has traditionally been disposed of in landfills or burned for boiler fuel. However, both methods are harmful to the environment (Hallerman 2019, Barai et al. 2020, Khandaker et al. 2024). These by-products can, however, be transformed into valuable resources through various approaches.

Jute (*Corchorus olitorius*) fiber waste can be repurposed into biodegradable composites, reducing dependence on synthetic materials and decreasing plastic pollution. Automobile manufacturers use jute fiber composites in parts like the structural bonnet of off-road vehicles (Alves et al. 2010, Naik et al. 2022). Moreover, jute waste can be reused in cement panels reinforced with recycled jute fibers (Ferrandez-García et al. 2020). This will improve properties, density,

flexural strength, and thermal conductivity. Jute waste is also beneficial to the agricultural sector because it produces biodegradable geotextiles that control soil erosion (Islam & Ahmed 2012). It is also used to develop biodegradable thermoplastic polyurethane semi-transparent films (Islam et al. 2024).

Developing eco-friendly fungicides from plant extracts, including jute, offers a sustainable solution to agricultural practices (Islam & Ahmed 2012, Hallerman 2019, Barai et al. 2020). In addition to contributing to sustainable development, jute waste can be converted into useful products, reducing environmental pollution, promoting a circular economy, and supporting rural livelihoods (Shanmugam 2021). The ecological benefits and innovative potential of jute waste utilization are driving forces for further research and development in this field (Rajan et al. 2022).

As mentioned above, developing new materials that meet specialized performance requirements while minimizing environmental impact is becoming an increasingly significant part of the research process with technological advancement (Regin et al. 2024). A promising class of such materials is polymer biocomposites, which combine a biopolymer matrix with a biodegradable filler to produce properties that are not possible with either component alone (Christian 2016, Elnashar et al. 2023). In this era, biocomposites are gaining attention due to both their economic and environmental benefits (Gurunathan et al. 2015, Bhat 2021, Andrew & Dhakal 2022). As a result of using biodegradable materials, plant fibers, and agro-industrial waste, biocomposites are a viable solution for reducing plastic waste and repurposing materials that would otherwise be disposed of in landfills or burned (Ortega et al. 2021, Moshood et al. 2022).

Poly(butylene succinate) (PBS) is a biodegradable aliphatic polyester, notable for its high processing ability, superior heat transfer characteristics, robust strength, and good compressive properties (Rafiqah 2021, Aliotta et al. 2022, Barletta et al. 2022). These attributes make PBS a suitable candidate for a wide range of commercial applications, including biomedical devices and food packaging, due to its biodegradability and non-toxicity (Łopusiewicz et al. 2021, Rafiqah 2021, Mtibe et al. 2023). However, the relatively high cost of PBS limits its widespread industrial use (Rafiqah 2021).

PBS composites with natural fibers not only reduce production costs but also minimize the environmental impact. These composites leverage renewable resources, decrease reliance on fossil fuels, and promote a reduction in plastic waste due to their biodegradability (Moshood et al. 2022). Consequently, PBS composites offer a more sustainable alternative for various industries, contributing to a greener

and more eco-friendly future (Mochane et al. 2021, Vázquez-Núñez et al. 2021, Barletta et al. 2022). Therefore, researchers focused on making PBS composites with natural fibers and agro-industrial wastes, aiming to reduce production costs while harnessing renewable resources.

Several natural fibers have been reported to be suitable for this purpose, including almond shell flour, rice husks, wheat bran, bamboo fibers, and sugarcane rind fibers. They offer low cost, availability, low density, a renewable and sustainable nature, biocompatibility, and environmental friendliness. Biocomposites prepared from natural fibers can exhibit enhanced mechanical, thermal, and tribological properties. In recent years, PBS has become a more readily available biodegradable material, allowing researchers to develop biodegradable composites made of PBS and natural fibers (Quiles-Carrillo et al. 2018, Yap et al. 2020, Jansiri et al. 2021, Sasimowski et al. 2021).

In recent studies, natural fibers are effective as reinforcement in polymer biocomposites. PBS composites containing almond shell flour have been shown to increase tensile strength, although elongation at break and tensile modulus were affected. The previous studies reported that biodegradation of PBS composites can be enhanced by sugarcane natural fibers (Quiles-Carrillo et al. 2018, Yap et al. 2020, Chamas 2021, Jansiri et al. 2021). The impact of rice husk and date palm fiber waste on the mechanical and biodegradable properties of biocomposites has also been studied, which showed important trade-offs between increased biodegradability and enhanced mechanical performance (Quiles-Carrillo et al. 2018, Yap et al. 2020, Chamas 2021, Jansiri et al. 2021).

A variety of factors influence the mechanical properties of biocomposites, including their fillers, processing conditions, and natural fiber dispersion (Mohammed 2023). Consequently, the selection of these factors is crucial when selecting natural fibers for biocomposite materials. Utilizing agrowaste as a filler in biocomposites has gained significant importance due to its potential to improve the physical, mechanical, thermal, and thermomechanical properties (Fayomi et al. 2020, Talabi et al. 2024).

Agro-waste materials, including plant fibers, agricultural residues, and food processing by-products, can enhance the strength, stiffness, toughness, and thermal stability of biocomposites, making them attractive for various applications (Das 2022). Moreover, incorporating agro-waste in biocomposites can yield economic and environmental benefits by reducing landfill waste and reliance on petroleum-based materials (Maraveas 2020, Ortega et al. 2021, Phiri et al. 2023). Recent research highlights the diverse applications and enhanced properties of biocomposites.

The addition of cassava pulp to poly (butylene succinate) (PBS) improves biodegradability and mechanical properties (Nithikarnjanatharn & Samsalee 2022).

Studies on poly(lactic acid) (PLA) composites filled with *Dalbergia sissoo* wood waste show significant improvements in physical, mechanical, and thermal properties (Singh et al. 2021). Agro-flour-filled PBS composites exhibit favorable biodegradability and mechanical performance (Sasimowski et al. 2023). PLA/PBS blends with cellulose fiber demonstrate potential for packaging applications. Hemp fibers and shives in PBS-based composites offer biodegradability, while bamboo and kenaf fibers enhance mechanical properties, revealing versatility and environmental benefits (Dönitz et al. 2023).

The present research study aims to develop and characterize composite materials composed of poly(butylene succinate) (PBS) bioplastic and fine jute fiber (JF) filler, focusing on their mechanical, surface morphological, and biodegradability properties. Biocomposites with varying JF content (0 to 20 wt%) will be synthesized to assess how different filler concentrations influence the material's properties. The goal is to identify the optimal jute fiber content that enhances the material's performance while maintaining superior biodegradability. This will contribute to sustainable material development and offer potential applications in environmentally friendly products.

MATERIALS AND METHODS

Collection of Material/Sample

In this study, a commercial injection grade of poly(butylene succinate) (PBS) (B3C03) from BioPBS Pvt Ltd, Gujarat, India, was used as the matrix material. This PBS has a melting temperature in the range of 120-200°C, a density of 1.25-1.50 g.cm⁻³, and a melt flow rate of 5 g.10 min⁻¹ at 120°C with a 2.0 kg load. The jute fiber filler was sourced from a local agricultural farm in Jaipur (Rajasthan), India.

Processing of the Sample

Before incorporation, the jute fibers were washed and dried at 80°C for 15 h. Jute fibers of various sizes, ranging from 50 µm to 80 µm, were mixed with the melted PBS matrix at varying concentrations, with a maximum of 20% jute fiber content.

In this study, PBS pellets were dried in a hot air oven at 80°C for 15 h to remove moisture. Jute fibers with diameters ranging from 50 µm to 80 µm were mixed with PBS to create composites with various weight ratios, from PBS100JUTE0 to PBS80JUTE20. The mixing process involved a twin-screw extruder operating at 150°C and a screw speed of

50 revolutions per minute, ensuring thorough blending of the PBS and jute fiber particles. After extrusion, the biocomposite strands were pelletized and cooled using a cold air circulation system. These pellets were then molded into biocomposite plates using a hand-operated injection machine, applying a pressure of 5 MPa at 150°C for two minutes. The injection rate for forming the biocomposites ranged from 20 cm³.s⁻¹ to 50 cm³.s⁻¹, facilitating uniform sample production for further testing and analysis (Singh et al. 2021).

Melt Flow Index

The melt (flow) index has become a widely established criterion for the extrudability of thermoplastic materials, especially polyolefins. It is standardized as the weight of polymer (polyolefin) extruded in 10 min at a constant temperature (190°C) through a tubular die of specified diameter (0.0825 inches) (Van Krevelen & Te Nijenhuis 2009). In this study, the Melt Flow Index (MFI) was measured as per ASTM D1238 standards (ASTM D1238-20). The measurement was performed using a melt flow index instrument (P.S.I Sales Pvt. Ltd., MFI-421/16) at 150°C with a load of 2.5 kg. The MFI value reported is the average of three independent measurements.

Composite Characterization

Mechanical testing: The mechanical evaluation will include tensile strength, compressive characteristics, and overall durability. Tensile strength and Young's modulus were measured according to the standard ASTM D638 (ASTM D638 [no date]). A universal testing machine (UTM) was used to evaluate the mechanical properties, with a cross-head speed of 50 mm.min⁻¹ and a load cell that could measure up to 10 kN. The test specimens were made according to the dimensions specified in the standard, with a total length of 115 mm, a thickness of 3.2 mm, and a middle length of 33 mm in the shape of dumbbells. An Izod impact strength test was conducted on PBS/JF biocomposite specimens according to the ASTM D256 standard (43), with the samples kept at room temperature. The test was conducted using a pendulum impact tester. In the present study, hardness was measured for the PBS/JF composites according to the ASTM D 2240 (44) international standard. The Shore D hardness value was determined using specimens with a length of 63.5 mm and a thickness of 3.2 mm.

Biodegradability properties: The biodegradability was examined through natural soil burial tests, assessing the degradation of the biocomposites by determining weight loss. The rectangular specimens with dimensions of 50 mm in length, 2 mm in thickness, and 10 mm in width were fabricated by the injection molding method with injection

temperature and pressure of 170-190°C and 25MPa. The prepared samples were buried in soil for 50 days according to the method described by Kim et al. (48). The specimens were placed and buried in the backyard at a depth of 25 cm below the surface of the topsoil. After being buried for the mentioned time: 0, 7, 15, 25, 30, 40, and 50 days (48), the specimens were taken out and washed with distilled water before being dried in an oven with hot air at 50°C for one day. The percentage of weight loss was then calculated using a digital scale, and the result was determined using the following equation:

$$W_{\text{loss}} = W_{\text{initial}} - W_{\text{final}} / W_{\text{initial}} \times 100\% \quad \dots(1)$$

Where initial and last represent the initial day and final day of the composite buried in the soil for testing.

Water absorption properties: In this study, the gravimetric method was used to determine the density and water absorption of pure PBS and PBS/JF composites. Three samples were tested for water absorption. The samples were weighed accurately on a scale and then submerged in water to determine their volume. For the water absorption test, test samples of biocomposites were soaked in water for 15 days. The samples were then allowed to drain for a minute before being dried with a towel and air-dried until they were completely surface-dry. The experiment was run in triplicate. The weight of each sample (Wt) was recorded, and the volume of water absorbed was calculated. The water absorption (WA) performance of PBS/JF biocomposite samples was evaluated by measuring the difference in weight between the composite and the pure PBS sample using the following equation.

$$\text{Biocomposite } W_{\text{absorption}} = \frac{W_{\text{final}} - W_{\text{initial}}}{W_{\text{initial}}} \times 100 \quad \dots(2)$$

Density determination of PBS/jute fiber composite: Density was determined according to the ASTM D 1895-17 (2017) standard. Five replicates were used for density determination. The density of the sample was calculated using the following equation:

$$\text{Density (g.cm}^{-3}\text{)} = m.v^{-1} \quad \dots(3)$$

Where, m represents mass and v for volume (Saffian 2021).

Statistical Analysis

The results are presented as mean $\pm$ SD of three replicates. Statistical analysis was done by one-way ANOVA. Further, significant results ($p < 0.05$) were compared by post-hoc test, like Tukey's Multiple Comparison Test and pairwise T-Test, significant (ANOVA Calculator | AAT Bioquest [no date]). For statistical analysis, two-way ANOVA is also performed to compare the results of PBS with its jute composites using SPSS software.

RESULTS AND DISCUSSION

Melt Flow Index

The melt flow index (MFI) of PBS/JF biocomposites with jute fiber diameter sizes of 50 to 80 μm was found to be in the range of 25~47 g.10 min^{-1} , which is shown in Fig. 1.

The results from the Melt Flow Index (MFI) tests reveal a notable decrease in the flow behavior of PBS plastic following

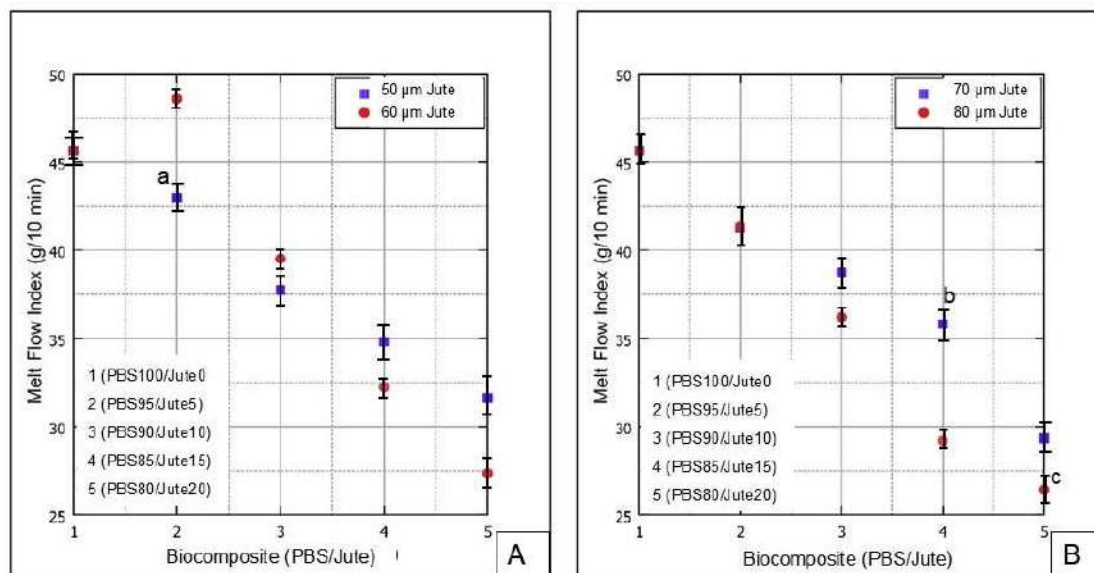


Fig. 1: Melt flow index of jute fiber reinforcement biocomposites (A) 50-60 μm (B) 70-80 μm . One-way ANOVA was used to test the treatment effect. Small letters indicate significant differences across treatments tested using post hoc Tukey's HSD test. All values were taken as significant at $p < 0.05$.

the introduction of jute fibers of different sizes, ranging from 50 to 80 microns. Fig. 1 illustrates the MFI values for Jute fibers at 50, 60, 70, and 80 μm . Notably, the size of the jute fibers correlates inversely with the MFI values, as depicted in Fig. 1(a) and 1(b). Specifically, the MFI is at its lowest when using 80 μm jute fibers, particularly evident in PBS80Jute20 composites. This trend of decreasing MFI with increasing amounts of jute fibers was observed. The minimum MFI was observed when the jute fiber percentage reached 20% (PBS80Jute20 composites). However, the size of jute particles did not affect the MFI significantly with respect to the particle sizes. This shows that the selected particle size range did not impose any significant effect on the developed composites.

Jute fibers with a smaller diameter exhibited higher homogeneity and compatibility within the polymer matrix, as demonstrated by their even distribution. This was supported by the result of the melt flow index test, which showed that the PBS80/JF20 sample with 80 μm particles had a minimum MFI of $26 \text{ g} \cdot 10 \text{ min}^{-1}$, significantly lower than that of pure PBS material ($p < 0.05$). This difference can be attributed to the mixing of jute fibers, which enhanced the discontinuous mixing of both PBS and jute fibers in the composite. A similar finding was reported in previous studies stating that increased fiber content of the composite resulted in lower MFI (Soleimani et al. 2008).

Mechanical Properties

The tensile strength and Young's modulus of the PBS/JF

biocomposites are shown in Fig. 2, which gives an idea of the mechanical properties of the composites. The pure PBS exhibited a tensile strength of 27.2 MPa. With particle sizes reduced to 50 μm , PBS/JF biocomposites showed a tensile strength between 21 MPa and 27 MPa, with jute fibers accounting for 10 to 20% of the mass. Adding jute fibers to PBS resulted in a significant ($p < 0.05$) decrease in tensile strength of 21 MPa. At 80 μm particle size, PBS80/Jute20 has low tensile strength. However, the lowest tensile strength, 21.4 MPa, was reported with the addition of 50 μm particle size of jute fibers (PBS80/Jute 20). This is possibly due to the smaller particle size of jute fibers being more compatible with the matrix composite. However, the selected particle sizes (50–80 μm) were too small to pose a significant effect on tensile strength ($p > 0.05$). Similarly, in previous reports, it was reported that there is a strong relationship between the tensile strength of composites and the fiber/matrix adhesion, and low adhesion resulted in a decrease in tensile strength and elongation at break (Liu et al. 2009).

The tensile strength of bioplastics decreased as the jute fiber percentage increased, regardless of jute fiber size. The tensile strength of pure PBS was superior to that of PBS/JF biocomposites. The decrease in tensile strength of the biocomposites when the jute fiber content increased may be due to the stiff interface between the fibers and matrix, which had less ability to deform (Terzopoulou et al. 2016, Jansiri et al. 2021, Vorawongsagul et al. 2021).

These findings are similar to those reported by (Kim et al. 2005) in their study on rice husk powder mixed with

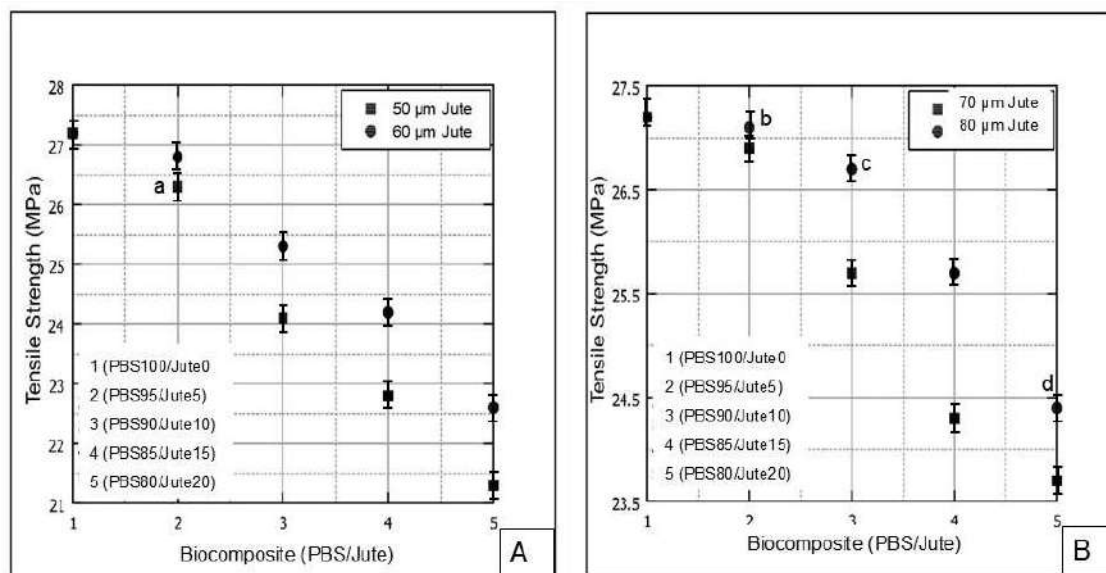


Fig. 2: Tensile strength of PBS/jute fiber compositions, a) 50 μm and 60 μm jute fibers, b) 70 μm and 80 μm jute fibers. One-way ANOVA was used to test the treatment effect. Small letters indicate significant differences across treatments tested using post hoc Tukey's HSD test. All values were taken as significant at $p < 0.05$.

PBS matrix materials. Similarly, our results showed that the tensile strength of the composite increased with decreasing the size of jute fibers.

Impact Strength and Energy

Impact strength is expressed in terms of impact energy. Impact energy describes the ability of biocomposites to absorb the force of an impact, which is related to the tenacity of biocomposite materials. The addition of jute fibers in PBS led to a significant decrease in impact energy and strength of biocomposites ($p > 0.05$). As shown in Fig. 3, the impact energy of PBS80/JF20 (2.7 KJ.m^{-2}) biocomposites containing $50 \mu\text{m}$ jute fibers was non-significantly ($p > 0.05$) lower than biocomposites containing $80 \mu\text{m}$ jute fibers (3.2 KJ.m^{-2}). However, PBS100/Jute0 possessed higher impact energy (4.2 KJ.m^{-2}) compared to the jute composite ($p < 0.05$).

The impact energy of each PBS/Jute fiber composite was lower than the impact energy of pure PBS, suggesting that pure PBS has good ductility due to its high impact strength. The fracture became more brittle when the concentration of jute fiber in the biocomposites increased up to a maximum of 20%. These results were found to be in contrast with the strength of treated jute fiber-based PBS composites developed by Liu et al. (2009). Fig. 3 shows the effect of particle size of jute fibers on PBS-based composites. The highest strength of PBS80/Jute20 was observed with $80 \mu\text{m}$ particles, revealing the strength of the polymer. This is possibly due to the high bonding ability of larger jute particles.

Density of PBS/Jute Fiber Composites

The densities of manually injected PBS/JF biocomposites are presented in Table 1. The average density of pure PBS was found to be 1.26 g.cm^{-3} . By the injection molding method, PBS molecules were aligned in the direction of flow, resulting in high density.

The addition of jute fiber with particle sizes of $50 \mu\text{m}$ to $80 \mu\text{m}$ resulted in a significant decrease in the density of the PBS matrix with increasing particle size ($p < 0.05$). The lowest density was observed with PBS95/Jute5, i.e., 1.27 g.cm^{-3} ($p < 0.05$) (ANOVA Calculator | AAT Bioquest [no date]).

The non-uniform molecular alignment of the molten polymer and Jute fibers of $80 \mu\text{m}$ is responsible for the formation of gaps within the biocomposites, leading to a decrease in composite density. In PBS biocomposites, the particle size of the reinforcing material, like jute fibers, affects the density of composites ($p < 0.05$). The small particles tend to have higher densities due to better packing efficiency and reduced porosity. The composite also has enhanced density and mechanical properties due to improved interfacial adhesion and a larger surface area for smaller particles (Saffian et al. 2021).

The highest density was observed with PBS80/Jute20, i.e. 1.68 g.cm^{-3} for $50 \mu\text{m}$ jute fibers. The trend of increasing density was observed with increasing the amount of Jute fibers. The possible reason is the uniform distribution of a high amount of jute fibers throughout the PBS matrix.

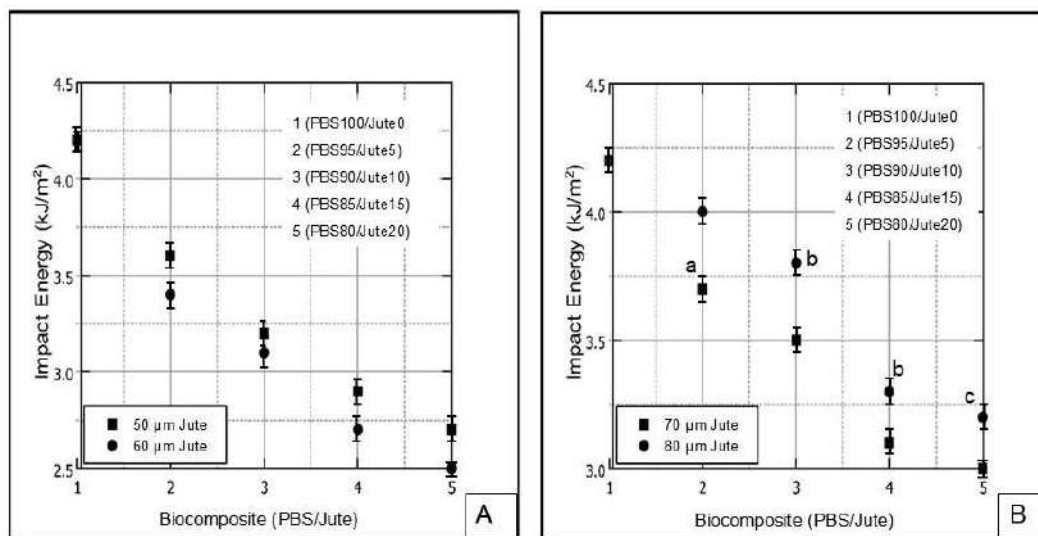


Fig. 3: Impact energy analysis of PBS/JF compositions: a) $50 \mu\text{m}$ and $60 \mu\text{m}$ jute fibers, b) $70 \mu\text{m}$ and $80 \mu\text{m}$ jute fibers. One-way ANOVA was used to test the treatment effect. Small letters indicate significant differences across treatments tested using post hoc Tukey's HSD test. All values were taken as significant at $p < 0.05$.

Table 1: Density of the PBS/Jute biocomposite.

PBS/Jute Ratio	50 μm	60 μm	70 μm	80 μm
PBS100/Jute0	1.26 $\pm$ 0.0	1.26 $\pm$ 0.0	1.26 $\pm$ 0.0	1.26 $\pm$ 0.0
PBS95/Jute5	1.35 $\pm$ 0.01	1.31 $\pm$ 0.01*	1.29 $\pm$ 0.01*	1.27 $\pm$ 0.01*
PBS90/Jute10	1.49 $\pm$ 0.01	1.39 $\pm$ 0.02*	1.34 $\pm$ 0.02*\$	1.32 $\pm$ 0.01*\$
PBS85/Jute15	1.54 $\pm$ 0.01	1.42 $\pm$ 0.01*	1.39 $\pm$ 0.02*\$#	1.37 $\pm$ 0.02*\$#
PBS80/Jute20	1.68 $\pm$ 0.02	1.58 $\pm$ 0.01*	1.45 $\pm$ 0.01*\$#	1.40 $\pm$ 0.03*\$#

Density ($\text{g}\cdot\text{cm}^{-3}$) ($p < 0.05$); * $p < 0.05$ density of 50 μm vs. Density of other particle size (60 /70/80 μm); \$ $p < 0.05$ density of others vs. Density of (PBS90/Jute10); # $p < 0.05$ density of others vs. Density of (PBS85/Jute15)

Table 2: Water absorption properties of Jute fiber-reinforced matrix.

PBS/Jute Ratio	50 μm	60 μm	70 μm	80 μm
PBS100/Jute0	0.25 $\pm$ 0.01	0.25 $\pm$ 0.01	0.25 $\pm$ 0.01	0.25 $\pm$ 0.01
PBS95/Jute5	0.38 $\pm$ 0.21	1.19 $\pm$ 0.31	1.23 $\pm$ 0.14	1.32 $\pm$ 0.2
PBS90/Jute10	1.45 $\pm$ 0.2	1.62 $\pm$ 0.23	2.1 $\pm$ 0.12	2.20 $\pm$ 0.21
PBS85/Jute15	1.89 $\pm$ 0.12	2.12 $\pm$ 0.24	2.36 $\pm$ 0.2	2.47 $\pm$ 0.13
PBS80/Jute20	2.18 $\pm$ 0.15	2.45 $\pm$ 0.12	2.78 $\pm$ 0.25	3.22 $\pm$ 0.26

$P < 0.5$ is significantly different compared to PBS (ANOVA Calculator | AAT Bioquest [no date])

Water Absorption

The pure PBS sample showed a non-significant increase (0.25%) in its weight due to the absorption of water (Table 2). Water absorption values of PBS/jute fiber

composites increased with increasing the size of jute fibers ($p > 0.05$), revealing the void spaces in the PBS matrix due to the large size of jute fibers (80 μm). Increasing the amount of jute fibers in the matrix, the water absorption increased ($p > 0.05$). The possible reason for this is the formation

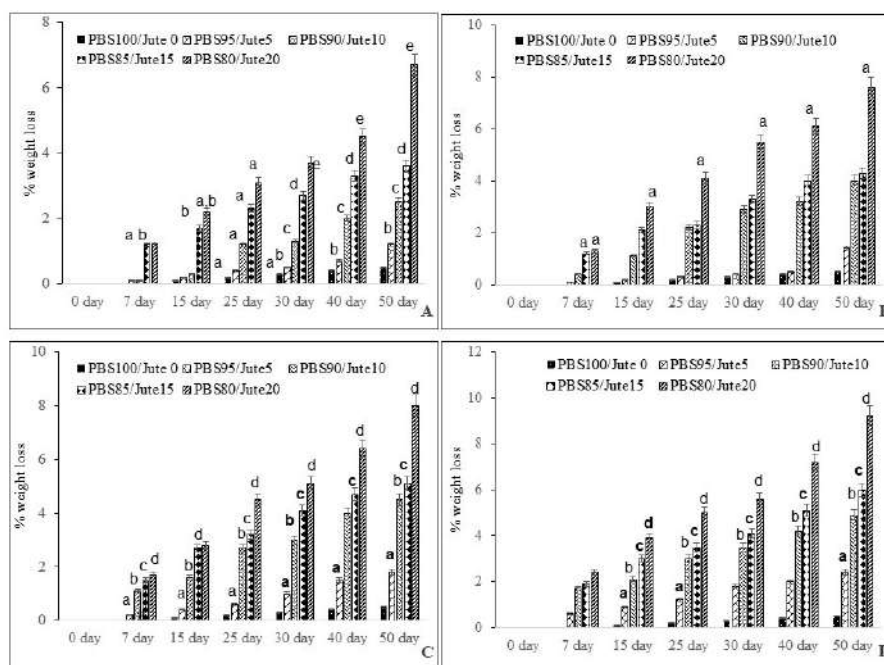


Fig. 4: The biodegradability of the PBS/JF composites is shown in Fig. 4 (a) to 4 (d) for 50 μm to 80 μm jute fibers. Two-way ANOVA was used to test the treatment effect. Small letters indicate significant differences across treatments tested using post hoc Tukey's HSD test. All values were taken as significant at $p < 0.05$.

of void spaces in PBS due to the addition of jute fibers (Table 2). However, with respect to PBS, the addition of jute fibers resulted in a significant increase in the water absorption properties. Water absorption increased significantly with an increase in the content of jute fibers ($p < 0.05$) (Table 2).

Biodegradability Study

The percentage of weight loss of PBS and jute fiber biocomposites after being buried in the soil for 0, 7, 15, 25, 30, 40, and 50 days for different particle sizes (50 to 80 μm) is shown in Fig. 4. There was a general trend where the percentage of weight loss of PBS/Jute fiber biocomposites increased with increasing jute fiber content in PBS/Jute-based biocomposites.

In this study, the weight loss of PBS/Jute fibers-based biocomposites was calculated for different particle sizes of jute fiber after being buried in the ground for 15 days. It was found that PBS/Jute fiber composites with a particle size of 80 μm had a slightly higher percentage of weight loss (9.5%) than those with a particle size of 50 μm (6.8%). This was due to insufficient interfacial adhesion between the jute fibers and the PBS matrix due to the large size (80 μm) of the jute fibers, resulting in a PBS composite that was easily degraded by microorganisms. However, the effect of fiber size is not significant. It was observed that the weight loss of PBS/Jute fibers biocomposites non-significantly decreased with a decrease in the size of jute fibers (50 μm) ($p > 0.05$). This was attributed to the superior internal structure of the composite reinforced with finer fibers (Sasimowski et al. 2021).

It was also found that the amount of weight loss was directly proportional to the amount of jute fibers added, with the highest weight loss observed at a jute fiber content of 20%, irrespective of fiber sizes (PBS80/Jute20). Biodegradability significantly increased with increasing jute fiber content with respect to PBS ($p < 0.05$). Jute fiber, a natural fiber with hydrophilic properties, facilitates water absorption and increases its biodegradability by making it susceptible to microbial action. The contact between water and PBS increased in the presence of jute fibers, leading to hydrolysis of the polymer and a reduction in weight due to the action of microorganisms in the natural soil burial process. This decrease in weight resulted in a weight loss percentage (Sasimowski et al. 2021, Nithikarnjanatharn & Samsalee 2022).

PBS-based biocomposites made from jute fiber waste have a wide range of applications. Since these composites are biodegradable and promote the circular economy, they play an important role in protecting the environment. Our study shows that the addition of jute fiber waste to PBS significantly enhances mechanical properties such as tensile

strength, impact energy, density, and water absorption properties, as well as biodegradability. The developed composite will address a key challenge in replacing traditional plastics. In addition to diverting waste from landfills, this approach will reduce dependence on natural resources. Jute waste-based biocomposites can be converted into useful products such as tableware, which promote the circular economy. By optimization of biodegradation, the lifecycle of these composites can be optimized with the minimization of waste. Using jute waste as a filler material in developing PBS-based biocomposites offers several cost advantages over synthetic alternatives. Jute waste, being an agricultural byproduct, is both abundant and relatively inexpensive compared to synthetic fillers such as glass fibers, carbon fibers, or mineral-based reinforcements, which often involve higher processing and transportation costs. Integrating jute waste into biocomposites not only minimizes material costs but also reduces the need for disposal methods like incineration or landfilling, leading to savings in waste management.

Jute-based composites are an important sector for biocomposite applications such as tableware. It is a suggested area where the findings of this study can be implemented due to its requirements for sustainability, biodegradability, and moderate mechanical properties. The major advantage of using jute waste processing generally requires lower energy and fewer chemical treatments, decreasing overall manufacturing expenses. In terms of product lifecycle, the biodegradability of jute-based composites can reduce costs associated with end-of-life disposal, especially for products with a limited lifespan. However, challenges in ensuring consistent mechanical properties, due to natural fiber variability, might necessitate additional quality control processes, which could offset some cost savings. Overall, adopting jute waste as a filler enhances the sustainability of biocomposites while providing an economical alternative to synthetic fillers, particularly in applications where moderate mechanical strength and biodegradability are prioritized over high-performance material requirements.

CONCLUSIONS

This study evaluated the mechanical properties and biodegradability of polybutylene succinate (PBS) composites reinforced with natural jute fibers of varying sizes (50–80 μm). It has been found that the incorporation of jute fibers into the PBS matrix non-significantly reduces the melt flow index, tensile strength, or impact energy compared to PBS. However, jute fiber-based composite significantly increases density and biodegradability when compared with PBS. Fiber size did not impose any significant impact on the melt flow

index, tensile strength, or impact energy. This study also examined the effect of jute fibers' size in the range of 50 to 80 μm to show the impact of larger fiber sizes (80 μm) on the mechanical properties of PBS/jute biocomposites. Larger fiber sizes (80 μm) resulted in non-significant decreases in melt flow index, tensile strength, density, and impact energy. However, a significant increase in water absorption and biodegradation rates was observed with the larger (80 μm) jute fibers. It has been found that adding natural jute fibers to PBS composites does not reduce some mechanical properties ($p > 0.05$), but significantly affects environmental performance properties, such as water absorption and biodegradability. The variations in fiber size indicated that smaller fibers may be preferable for maintaining mechanical integrity, whereas larger fibers enhance biodegradability. These insights are valuable for designing and developing sustainable biocomposite materials and balancing mechanical performance with environmental benefits.

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Assessment of the Carbon Credit Potential of an Electric Vehicle Charging Station

Shrawani Uday Chaudhari^{1†}, Abdulwahhab Jasim Mahdi² and Ashish Vilas Mane¹

¹Department of Environmental Science, Fergusson College, Pune-411004, Maharashtra, India

²Environmental pollution Department, College of Environmental Sciences, Al-Qasim Green University, Babylon 51013, Iraq

†Corresponding author: S. U. Chaudhari; shrawanichaudhari293@deccaneducationsociety.org

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ABSTRACT

The electric vehicle charging station (EVCS) based carbon credit potential in the present study was evaluated for the Shell Recharge Charging Station at Pimple Nilakh, Pune, Maharashtra. The objective was to determine whether carbon credits could provide an incentive for the establishment of EVCSs. The calculations were carried out with the Verified Carbon Standard (VCS) Methodology VM0038 for Electric Vehicle Charging Systems, Version 1.0. We show that the system can produce about 191 carbon credits per year. These credits are worth a potential monetary value of up to ₹ 7,96,947.50 in the Indian voluntary market, and up to ₹ 9,92,372.74 in the international compliance market, depending on the prevailing carbon credit values. This is at a maximum of approximately 31% more value than the annual profit generated from the charging station in the Indian voluntary market. Therefore, these carbon credits have the potential to be a useful way to speed up the adoption of green mobility by rewarding the creation of electric vehicle charging infrastructure. The results of this assessment showed the potential of EVCSs to provide sustainable transportation support and generate economic benefits through carbon credit monetization. The present work highlighted the need to standardize the validation and trading of carbon credits generated from green mobility.

INTRODUCTION

There is unequivocal warming of the climate system (Qin et al. 2013). In the past century, NASA has reported a 1°F temperature rise, resulting in things like droughts and intense hurricanes (Kaddo 2016). Since 1750, human activities, specifically fossil fuel use, have caused a 50% CO₂ increase (UCAR Center for Science Education 2024, NASA Science 2024, Riebeek & Simmon 2010). In 2019, the transport sector was responsible for 2,277.63 million metric tons of CO₂ in India, up 375% from 1990. By 2030, emissions could increase by 65 percent and 197 percent by 2050, with road transport the largest emitter. As a result, (Riehle et al. 2023) to overcome this challenge, the Indian government also introduced initiatives such as FAME II with greater EV subsidies, the PLI scheme for Advanced Cell Chemistry (ACC) battery storage for increased adoption of EVs (Sinha & Sharma 2021). India's current energy investments take up about 3% of its GDP. This is especially notable considering that corporate and financial institutions have also funded renewable energy initiatives at unprecedented levels, rising to a record of USD 14.5 billion in the fiscal year 2021–2022, a 72% increase from the pre-pandemic period of 2019–2020. EVs emit less than gasoline cars even when manufacturing and charging are taken into account (Annual G20 Report 2022). Renewable sources of energy, such as wind and solar, can still reduce the amount of greenhouse gas emissions generated by electricity generation for EVs, but that doesn't necessarily mean they'll eliminate it. The emission reductions from EVs

may exceed those of gasoline cars as renewable energy costs fall (EPA US 2023, Moseman 2022).

Carbon trading is a carbon market that allows businesses to buy and sell carbon credits. One credit equals a ton of CO₂ or equivalent gases. The Chicago Climate Exchange, European Climate Exchange, and soon India's MCX are all major exchanges. The United Nations' Kyoto Protocol began officially in 1997 with the introduction of Certified Emission Reduction Certificates (CERs) (Yuvika 2011). The Carbon Credit Trading Scheme (CCTS) was born in 2023, and the Green Credits Program is currently in draft and operates voluntarily. There are two primary carbon markets (Dhrishti IAS 2023). In Voluntary Markets, companies are willing to offset their carbon emissions to meet sustainability goals. In 2020, verified credits brought in over \$2 billion, and big names like Google, Apple, and Microsoft took part. (CHOOOSE 2023, Dhrishti IAS 2023). Policies such as the 'cap and trade' system govern Compliance Markets. In Q1 2021, Tesla sold carbon credits to traditional manufacturers, earning USD 518 million. Companies might buy billions of carbon credits per year to restore trust, pushing prices to \$238, market value over \$1.1 trillion by 2050 (Jennifer 2024), and 53% of Forbes 2000 net-zero companies are planning to use carbon credits (Eikelenboom et al. 2024). BEE and MoEFCC will manage India's Compliance Carbon Market, which is expected by 2026, covering 11 sectors (Yin 2023). Carbon credit values are higher in compliance markets than in voluntary ones (Carbon Offset Guide 2024). This is supplemented by the Green Credits Program, which promotes eco-friendly activities (Shukla 2024). In 2023, Evansa installed South Australia's first carbon credit-generating EV charging station in accordance with the Verra measurement standard (Noodoe 2023). Unlike other EV charging stations that require grid upgrades to handle the load, the Indian Oil solar-powered EV stations reduce load by 80% and tap into a \$15 billion carbon credit market (Gupta 2020).

This research determines the potential use of an EV charging station (EVCS) in Pune to mitigate greenhouse gas emissions and generate carbon credits. It quantifies CO₂e emissions avoided and evaluates whether the credits can provide an incentive to more stations to support sustainable transportation and clean energy infrastructure in India. Davis et al. (2010) point out that existing energy infrastructure is poised to emit a considerable amount of carbon dioxide by 2060, and substantial warming is unavoidable in the absence of extraordinary efforts to develop alternative solutions and to constrain the expansion of CO₂-emitting infrastructure. As we showed in Gupta et al. (2019), even with a high investment cost, low-carbon scenarios (such as EVs) can achieve performance close to or better than our business-as-usual scenario, and accommodate yearly economic growth of

5.8% from 2013 to 2050. Srikanth (2018) recommended that accelerating the progress of electric vehicles in India will lead to job creation in manufacturing, with a substantial lowering of vehicular emissions. Maharashtra's growing number of EV charging stations, due to government incentives, was mentioned by Jadhav et al. (2023). India's challenges to adopting EVs, such as high costs and limited infrastructure, were also highlighted by Kumar et al. (2020). To address deployment concerns, Pareek et al. (2020) reviewed EV charging station guidelines and technologies. Paul Sathiyar et al. (2022) suggested high high-polluting vehicle segments be targeted for EV adoption. According to Munshi et al. (2022), policy incentives are important, while Jois and Thimmaiah (2023) examined the role of carbon credits in developing economies. Carbon credits can boost EV industry revenue, according to Singh (2024). India's carbon trading future was explored by Paul (2010) and Sarkar & Dash (2011), while Rajput et al. (2018) and Garg & Arya (2015) have advocated for public awareness and a particular statute governing carbon credit contracts. According to Prabhakant & Tiwari (2009), the Government of India can earn ₹1901 million annually, while Subramanian & Balasubramanian (2022) suggest the use of carbon credits for renewable charging infrastructure.

Carbon Footprint of EV Charging Stations

Production and transportation of construction materials (concrete, steel) and equipment during station setup cause carbon emissions. The energy source used to power the station determines how the station will emit. The fact is that renewable energy reduces emissions, while fossil fuel-based electricity increases the footprint substantially. Additionally, carbon emissions are generated from the periodic repair, replacement, and energy for diagnostic testing and maintenance activities.

The objective of this work is to evaluate the carbon credit potential of an electric vehicle charging station (EVCS) through the application of the Verified Carbon Standard (VCS) methodology. The importance of carbon credits as financial rewards to accelerate the development of EV infrastructure is a focus. The research demonstrates the importance of this work to India's energy policies and sustainability goals, in line with the country's carbon reduction and economic growth commitments related to green mobility.

MATERIALS AND METHODS

Data were collected from multiple sources for this study. Carbon credits and EV charging stations (EVCSs) were reviewed in the context of research papers. The electricity

consumption data was based on visits to the Shell Electric Recharge Station. Emission factors were obtained from the India GHG Program (2023) and the Ministry of Power reports. We obtained EV sales data for 2023 from autopundit.com, battery size, ARAI range for EVs, and petrol vehicle mileage from manufacturer websites.

Carbon credits were calculated based on the Climate Neutral Business Network's use of the Verified Carbon Standard (VCS) Methodology, VM0038 for EV Charging Systems, in 2018. Since September 2018, this methodology has been available for study by scientists from various fields. In July 2020, SCS Global Services announced the first EV charger carbon offset project validated using this methodology. The VCS program is now managed by Verra, and is the most widely used voluntary greenhouse gas program, having reduced over 200 million tonnes of emissions, and is the only voluntary greenhouse gas program to be recognized as independent by the climate community.

EV charging systems are the focus of the VM0038 methodology, which quantifies emission reductions by replacing fossil fuel vehicle emissions with electricity. The monitoring parameters are based on CDM tools for additionality and emission reductions, and India is qualified under additionality as its EV market penetration is low. EV miles driven are calculated based on kWh consumption, and electricity delivered from project chargers is measured. Emissions and fuel consumption are estimated using comparable fossil fuel vehicles. The regional fuel emission factors are used to calculate GHG emissions avoided, while project emissions are calculated by multiplying the electricity consumed by the EVs with regional GHG factors. Finally, the carbon credits are calculated as the difference between the baseline and project emissions.

In this paper, we assume the use of average vehicle models that represent Indian traffic and MPG ratings from government/industry data. Baseline emissions are calculated from standard factors for internal combustion engines, and project emissions for EVs from the grid energy mix. They allow for a consistent comparison and more accurate data and ground reality will deliver better results.

$$BE_y = \sum_{i,f} ED_{i,y} * EF_{f,y} * 100 * IR_i^{y-1} / (AFEC_{i,y} * MPG_{i,y}) \quad \dots(1)$$

Where:

BE_y = Baseline emissions in year y (tCO₂e)

$ED_{i,y}$ = Electricity delivered by project charging systems serving applicable fleet i in project year y (kWh)

$EF_{f,y}$ = Emission factor for the fossil fuel f used by comparable fleet vehicles j in year y (tCO₂e/gallon)

IR_i^{y-1} = Technology improvement rate factor for applicable fleet i

$AFEC_{i,y}$ = Weighted average electricity consumption per 100 miles rating for EVs in applicable fleet i in project year y (kwh/100 miles)

$MPG_{i,y}$ = Weighted average miles per gallon rating for the fossil fuel vehicles comparable to each EV in the applicable fleet i, in project year y (miles per gallon)

$$AFEC_{i,y} = \sum_a (EV_{a,i,y} * EVR_{a,i,y}) / \sum_a EVR_{a,i,y} \dots(2)$$

Where:

$EVR_{a,i,y}$ = Electricity consumption per 100 miles rating for model a EV in applicable fleet i in project year y (kWh/100 miles)

$EVR_{a,i,y}$ = Total number of model a EVs in the applicable fleet i on the road by project year y (cumulative number of EVs)

$$MPG_{i,y} = \sum_a (MPG_{a,i,y} * EVR_{a,i,y}) / \sum_a EVR_{a,i,y} \dots(3)$$

Where:

$MPG_{a,i,y}$ = Mile per gallon rating for the fossil fuel vehicle model deemed comparable to each EV model from applicable fleet i in project year y (miles/gallon)

$EVR_{a,i,y}$ = Total number of EV models within applicable fleet i on the road by project year y (cumulative number of EVs)

Project Emissions

$$PE_y = \sum_{i,j} EC_{i,j,y} * EFkw_{i,j,y} \quad \dots(4)$$

Where:

PE_y = Project emissions in year y (tCO₂e)

$EC_{i,j,y}$ = Electricity consumed by project chargers sourced from region j serving applicable fleet i in project year y (kwh/year)

$EFkw_{i,j,y}$ = Emission factor (average) for the electricity sourced from region j consumed by project charging systems serving applicable fleet i in year y (tCO₂e/kWh)

Net GHG Emission Reductions and Removals

Net GHG emission reductions must be calculated as follows, including the application of a discount factor. D_y to adjust pro rata where EV fleet credits have been issued within the project region:

$$ER_y = (BE_y - PE_y - LE_y) * D_y \quad \dots(5)$$

Where:

ER_y = Net GHG emissions removals/reductions in year y (tCO₂e)

LE_y = Leakage in year y (tCO_2e)

D_y = Discount factor to be applied in year y (%)

Where:

$$D_y = ERC_y(ERF_y + ERC_y) \quad \dots(6)$$

Where:

ERC_y = Sum of GHG credits issued by all projects under this methodology (or others which support the introduction of EV charging systems) across this project's applicable fleet i categories within this total project region in project year $y-1$ (tCO_2e)

ERF_y = Sum of GHG credits issued by all projects under methodologies that support the introduction of EV fleets located within this project's total region, where the applicable fleet i categories are the same for both this EV charging system project and projects introducing EV fleets 21, in project year $y-1$ (tCO_2e)

Where no GHG credits have been issued for projects that introduce EV fleets in the EV charging system project's region, D_y will be 1 (i.e., there is no discount applied).

Case Study Design

In this work, we assume an electric vehicle charging station in Pune. This station was selected because of its simple design, high visibility, and consumer-trusted brand. In addition, the charging station has minimal staff requirements as it is a self-service charging station for the customer.

Pimple Nilakh, Pune, Maharashtra, India; Shell Recharge Charging Station. The Shell fuel pump is in the same premises, and the charging station is alongside it in Fig. 1. The car parking bays and two ports for charging with one DCFC occupy roughly a 200 sqft area, as shown in Fig. 2.



Fig. 1: Shell Recharge Charging Station Area for Cars.

Table 1: Chargers at Shell recharge charging station.

Vehicles Charged	Charger	Max power	Quantity
Cars supporting CCS 2	DCFC	120kW	1 (with 2 ports)
2-wheelers	AC	3.3kW	2
3-wheelers	AC	3.3kW	1

Referring to Table 1 and Fig. 3., for our system, we shall consider only the 120kW DC fast charger. It has two CCS 2 (Combined Charging System Type 2) ports. This charger can share the load among the ports, i.e., it can split power to charge two cars simultaneously.

The charging station operates 24/7 but serves about 40 cars daily, with varying power usage per charge based on battery size. Assuming each car charges for 100 miles, daily consumption is 788.4 kWh, equating to 6.57 h of DC charging. The 120 kW charger operates for 6 hours daily.

Several key components are covered by the project boundary according to the VM0038 methodology. It includes the electric vehicle (EV) car models used for calculating the average electricity consumption (AFEC) and the miles per gallon (MPG). Second, it indicates the geographical area in which the EV charging station is located. Third, it includes the EV charging system itself (including electricity supply sources and related infrastructure for its operation). Lastly, Table 2 shows the scope of emissions considered.

Assumptions and Limitations

The analysis is conducted for simplicity, assuming the fleet is made up entirely of BEV LDV 4-wheelers. The lack of data on road EV models in Pune and India makes the 2023 national sales data (India is a nascent EV market with less than 5% penetration) used. If a given model is a petrol



Fig. 2: 3.3 kW AC Chargers for 2-wheelers and 3-wheelers.



Fig. 3: 120 kW DCFC with 2 CCS 2 ports.

manual, petrol manual vehicles are chosen as comparable models, and if a given model is not a petrol manual, a similar-sized model from the same manufacturer is used. MPG and AFEC values have been derived from ARAI ratings and are adjusted by 30 percent to factor in the difference in real-world performance. CO₂ emissions are used to calculate baseline emissions, and equivalent factors are not available for other gases. The discount factor is set equal to one, as no GHG credits are issued for EV fleets, and leakage is considered zero, as per VM0038 methodology. For LDVs, the technology improvement rate is set to the default value of one, and the petrol emission factor from 2015 is used, as these factors are not overly sensitive to time.

RESULTS AND DISCUSSION

AFEC and MPG Calculation

Table 3 shows the highest-selling EV models and their numbers in 2023.

Table 2: Gases considered for baseline emissions.

Source	Gas	Included?	Justification/Explanation
Fossil fuel combustion of vehicles displaced by the project activities	CO ₂	Yes	Main emission source
	CH ₄	No	May be excluded for simplification
	N ₂ O	No	May be excluded for simplification
	Other	No	Not Applicable

Table 4 gives the calculated values of kWh/100 based on the battery size and ARAI Range of the EV models.

$$\text{kWh/100 miles rating} = \text{Battery size/Range} * 100 * 0.7 \quad \dots(7)$$

The factor 0.7 is because the value obtained was reduced by 30%, as the real-world range is considered 30% less than ARAI. (MotorBeam Team 2014).

Table 5 gives the calculated comparable MPG values based on the ARAI mileage of the comparable manual petrol vehicle.

$$\text{MPG rating} = \text{Comparable kmpl Rating ARAI} * 2.3524 * 0.7 \quad \dots(8)$$

Table 3: EV Sales 2023.

Sr. No.	EV Model	Total Sales in 2023
1.	Tata Tiago Electric	34853
2.	Tata Nexon Electric	21072
3.	Tata Tigor Electric	10765
4.	MG Comet	6628
5.	Mahindra XUV 400	6231
6.	MG EZS	5970
7.	Citroen eC3	2360
8.	BYD Atto 3	2078
9.	Hyundai Ioniq 5	1228
10.	Kia EV6	658
11.	Hyundai Kona	501

Source: (Team Auto Punditz 2024)

Table 4: kWh/100 Miles Rating.

Sr. No.	EV Model	Battery size [kWh]	ARAI Range [km]	kWh/100 miles
1.	Tata Tiago Electric	19.2	250	17.65314332
2.	Tata Nexon Electric	30	325	21.21772033
3.	Tata Tigor Electric	26	315	18.97245892
4.	MG Comet	17.3	230	17.28936704
5.	Mahindra XUV 400	34.5	375	21.1469946
6.	MG EZS	50.3	461	25.08002047
7.	Citroen eC3	29.2	320	20.97460062
8.	BYD Atto 3	60.48	521	26.68301413
9.	Hyundai Ioniq 5	58	631	21.12805221
10.	Kia EV6	77.4	708	25.1286137
11.	Hyundai Kona	39.2	452	19.93464285

(Source for battery size and ARAI range – Data from manufacturer websites. ARAI in MIDC for simplicity in comparison)

Table 5: Comparable MPG Rating.

Sr. No.	EV Model	Comparable Model	Comparable kmpl Rating ARAI (petrol manual)	Comparable MPG Rating
1.	Tata Tiago Electric	Tata Tiago	20.09	33.0818012
2.	Tata Nexon Electric	Tata Nexon	17.44	28.7180992
3.	Tata Tigor Electric	Tata Tigor	19.28	31.7479904
4.	MG Comet	MG Hector	13.79	22.7077172
5.	Mahindra XUV 400	Mahindra XUV 300	18.24	30.0354432
6.	MG EZS	MG Astor	15.4	25.358872
7.	Citroen eC3	Citroen C3	19.3	31.780924
8.	BYD Atto 3	Kia Carens	21	34.58028
9.	Hyundai Ioniq 5	Hyundai Creta	17.4	28.652232
10.	Kia EV6	Kia Seltos	17	27.99356
11.	Hyundai Kona	Hyundai Venue	17.5	28.8169

(Source for kmpl rating – Data from manufacturer websites. ARAI in MIDC and petrol manual for simplicity in comparison)

$$\text{MPG} = 235.24/\text{litres per 100 km}$$

Conversion factors: 1 gallon = 3.785 liters, 100 km = 62.15 miles

The factor 0.7 was used because the obtained value was reduced by 30%, as the real-world range is considered to be 30% less than ARAI. (MotorBeam Team 2014). Thus, we can calculate the AFEC and MPG values using the data in Tables 4 and 5, respectively, and equations (2) and (3), respectively:

$$AFEC_{i,y} = 19.71 \text{ kWh/100 miles} \quad \dots(9)$$

$$MPG_{i,y} = 30.36 \text{ miles per gallon} \quad \dots(10)$$

The comparison of the calculated kWh/100 miles and comparable MPG Rating is shown in Fig. 4 for each of the car models considered.

The values in Table 6 put into equation (1) give us the value of baseline emissions:

$$BE_y = 377.62 \text{ tCO}_2\text{e} \quad \dots(11)$$

The values in Table 7 put into equation (4) give us the value of the project emissions are:

$$PE_y = 186.59 \text{ tCO}_2\text{e} \quad \dots(12)$$

Carbon Credit Potential

The leakage is assumed to be zero as the VM0038 methodology does not consider it. Applying the discount factor of one, the carbon credits generated per charger can be calculated.

The ER value obtained using equation (5) and the calculated BE and PE values:

$$ER_y = 191.03 \text{ tCO}_2\text{e} \quad \dots(13)$$

$$\text{Rounding up, } ER_y = 191 \text{ tCO}_2\text{e} \quad \dots(14)$$

$$\text{Carbon credits generated/year} = 191 \quad \dots(15)$$

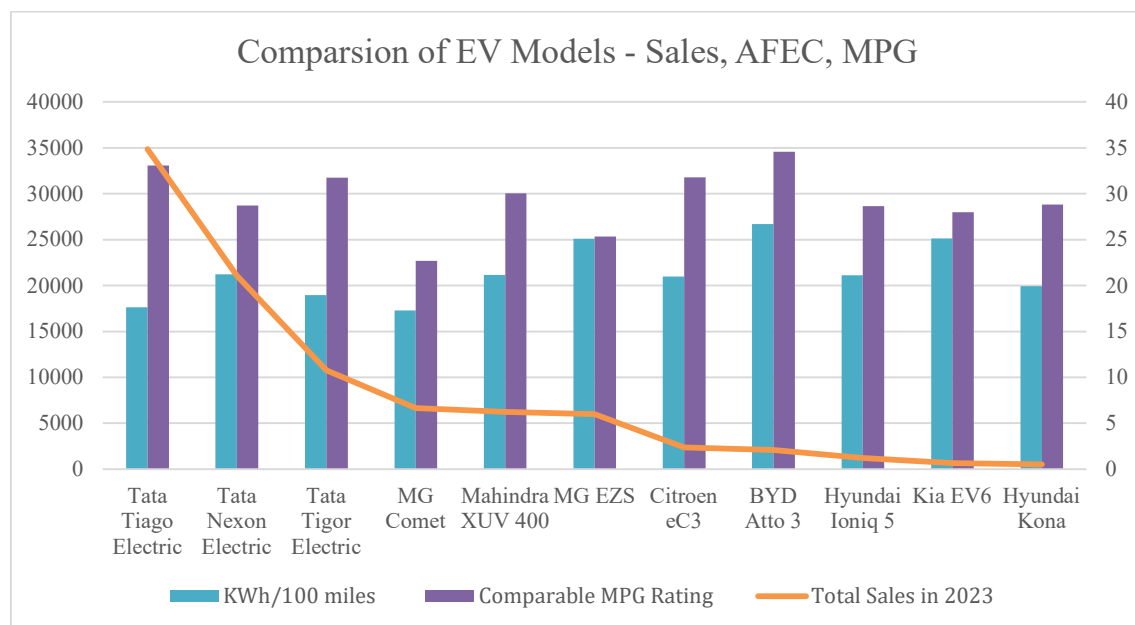


Fig. 4: Comparison of EV Models - Sales, AFEC, MPG.

Table 6: Baseline and project emissions calculation.

377.62	BE _y	Baseline emissions in year y (tCO ₂ e)
262800	ED _{i,y}	Electricity delivered by project charging systems serving applicable fleet <i>i</i> in project year y (kwh)
0.0085	EF _{j,f,y}	Emission factor for the fossil fuel f used by comparable fleet vehicles <i>j</i> in year y (tCO ₂ e/gallon)
1	Iri,	Technology improvement rate factor for applicable fleet <i>i</i>
19.7	AFEC _{i,y}	Weighted average electricity consumption per 100 miles rating for EVs in applicable fleet <i>i</i> in project year y (kWh/100 miles)

Table 7: Values for calculating project emissions.

Value	Parameter	Explanation
262800 kWh	$EC_{i,j,y}$	Taking the assumption that $EC_{i,j,y} = ED_{i,y}$
0.00071 tCO ₂ /kWh	$EFkw_{i,j,y}$	According to the Indian Power Sector's CO ₂ Baseline Database, the weighted average emission factor of the Indian grid FY2021- 22, considering renewable energy, is 0.71 tCO ₂ /MWh (India GHG Program, 2015)

Voluntary Market Value

As carbon credit values in the Indian Voluntary Market vary from US\$2 to US\$50 (Thomson Reuters Foundation 2023) and no standard average exists, we have analyzed compliance markets in different countries. However, Table 8 shows the range of estimated values in the Indian Voluntary Market.

Compliance Market Value

Table 9 shows the potential monetary value that can be generated by the system under different compliance markets across the globe. The latest carbon credit values have also been applied, which have been sourced from (CarbonCredits.com 2024). The latest currency conversion factors have been used.

Financial Analysis

The financial analysis reflects current trends, which show increasing carbon credit values in both voluntary and compliance markets. This is because of the increased demand for decarbonization solutions. It should be kept in mind that credit values are highly volatile, and influenced by policy changes, market dynamics, and regional demand-supply

Table 8: India Voluntary Market – Potential Carbon Credit Value.

India Voluntary Market	Value per CC	Potential Value Generated per Year
Lower range	US\$2	₹ 31,877.90
Higher range	US\$50	₹ 7,96,947.50

Table 9: Compliance Markets – Potential Carbon Credit Value.

Compliance Markets	Value per Carbon Credit	Potential Revenue
European Union	€ 57.36	₹ 9,92,372.74
UK	£ 34.39	₹ 6,94,026.65
California	\$ 28.66	₹ 4,56,810.31
Australia	\$ 33.75	₹ 5,37,939.56
New Zealand	\$ 58.50	₹ 9,32,428.58
South Korea	\$ 6.11	₹ 97,386.98
China	\$ 12.12	₹ 1,93,180.07

factors. This may cause changes in revenue generation and affect the financial planning of EVCS operators. In such instances, stable credit pricing and demand with strong policies are decisive for sustained revenue generation.

Annual Revenue Through Charging

As per the pricing given at the Shell Recharge Charging station, the price charged is ₹ 24/kWh. However, the discount for a long-term subscription may cost 17.5-19.99/unit. Thus, we assume that on average the price charged is 20/kWh. Since our system is expected to produce 2,62,800 kWh per year, we find that at a competitive price,

$$\text{Annual Revenue} = ED_{i,y} * \text{Price per kWh} \quad \dots(16)$$

$$\text{Annual Revenue through charging} = \text{INR ₹ 52,56,000} \quad \dots(17)$$

Initial Investment Cost

We shall estimate the minimum one-time cost for setting up an EVSC with a single CCS Charger, with the major cost components shown in Fig. 6. Thus, we estimate that the minimum initial investment totals about ₹ 33,00,000.

Operation Cost

The operation cost is the yearly cost required for the operation of the charging station, with its components shown in Table 10. The electricity tariff in Pune is ₹ 6/unit, according to (TATA AIG Team 2023). Thus,

$$\text{Electricity cost} = EC_{i,j,y} * \text{Electricity tariff} \quad \dots(18)$$

Net Profit

Ignoring inflation and growth, the average annual profit for 10 years = ₹ 25,78,491.87. ..(19)

This is without considering the value generated from carbon credits.

Scope in Maharashtra

According to a Ministry of Power Report 2024, India has 12,146 public charging stations, with Maharashtra having 2,782 (excluding heavy-duty vehicle stations). If all stations are like the one studied, each generating 191 carbon credits, Maharashtra can save about 5,31,362 credits, which will amount to 5,31,362 tCO₂ emissions avoided and earn ₹ 53,74,26,965 in revenue.

The carbon credit potential of the electric vehicle (EV) charging station analyzed in this study provides important insight into the financial viability and environmental impact of such infrastructure. The potential to generate carbon

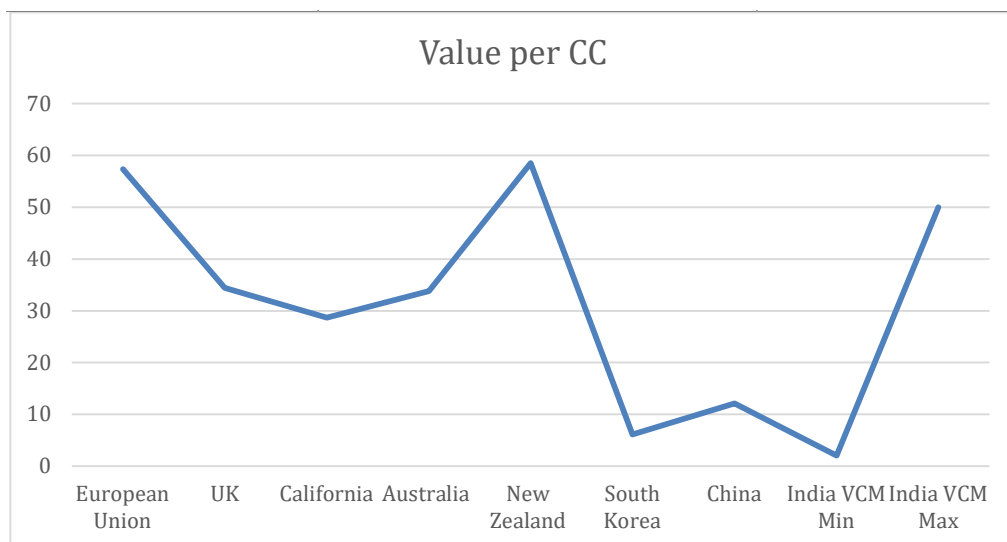


Fig. 5: Value per CC in Different Markets.

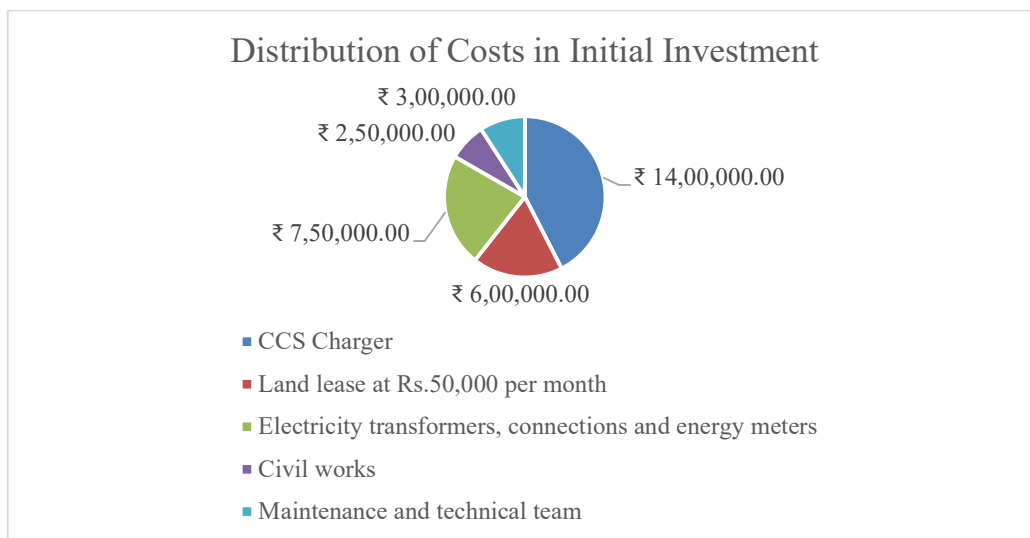


Fig. 6: Distribution of costs in initial investment (Acharya 2024).

Table 10: Operation Cost.

Activity/Infrastructure	Cost
Electricity Cost	₹ 17,08,342.36
Land lease at Rs. 50,000 per month	₹ 6,00,000.00
Maintenance and technical team	₹ 3,00,000.00
Total	₹ 26,08,342.36

credits is evident based on the calculated emissions reduction (ER) of 191 tCO₂e per year per charger. The finding reinforces the importance of EV charging stations in helping to mitigate greenhouse gas (GHG) emissions.

We then considered both voluntary and compliant carbon markets in evaluating the financial implications. Carbon credits in the Indian Voluntary Market were estimated to be in the range of ₹ 31,877.90 to ₹ 7,96,947.50 per year for the system, depending on the value per credit. Carbon credit in the Indian Voluntary Market generates an extra profit of up to 30.9%. It would encourage more charging stations to be built. Nevertheless, for more precise estimations, we used the values of carbon credits in the compliance markets of different countries. The system valued the compliance market from ₹ 97,386.98 to ₹ 9,92,372.74 per year and is of significant revenue generation potential.

In addition, the financial analysis showed potential for charging revenue, with an annual revenue of ₹ 52,56,000. But that must be weighed with operating costs, including electricity costs and initial investment. The total initial investment made was ₹ 33,00,000, and operating costs (electricity and maintenance) were approximately ₹ 26,08,342.36. These costs, however, amounted to ₹ 25,78,491.87, on average, over 10 years, accounting

for inflation and growth, and the charging station was profitable. Additionally, given the market size, the potential contribution of such charging stations to emissions reduction is significant, for example, in Maharashtra.

The carbon footprint of EV charging stations varies with charging habits such as frequency, time of day, and charging duration. Charging during peak grid demand often increases emissions if fossil fuels are used to meet the load. These variations make it challenging to standardize emissions reductions, as they depend on user behavior and grid energy mix.

Our findings highlight the potential of carbon credits to incentivize EVCS development. There is an urgent need to create supportive carbon credit monetization as part of green mobility initiatives. Standard guidelines for carbon credit validation are the need to implement and attract investment. Public-private collaborations are the key to this. Private entities can contribute to innovation with required capital investments. Ultimately, it's the base to achieve sustainability goals, targeting both economic and environmental benefits.

CONCLUSIONS

The research objectives of the study were successfully achieved, as the study offers key insights into the potential of electric vehicle (EV) charging stations to reduce emissions and be financially viable. Carbon credit potential from charging stations is high, the study revealed. Although the initial investment costs and operating expenses are incurred, the charging station is profitable. More carbon credits and government subsidies would make financial viability even better. Particularly, Maharashtra has great

potential in carbon credit generation, which is one of the broader impacts of EV charging stations, not only reducing emissions but also generating substantial financial returns. We show how charging systems could earn carbon credits, and therefore promote sustainable transportation and reduce emissions. This enforces the role of carbon credits in incentivizing infrastructure and expert consultation on registering, monetizing, and trading credits on global markets. Standardizing carbon credit calculations, exploring financing mechanisms for green mobility, and integrating blockchain for transparent trading, alongside optimizing EV chargers for energy efficiency, are future research foci. Further, infrastructure deployment and renewable energy integration models should be scalable.

The findings highlight the potential of carbon credits in promoting EVCS development. It demands need-based changes in the future renewal of policies. The supportive framework is the key to the integration of carbon credits into India's sustainability strategies. Standard trading mechanisms are required for widespread adoption to get carbon credits. Collaboration between the public and private sectors is essential to expand the EVCS network. These efforts will lead to reducing carbon emissions, nurturing a synergistic way for sustainable transportation and economic growth.

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Aqueous Phase of Hydrothermal Liquefaction as a Concrete Modifier

Yuliya Kulkova^{1†} , Ibragim Garifullin² and Maria Dmitrieva²  and Olga Babich¹ 

¹Institute of Living Systems, Immanuel Kant Baltic Federal University, Kaliningrad, Russia

²Institute of High Technologies, Immanuel Kant Baltic Federal University, Kaliningrad, Russia

†Corresponding author: Yuliya Kulkova; kulikova.nipu@gmail.com

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ABSTRACT

The processes of liquid fuel production from organic wastes by hydrothermal liquefaction lead to the formation of wastewater characterized by a high content of organic compounds, including lignosulfonates and their derivatives. The paper presents the results of a study designed to assess the possibility and feasibility of using such wastewater as concrete modifiers. The study confirmed the hypothesis that the use of HTL-AP slows down concrete curing processes (curing time increased 1.9 times compared to the control sample). It was found that the modifying properties of HTL-AP are higher than those of the commercial concrete modifier because even at higher curing retardation rates (curing time is 12.2% higher compared to concrete modified with the commercial solution), the use of HTL-AP results in minimal reduction in the strength properties of the concrete. The application of HTL-AP and commercial modifier results in a 7.1% and 14.5% reduction in compressive strength, respectively, and a 6.2% and 12.2% reduction in tensile strength, respectively. Based on the results of the study, it is concluded that the use of HTL-AP as a retarder in concrete curing processes is well justified, as the positive effect has been experimentally confirmed. Using HTL-AP as a concrete modifier will improve the environmental efficiency of HTL processes and reduce the cost of frost-resistant concrete by eliminating the use of traditional expensive modifiers.

INTRODUCTION

The issues of waste recycling and their beneficial use for solving technological problems in the construction industry and for solving energy-saving problems are quite actively being considered by scientists from various countries (Achi et al. 2024, Gupta et al. 2023).

Hydrothermal liquefaction technology is very effective for processing organic wastes with high moisture content (up to 75-95%) at temperatures of 200-400°C and pressures of 4-22 MPa, resulting in valuable energy sources (bio-coal, bio-oil, and biogas), the ratio and yield of which depend on the conditions of the technological process (Mahima et al. 2021, Yu et al. 2022).

There is considerable interest in this technology worldwide, as it offers a number of significant advantages, including the elimination of pre-drying of raw materials, resulting in higher energy efficiency (Mishra & Mohanty 2019). One of the key problems of hydrothermal liquefaction processes is the formation of wastewater (HTL-AP) up to 80% by mass of the processed feedstock (Watson et al. 2020, Leng et al. 2020, SundarRajan et al. 2021).

HTL-AP is characterized by a high content of organic compounds and nutrients (organic carbon content up to 25 g.L⁻¹) (Thomsen et al. 2024, Cheikhwafa et al. 2024, Shen et al. 2018, Yang et al. 2018). According to some studies (Watson et al. 2020, Shen et al. 2018, Kulikova et al. 2023), heavy metals can be detected in the composition of HTL-AP, which is characteristic of wastewater from the processing of excess activated sludge. Thus, ensuring the environmental and

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economic efficiency of HTL-AP treatment, utilization, or beneficial use is a critical task.

Based on literature data, HTL-AP was found to potentially contain polyaromatic compounds such as lignosulfonates or their analogs (Kulikova et al. 2023, He et al. 2019, Si et al. 2019, Chen et al. 2018, Jokinen et al. 2023). Several studies aimed at finding technical and technological solutions for the beneficial use of HTL-AP are known, for example, as a nutrient medium for the cultivation of microalgae (Cordova et al. 2020, Shende et al. 2017), for the production of biogas (Seyedi et al. 2021), biohydrogen (Si et al. 2018, Torri et al. 2021), and as a fertilizer for plant cultivation (Jesse et al. 2019). The literature does not describe the use of HTL-AP in the construction industry as a source of lignosulfonates for concrete modification.

At the same time, lignosulfonate preparations are widely used as modifying additives for concrete, providing improvement of concrete technical properties such as strength, water, and frost resistance (Breilly et al. 2021). Due to the disruption of global supply chains, the Russian construction materials market is currently experiencing a need to seek out and develop innovative technologies for the production of such modifiers.

An important aspect of using fine-grained concrete is not only its strength and durability but also its environmental safety. Lignosulfonate-based modifiers have low toxicity and no harmful effects on the environment, making them a preferred choice for use in construction (Liu et al. 2018).

Considering the relevance of the search for innovative, ecologically and economically effective ways of HTL-AP utilization, a hypothesis was made about the possibility of using the above-mentioned wastewater for concrete modification. The paper presents the results of testing the hypothesis that the addition of HTL-AP to concrete mixtures will increase their mobility, increase the curing time while maintaining the strength properties of products.

Currently, there are no studies in the literature showing the feasibility of using HTL-AP for concrete modification. This work will open a new direction for processing this type of wastewater, which will give impetus to the development of HTL technologies and will reduce the cost of certain types of building materials and products.

MATERIALS AND METHODS

Conditions for Obtaining the Water Aqueous Phase of Hydrothermal Liquefaction (HTL-AP)

Excess activated sludge from the municipal biological wastewater treatment plant in Kaliningrad (Russia) was

used as raw material. The raw materials were delivered to the laboratory in their natural wet state.

The process was conducted at 280 °C in an autoclave reactor under a pressure of 3.6 MPa, with a total processing time of 30 minutes. Hydromodule 1:10. After HTL, the reactor was discharged to a filter system where the solid phase was separated from the liquid phase. The liquid phase was then mixed with dichloromethane in a ratio of 1:2, and the extraction of the oil fraction (biofuel) was performed. The mixture of dichloromethane and water was separated in a separating funnel. The aqueous phase (HTL-AP) was stored in a refrigerator until use.

Methods for Studying HTL-AP Composition

The density of the additive was determined by the weight method. A photometric method was used to determine the concentration of lignosulfonates based on their interaction with nitric acid and the formation of yellow nitro derivatives in an alkaline medium. The interfering influence of phenols and aromatic amines was eliminated by steam predistillation without changing the pH of the medium. Then, 2 cm³ of sodium nitrite solution and 2 cm³ of acetic acid solution were added to the 100 cm³ of diluted sample and stirred. After 15 minutes, 4 cm³ of ammonia solution was added. The optical density was measured at a wavelength of $\lambda = 430$ nm and then subtracted from this value the optical density of the blank, which was treated in the same way as the test sample, but with the addition of 2 cm³ of distilled water instead of the sodium nitrite solution. The content of the substances was determined using the calibration curve. The graduation curve was plotted against tannin (Khabarov 2004).

Composition of Mortar Mixture

The composition of the tested concretes is presented in Table 1. Portland cement (PC 300-D0-B), quick-setting cement with compressive strength at 28 days (M-300), without additives (produced by Nargit, Russia, Mytishchi), was used. Sand was purchased from the supplier NerudStroy-M, Moscow (the origin of the sand is not indicated on the package). Composition of mortar mixture was selected in accordance with the state standard of the Russian Federation GOST 27006- 2019 "Concretes. Composition selection rules" (GOST 2019).

Determination of Rheological Characteristics

The consistency of the mortar mixture was determined according to GOST 5802-86 using the device presented in Fig. 1.

Table 1: Compositions of tested fine-grained concretes.

Component	Control sample	Sample with HTL- AP	Sample with commercial lignosulfonate
HTL-AP	-	92 mL	-
SAVEMIX4000 lignosulfonate, mL	-	-	8 mL
Portland cement (PC 300-D0-B)	1400 g	1400 g	1400 g
Quartz sand fr. 1.0-1.5 mm, quartz sand with a size modulus	3360 g	3360 g	3360 g
Water	700	608	692

The device is positioned on a horizontal surface, and the degree of freedom of sliding of the rod 4 within the guides 6 is evaluated. The vessel 7 is filled with the mortar mixture to 1 cm below the edges, the mortar mixture is then rammed by poking with a steel rod 25 times and by tapping 5-6 times lightly on the table, then the vessel was placed on the site of the device. The tip of the cone 3 is brought into contact with the surface of the solution in the vessel, the cone rod is fixed with the locking screw 8 and the first reading on the scale is made. Then the locking screw is released. The cone must sink freely into the mortar mixture. The second reading is taken on the scale 1 minute after the beginning of the cone immersion. The depth of cone immersion, measured with an error of up to 1 mm, is determined as the difference between the first and second readings. The normal consistency of the mortar mixture was determined according to ASTM C191-08 using a Vika OGC-1 device mark (Laborkomplekt, Moscow, Russia).

When the mixing is finished, the ring is quickly filled with cement paste in a single step and shaken 5-6 times, tapping the plate against a hard surface. The surface of the paste is leveled with the edges of the ring by cutting

off the excess paste with a knife wiped with a damp cloth. Subsequently, the instrument pestle is brought into contact with the surface of the paste situated in the center of the ring, and the rod is secured with a locking device. The rod is then released rapidly, allowing the pestle to plunge into the paste. Following the release of the rod, the immersion is measured on the scale after 30 seconds. The ring with the paste must not be jolted when measuring. In the event that the consistency of the cement paste is deemed inappropriate, the quantity of water should be adjusted and the paste re-mixed, ensuring that the pestle is immersed to a greater depth. The quantity of water required to create a paste of standard consistency is calculated with a precision of 0.25%.

The setting time of the concrete mortar was determined in accordance with the ASTM C191-08 standard using the Vika OGC-1 device. To determine the onset of setting, the needle is lowered until it touches the surface of the cement paste, and the rod is secured in this position with a locking device. After 1-2 seconds, the rod is released, and the metal needle sinks without barriers into the cement paste. The depth of needle penetration was measured after 30-45 seconds. Measurements were repeated in the same way every 10 minutes, but the ring was shifted by 10-15 mm after each measurement. Care should be taken to ensure that the needle does not hit the site of the previous needle injection. After each immersion, the needle was cleaned carefully with a tissue. Between the measurements cement paste on the plate was covered with the textile material, but without contact with the cement paste. The temperature in the room should be 20-25° and the humidity should be not less than 65%. The setting time is defined as the time from the moment of mixing water with cement powder to the moment when the needle does not penetrate the cement paste or penetrate by less than (4 ± 1) mm.

Determination of Mechanical Characteristics of Concrete

To evaluate the influence of the modification on the quality of the products, 40 x 40 x 160 mm prisms were produced and cured under normal conditions at a temperature of 20±2 °C and humidity of 95-95%. The deflection and compressive

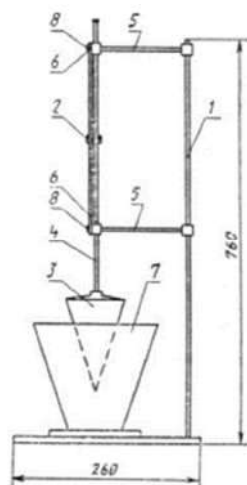


Fig. 1: Device for determining the mobility of the mortar mixture: 1 – stand rod, 2 – scale, 3 – reference cone, 4 – stand rod, 5 – holders, 6 – guides, 7 – vessel of the mortar mixture, 8 – locking screw.

strength of the specified samples were investigated. The deflection and compressive strengths were determined using a ToniPRAX unit (Berlin, Germany) according to DIN EN 196-1-2016 (Taher 2024). The deflection tests were performed at 1 day, 7 days, and 28 days intervals.

RESULTS AND DISCUSSION

Analysis of Physicochemical Properties and Composition of HTL-AP

Composition analysis using NMR spectroscopy was previously performed for the above wastewaters (Kulikova et al. 2023). Carbon in aliphatic chains (34.05–41.82%) was found to be predominant in the wastewater composition, followed by carbon in bonds with carboxyl groups and aromatic rings (26.42–34.44%). Based on these data, it was concluded that the predominant components are oxygenated aromatic compounds, including lignosulfonates (Fig. 2).

Since there was no clear confirmation of the presence of lignosulfonates in previous works, it was decided to quantitatively analyze their content using the photometric method and tannin as a standard. The results of the evaluation of HTL-AP properties are summarized in Table 2. Since the content of lignosulfonates in HTL-AP is about 30 times lower than in the commercial modifier solution, this fact must be taken into account when formulating the test concrete mixtures.

Analysis of published data showed that the HTL-AP in most cases is characterized by high COD values (in some cases over 100 g.L⁻¹) and contains large amounts of organic carbon (SundarRaj.2an et al. 2021). The quality of wastewater significantly depends on the type of raw materials

Table 2: Main physicochemical parameters of HTL-AP.

Parameter	HTL-AP	SAVEMIX4000
Density	1.0112	1.1488
Lignosulfonate concentration	2.89±0.14 g.L ⁻¹	300 g.L ⁻¹
pH	6.5	4.5–5.5

used. According to the literature data, the content of organic compounds in HTL-AP from algae biomass processing is 20–65 g COD.L⁻¹, from manure processing 10–55 g COD.L⁻¹, sludge processing 5–15 g COD.L⁻¹, and lignocellulose biomass processing 10–22 g COD.L⁻¹ (Fan et al. 2022, Gu et al. 2019, Leng et al. 2021, Usman et al. 2019).

This variability in the composition of wastewater, depending on the raw materials, creates certain difficulties in ensuring the consistency of the concrete modifier composition planned for production. However, this problem can be overcome by averaging, adjusting, and constantly monitoring key parameters of manufactured products. In case the composition of the raw materials is constant (if the HTL equipment is aimed at processing a specific type of waste), the consistency of the HTL-AP composition will also be achieved.

Evaluation of Rheological Properties of Concrete Mixtures

As mentioned above, 3 types of concrete mixtures were tested: unmodified concrete (sample 1), modified with HTL-AP (sample 2), and modified with the commercial modifier SAVEMIX4000 (sample 3). The results of the evaluation of rheological properties are presented in Table 3.

From the experimental results, it can be concluded that the mixture modified with HTL-AP is 31.6% more mobile than the unmodified control sample. At the same time, the mobility of the concrete mix modified with commercial composition is inferior to the sample with HTL-AP modification (mobility is lower by 6.3%).

The initial density of the concrete paste, determined by analyzing the immersion depth of the needle of the Vika device, with the same amount of liquid for sample 2, was a maximum of 40 mm, which is 8 times higher than that of the control sample. At the same time, the setting time of sample 2 is 1.9 times higher than that of the control sample and 12.2% higher than that of the sample modified with the commercial solution. It can be concluded that HTL-AP has modifying properties that significantly increase the mobility of the concrete paste and the setting time.

An important parameter in this case is the preservation of the mechanical properties of the modified concrete with the increase of the setting time. In this connection, in the

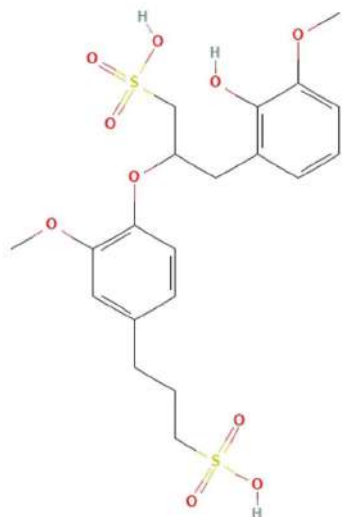


Fig. 2: Structure of lignosulfonates (PubChem 2024).

Table 3: Results of evaluating the rheological properties of concrete.

Parameter	Sample 1	Sample 2	Sample 3
Concrete consistency, ΔP , cm	6.5	9.5	8.9
Depth of immersion of the Vika device when evaluating the density of concrete paste, mm	5	40	34
Setting time	2 h 10 min	4 h 5 min	3 h 35 min

second stage of the research, the mechanical properties of the products from the studied concrete samples were evaluated.

Mechanical Properties of Concrete

The results of the evaluation of the tensile strength of the products made from the concrete samples tested are presented in Table 4 and Fig. 3. Based on the data, the following conclusions can be drawn: on the first day, the tensile strength of the modified concrete is 20% lower than that of the control, which indicates that lignosulfonates acts as a retarder in the hardening of the concrete mix. Further testing showed that the rated tensile strength of the modified concretes (at 28 days) exceeded that of the control mix by 6.2% for the

HTL-AP modified mix and 12.3% for the mix modified with commercial lignosulfonates (SAVEMIX4000).

The results of the compressive strength measurements are shown in Table 5 and Fig. 4. Similar results were obtained in the compressive strength tests. It can be seen that on the first day, the strength of the modified concretes is 20% lower than that of the control concrete, indicating that lignosulfonates act as a retarder in the hardening of the concrete mix. Further tests showed that the ultimate strength of the modified concretes (at 28 days) was lower than that of the control mix by 7.1% for the mix modified with HTL-AP and by 14.5% for the mix modified with commercial lignosulfonates (SAVEMIX4000).

Table 4: Tensile strength test data.

Sample	Period of testing	Sample N	Weight, g	V, cm ²	p, g.cm ⁻²	G, tensile, kN	R, tensile, MPa
Control	24 h	1	560	256	2.19	1	3.88
		2	558	256	2.18	1.1	
		3	555	256	2.17	1	
	7 days	4	550	256	2.15	2.1	7.88
		5	540	256	2.11	2.1	
	28 days	6	551	256	2.15	2.6	10.13
		7	575	256	2.25	2.6	
		8	584	256	2.28	2.9	
Modification with HTL-AP	24 h	1	560	256	2.19	0.8	3.13
		2	560	256	2.19	0.9	
		3	547	256	2.14	0.8	
	7 days	4	530	256	2.07	2	8.00
		5	535	256	2.09	2.2	
		6	525	256	2.05	2.2	
	28 days	7	529	256	2.07	2.6	9.50
		8	541	256	2.11	2.6	
		9	538	256	2.10	2.4	
Modification with SAVEMIX4000	24 h	1	569	256	2.22	0.8	3.25
		2	571	256	2.23	0.9	
		3	565	256	2.21	0.9	
	7 days	4	572	256	2.23	2.1	8.06
		5	564	256	2.20	2.2	
	28 days	6	552	256	2.16	2.5	8.88
		7	541	256	2.11	2.4	
		8	545	256	2.13	2.2	

The data presented above confirm the technological feasibility of using HTL-AP as a modifier. Besides that, the beneficial use of HTL-AP will reduce the environmental burden created by wastewater from the hydrothermal liquefaction process. In addition, the use of this wastewater will reduce the

resource intensity of the construction industry, as primary resources will not be consumed for the production of concrete modifiers. Ultimately, the introduction of this technology will ensure a reduction in the resource intensity of the ecological and climatic footprint of human industrial activity.

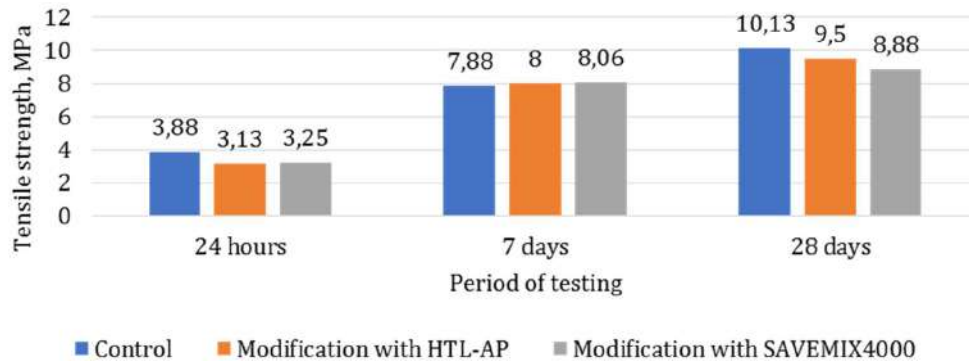


Fig. 3: Tensile strength.

Table 5: Compressive strength test data.

Sample	Period of testing	Sample N	Weight, g	V, cm ²	p, g.cm ⁻²	P, compr., kN	N, compr., MPa
Control	24 h	1	560	256	2.19	34.53	21.58
		2	558	256	2.18		
		3	555	256	2.17		
	7 days	4	550	256	2.15	95.03	59.39
		5	540	256	2.11		
	28 days	6	551	256	2.15	135.70	84.81
		7	575	256	2.25		
		8	584	256	2.28		
Modification with HTL-AP	24 h	1	560	256	2.19	25.05	15.66
		2	560	256	2.19		
		3	547	256	2.14		
	7 days	4	530	256	2.07	89.57	55.98
		5	535	256	2.09		
		6	525	256	2.05		
	28 days	7	529	256	2.07	126.00	78.75
		8	541	256	2.11		
		9	538	256	2.10		
Modification with SAVEMIX4000	24 h	1	569	256	2.22	24.13	15.08
		2	571	256	2.23		
		3	565	256	2.21		
	7 days	4	572	256	2.23	97.15	60.72
		5	564	256	2.20		
		6	552	256	2.16	116.07	72.54
	28 days	7	541	256	2.11		
		8	545	256	2.13		

Table 6 shows a comparison of the economic parameters of commercial concrete modifiers with HTL-AP. As we can see, replacing traditional modifiers with HTL-AP will not only reduce the environmental impact on the environment, but will also reduce the cost of concrete by 1.46 USD per ton of mortar mixture.

Thus, several important conclusions can be drawn from the research conducted:

- Lignosulfonates and their derivatives are present in significant amounts in the composition of the aqueous phase of hydrothermal liquefaction processes.
- The aqueous phase from the hydrothermal liquefaction of excess sludge has significant resource potential and can potentially be used as a modifier in fine-grained concrete.
- The results of the tests on the use of HTL-AP as a concrete modifier show an increase in the mobility of the mixture by 40% in comparison with the control sample. At the same time, the admixture has a retarding effect on the concrete hardening process, which is confirmed by the results of tests on the strength of samples after 1 day.
- The application of HTL-AP leads to a slight decrease in the strength properties of the concrete (compressive strength by 7.1%, fracture strength by 6.2%), while the decrease in the strength properties when using commercial solutions was much more significant at 14.5 and 12.2%, respectively.

- The comparison of the effectiveness of the commercial solution with the studied solution showed that HTL-AP has a higher effectiveness in retarding the setting, as the setting rate of the HTL-AP modified concrete was 6.3% lower.

CONCLUSIONS

It can be concluded that the use of HTL-AP as a curing retarder is well justified, as the positive effect on the concrete curing process has been confirmed. This technological solution will allow solving a whole complex of problems, including ensuring the use and maximum utilization of the resource potential of HTL-AP; it will provide valuable and at the same time cheap concrete modifiers.

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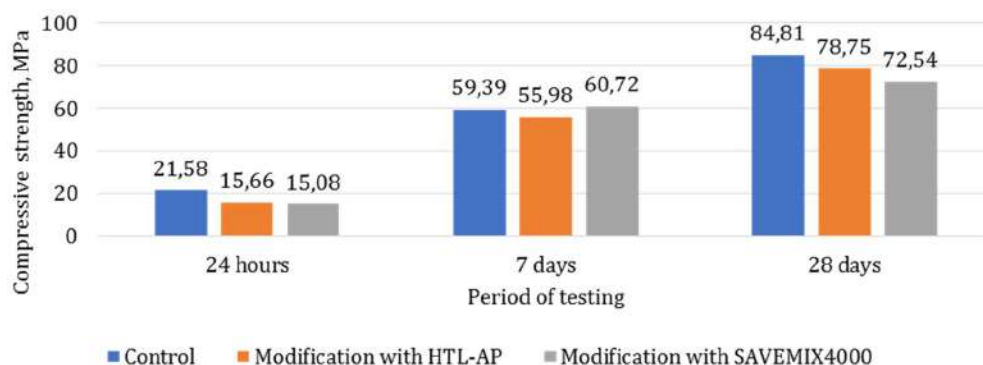


Fig. 4: Compressive strength, MPa.

Table 6: Comparison of economic parameters of various concrete modifiers.

Name of modifier	Origin	Price	Consumption, % of the mass of cement
SikaPlast-520N	Sika, Russia	1.9 USD.L ⁻¹	0.3
Sodium Lignosulfonate	SIDLEYCHEM LTD., China	2 USD.kg ⁻¹	0.1
Concrete Plasticizer	A.S.Chemical, India	0.9 USD.L ⁻¹	0.2-0.4
HTL-AP	Depends on the place where the process is implemented	0	6.6

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Assessing Climate Sensitivity to Urban Land-Use Changes in Iraq

Sara Ali Muter¹, Yasmin Q. Tawfeek¹, Monim H. Al-Jiboori¹ and Jamal S. Abd Al Rukabie²

¹Atmospheric Sciences Department, College of Science, Mustansiriyah University, Baghdad, Iraq

²Department of Science, College of Basic Education, University of Sumer, Thi-Qar, Iraq

†Corresponding author: Monim H. Al-Jiboori, mhaljiboori@gmail.com

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ABSTRACT

Iraq has been suffering from a continuing rise in surface air temperature, causing a general deterioration in ecosystems. Land-use climate sensitivity focuses on how changes in land cover (e.g., deforestation, urbanization, or agricultural expansion affect local or regional climate conditions, particularly surface temperatures. Using yearly data on built-up areas and air temperature over the period (1971-2022), this sensitivity is assessed based on alterations in built-up areas for three Iraqi cities-Basrah, Baghdad, and Mosul, as well as for the entire country of Iraq. The time series of the areal yearly averages for air temperatures was analyzed. Standardized Euclidean distance and linear regression models were used to assess the effect of built-up changes and temperature trends, respectively. The results revealed that the trend in Iraq has positively increased, with a value of 0.07°C/year. The results also illustrate that alterations in built-up areas have contributed to the increase in yearly temperature in Basrah and Baghdad, but not in Mosul, which seems to be more affected by global warming and land cover changes. Urbanization plays a significant role in shaping the social, economic, and environmental landscapes of Baghdad and Basrah.

INTRODUCTION

Continuous urbanization in the urban environment, including increasing built-up areas and other human activities, has caused a significant modification in substantial surface properties and then altered the urban climate (Mahmood 2014, Mohowes & Al-Jiboori 2023, Pushpalatha et al. 2024). Anthropogenic changes in land cover, such as impervious surfaces of asphalt and other dark materials, have contributed to local or regional warming through changes in the properties of biophysical surfaces, land fragmentation, and biodiversity (Novio et al. 2024). The amount of sunlight absorbed by the Earth is dramatically increased by these surfaces, which is re-radiated longwave radiation heating the atmosphere by its interaction with molecules of greenhouse gases (GHGs) that subsequently re-emit a portion of this heat to the surface, leading to increased average air temperature (Mahmood et al. 2024).

Climate sensitivity (CS) is a quantitative metric of the climate to human action and is defined as the response of the local near-surface temperature (NST) to different physical and human influences (Xian et al. 2020). Accurate estimates of the climate response are needed to assess the impacts of climate change and to inform policy. Generally, several natural factors influence NST at a certain place, like latitude, elevation, distance from water bodies, synoptic pressure systems, and local aspects (Ahrens & Henson 2022). Radiative forcing, which is the difference between incoming and outgoing radiation at the top of the Earth's atmosphere, can be linked to temperature changes by comparing it to a reference level of forcing. Human activities, such as fossil fuel combustion, transportation, land-use changes, and elevated air pollution levels, have altered radiative forcing (Anad et al. 2022).

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For instance, increased atmospheric carbon dioxide (CO₂) enhances net radiative forcing by reducing atmospheric transparency to thermal radiation, ultimately contributing to human-induced global warming (Otero 2020). It leads to increasing NSTs and then to changes in climate patterns.

CS cannot be measured directly, but it must be estimated using historical instrumental records, palaeoclimate data, or mathematical models. Over the past years and in the context of ongoing global warming, Iraq has become increasingly concerned about the frequent consequences of rising air temperatures. These include extreme temperatures, heat waves, severe drought, sand and dust storms, rainfall variability, water scarcity and salinity, especially in the central and southern parts of the country (Zahraa et al. 2020). There is an urgent need to explore estimates of CS for future action to avoid exacerbating these threats.

Many studies have described the warming climate over Iraq in terms of temperature trends (Salman et al. 2017, Ibraheem et al. 2023, Al Rukabie et al. 2023) and increased CO₂ over different long periods, but up to our knowledge, there are a few studies carried out on rising temperature caused by land use changes in urban areas. Two studies were performed by Abdaki et al. (2021) and Binder et al. (2022) for predicting long-term climate change in which the future annual mean temperature was projected for four years: 2025, 2050, 2075, and 2100 using coupled model inter-comparison

project phases under different scenarios of representative concentration pathways, and they expected that annual mean temperature to increase in the range 1-4°C. Based on historical real data of NST and its connection to surface built-up changes and temperature trends, future studies can provide a crucial localized perspective, bridging the gap between urbanization and Iraq's warming climate. In this paper, the data for urban expansion in three major cities of Iraq: Basrah (in the south), Baghdad (in the middle), and Mosul (in the north) were collected. The main objectives are to 1) examine the local sensitivity to built-up areas under different climate conditions, 2) determine the reliable value of CS resulting from changes in built-up areas, highlighting the need for mitigation and adaptation strategies for future alterations, and 3) compare the sensitivity variables and analyze their correlations.

MATERIALS AND METHODS

Description of the Site and Data Collection

The presented study was carried out in Iraq, located in the Middle East, and a country in southwestern Asia (Fig. 1). The total area is 437072 km² and consists of agricultural land (23%), urban and suburban areas (3%), and water bodies (1%), with the remainder being about 73% desert (World Data 2023). The latter percentage reflects the common

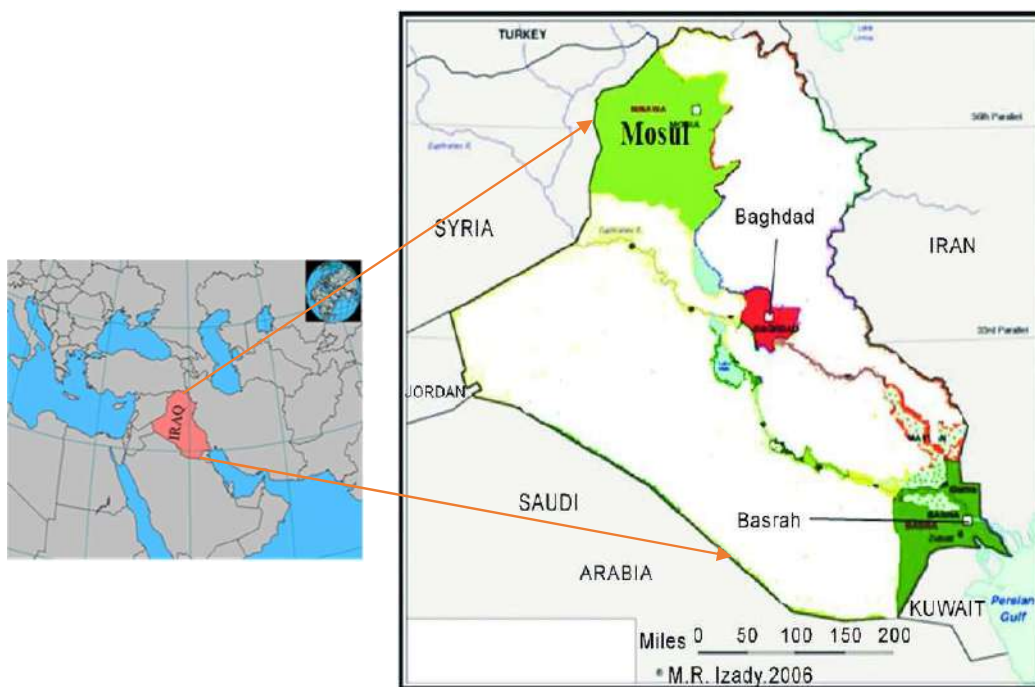


Fig. 1: Iraq's map on which the studied areas are: Basrah, Baghdad and Mosul.

Table 1: Geographic features for the three cities: Basrah, Baghdad, and Mosul.

Province	Latitude [°] E	Longitude [°] N	Elevation [m]	Pop. density [pop.km ⁻²]	Total area [km ²]	% of Iraq
Basrah	30.52	47.82	5	160.6	19070	4.4
Baghdad	33.3	44.43	34	187.9	894.2	0.2
Mosul	36.31	43.15	223	105.2	180	0.04

influence of drought. Iraq's topography is characterized by mostly broad plains, with large desert plateaus dominating the west of the country. To prevent these threats from worsening, there is an urgent need to explore estimates of CS for future policies. The elevation above sea level has a wide variation, from remnants of sea level in the southeast to mountains with heights of 3583 m in the north and northeast along the borders with Iran and Turkey (Al-Ansari 2021). Iraq's terrain comprises four main regions: highlands (north), alluvial plains (middle), deserts (west and southwest), and rolling uplands (northwest) (Khadduri et al. 2003). From the latter region, two rivers (Tigris and Euphrates) flow south-east. They join the Shatt-Al-Arab.

The study area was divided into three parts: south, central, and north, each represented by the major cities of Basrah, Baghdad, and Mosul. These are the most densely populated cities and contribute significantly to the total population (World Population Review 2023). Geographic coordinates such as latitude, longitude, and elevation above mean sea level (msl), areas and their ratios to the total area of Iraq, and the population density in 2022 (in pop.km⁻²) for the cities above are presented in Table 1. Iraq's climate in general ranges from semi-arid to arid, with dry, hot summers and cooler winters. The average yearly NST ranges from 0°C (winter) to 38°C (summer). Rainfall is a seasonal event that occurs between November and May, with an annual average ranging from 150 mm in the south to 400 mm in the northeast parts (Muter et al. 2024).

Yearly means of NST at the three cities obtained from the Iraq Meteorological Services and Seismology for 52 years running from 1971 to 2022 were analyzed. In addition, the built-up areas for the three Iraqi regions: Basrah, Baghdad,

and Mosul, were obtained from the most recent studies conducted for each city, as displayed in Table 2, using Landsat images with 30x30 m resolution and very good accuracy. During the interval 2014-2016, the political situation in Baghdad was unstable due to military operations, so the built-up area in 2015 (437.8 km²) was less than that in 2008 (621.7 km²).

CS Variables

CS to changes in land cover can be viewed as radiative forcing (ΔF) factors for assessing climate change. These factors can directly and indirectly affect the change in mean near-surface temperature (ΔNST) through a simple equilibrium model (Sherwood et al. 2020)

$$\Delta NST_k = - \frac{\Delta F_k}{\phi} \quad \dots(1)$$

ϕ represents the climate feedback variable, and k denotes the forcing agent. Radiative forcing encompasses contributions from both anthropogenic and natural factors. The latter generally has a minor effect on the yearly mean temperature trend in most regions (Rälsänen 2021). On the other hand, the former has significant effects as reported in many studies (Egorova et al. 2018, Sherwood et al. 2020). In this study, the changes in the urban areas as a function of the anthropogenic forces are studied. One of the above forcing agents is commonly used to estimate the NST response through modeling simulations, such as general circulation models for the global climate system and regional climate models for specific areas. However, studying each agent in isolation introduces uncertainty into this estimate. For future years, these data can be derived by interpolation using the trend analysis over a given period (Mahmood et al. 2024). In a review paper, Sherwood et al. (2020) show that an estimate of climate sensitivity can be achieved not only by future climate projections, but also by analysing relevant observational data (e.g., Goodwin & Cael 2021). Therefore, the present study tries to determine the urban surface changes that can account for the regional effects in a global climate without relying on climate models. Subsequently, their results are correlated, as historical land cover changes can contribute significant amounts of CO₂ (Mahowald et al. 2017).

Human-induced changes in land cover, such as the expansion of built-up areas, have greater values than changes

Table 2: Built-up areas of the three cities in different years.

Provinces	Year	Build-up area [km ²]	Reference
Basrah	1990	3280	Hadeel et al. (2009)
	2003	3756.8	
	2000	192.8	Al-Jiboori et al. (2020)
	2015	437.2	
Baghdad	2008	621.7	Tawfeek et al. (2020)
	2013	706.3	
	2013	706.3	Tawfeek et al. (2020)
	2019	727.3	
Mosul	2013	132	Saleh & Ahemd (2021)
	2021	115.4	

in NST (Al-Jiboori & Mohowes 2023). To standardize the absolute difference in their values, we subtract the original values from their mean and divide by the standard deviation. This process is necessary to balance the contributions to CS. For easier comparison, we center the variables at their means, ensuring all variables have a mean of zero. Therefore, the Standardized Euclidean Distance (SED) was chosen to measure the rate of change of climate variables in response to built-up areas, A (Ordonez et al. 2014, Xian et al. 2020). To assess the impact of built-up area changes on the regional climate, the difference in built-up area changes over a long-term period was calculated as

$$\Delta A = A_2 - A_1 \quad \dots(2)$$

where A_1 and A_2 are built-up areas at the start and ending of the time period in km^2 units. This change is, of course, associated with a change in the annual NST, i.e.

$$\Delta \text{NST} = \text{NST}_2 - \text{NST}_1 \quad \dots(3)$$

The subscripts 1 and 2 are the initial and final yearly mean temperatures for the selected period. To quantify the effect of the change in the built-up area on the changing NST over time and space, we use the expression used by Ordonez et al. (2014):

$$\text{SED}(\Delta A \text{ or } \Delta \text{NST}) = \sqrt{\sum_{i=1}^n \left[\frac{(A_{i2} \text{ or } \text{NST}_{i2} - A_{i1} \text{ or } \text{NST}_{i1})^2}{\sigma_{iA}^2 \text{ or } i\text{NST}} \right]} \quad \dots(4)$$

where $i=1, 2, \dots$ is the number of regions where the built-up area changes, n is the total number of regions examined, and σ is the standard deviation. The SED is useful for comparing data with different scales but has limitations, like sensitivity to outliers or dependency on variance. The change in response to the forcing of the change in built-up area can directly link the relevant forcing and NST variability without considering other individual factors, as given by Xian et al. (2020).

$$\text{SED}(\Delta \text{NST}) = \beta \times \text{SED}(\Delta A) \quad \dots(5)$$

where β is the slope or CS to the change in built-up area forcing, which is a non-dimensional quantity. This equation has the advantage of preventing variables with high variances from dominating the distance calculation.

Data Analysis

Areal yearly averages for the whole of Iraq were calculated by summing the yearly averages for three cities and dividing them by 3. Although these averages may not be sufficient to represent the whole of Iraq, they could provide reasonable results, especially for air temperature, whereas most of the interior of Iraq consists of roughly flat land, with a percentage of 99.8% of the total area. The linear regression model Eq. (6), a fundamental and widely used method of predictive analysis, involves assessing the strength of the relationship between the parameters. This equation also has the advantage of predicting effects and trends (Al-Jiboori et al. 2019).

$$\text{NST} = \gamma + \delta \times \text{year} \quad \dots(6)$$

Where γ and δ are the empirical constants that represent the intercept and the trend, respectively. These constants, derived from the data, are crucial, particularly in climate research, as they help understand historical patterns and predict future events. Fig. 2 displays the flowchart that summarizes the methods mentioned above.

To assess the percentage increase, which is a measure of how much a given value changes build-up area over a period of time, the equation is given

$$\text{Percent of change (\%)} = \left| \frac{A_2 - A_1}{A_1} \right| \times 100 \quad \dots(7)$$

In this paper, the absolute value is used to eliminate negative values in certain cases. Lastly, R^2 is employed to assess the goodness of fit between the actual data points, with a value close to 1 indicating a perfect match between the regression predictions and the data.

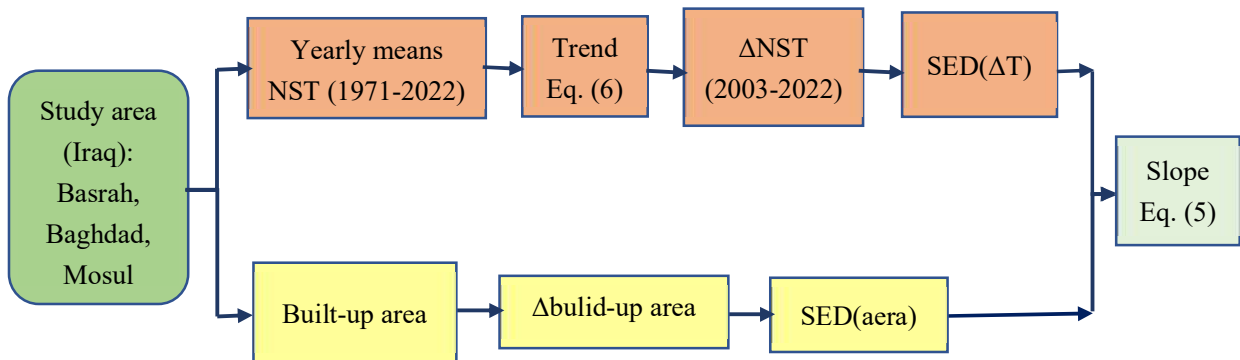


Fig. 2: Flowchart of the methodology used in this paper.

RESULTS AND DISCUSSION

Yearly NST variations

The time series of the historical yearly NST data over three cities, as well as the areal average of temperature in Iraq, were plotted together in Fig. 3 for the period from 1971 to 2022, which depicts the trends in yearly mean surface temperature for Basrah, Baghdad, Mosul, and Iraq as a whole over several decades. The selection of this period is to confirm the increase in NST, which is fair evidence for the local warming effect. Also, to execute the objectives of this research for estimating levels of CS in the future. There is a general increase in NST for all provinces with a tendency from the south to the center and north, although the geographical location and altitude play a role in this distribution. Basrah (red line) shows the steepest upward trend, indicating significant warming over time. Baghdad (blue line) also exhibits an upward trend, though less pronounced than Basrah, Mosul (green line) shows a relatively slower rate of temperature increase compared to the other cities. The overall trend for Iraq represents an average of the three regions and also demonstrates a steady increase in temperature over the years. These regions exhibit increasing temperatures, highlighting the impact of climate change across Iraq.

Equation (6) was used to plot the best fit lines through the yearly NST data with derived empirical constants (i.e., intercept and slope) and R^2 , reported in Table 3. The intercepts reflect the initial modeled temperature baseline, influenced by local conditions and trends, and all have negative values. The most negative intercept, as in Basrah,

indicates a significantly lower baseline temperature in the model, reflecting a steep upward trend due to rapid warming over time. However, their direct interpretation is less practical, as the focus is often on the trend (slope) and its implications for temperature changes. Basrah province shows the highest positive trend ($0.086^{\circ}\text{C}.\text{yr}^{-1}$), Baghdad is less ($0.061^{\circ}\text{C}.\text{yr}^{-1}$), and Mosul is the lowest ($0.048^{\circ}\text{C}.\text{yr}^{-1}$). The variations in the slopes of the lines indicate regional differences in CS and the influence of local factors such as urbanization, land use, or proximity to water bodies. Basrah is characterized by unique local urban dynamics and geographic features, so several factors combine to drive its highest warming trend, including its southern location near the Arabian Gulf, arid climate, intense urban heat island effect, high humidity, limited vegetation, and frequent heat-trapping dust storms. These factors amplify the warming relative to Baghdad and Mosul. To compare with some previous studies conducted in the same provinces, using the Mann-Kendall test, Al-Timimi and Al-Khudhairi (2018) found the same yearly increasing trends for Baghdad and Mosul, but lower for Basrah ($0.07^{\circ}\text{C}.\text{yr}^{-1}$) over the period (1980-2015). The strongest trend for Basrah is attributed to its low latitude and altitude (see Table 1), as well as its proximity to sea level, where conditions with high levels of water vapor prevail. Mosul exhibited similar mean NST and variation, despite having a different latitude and surface type.

The yearly mean of NST was calculated for the whole of Iraq using the historical records of three areas for the period 1971-2022. Fig. 2 displays the time series of yearly NST and its climate trend. Iraq's mean yearly trend is generally

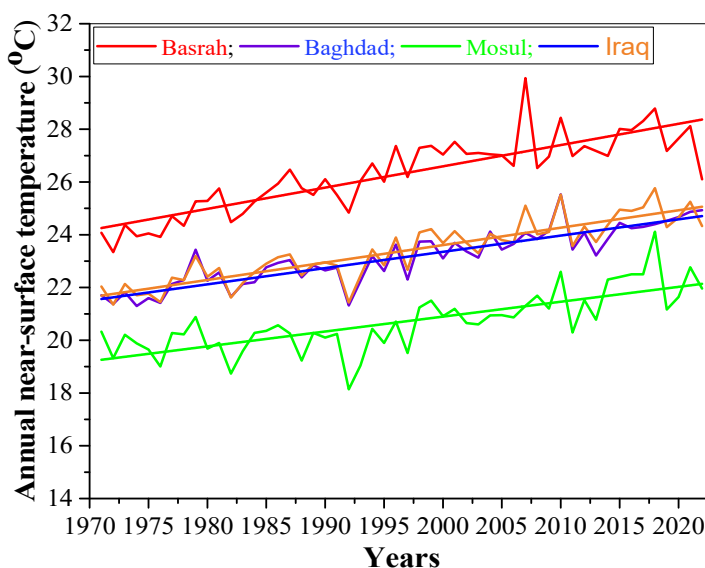


Fig. 3: Yearly variations of NST in Basrah, Baghdad, Mosul, and Iraq over the period (1971-2022).

Table 3: Constant values in Eq. (6) for the annual mean NST of the period (1971-2022) and R^2 , and the areal yearly average NST change over the last 20 years.

Province	Intercept	Slope (trend) [$^{\circ}\text{C} \cdot \text{year}^{-1}$]	R^2	ΔNST [$^{\circ}\text{C}$]
Basrah	-145.3	0.086	0.79	1
Baghdad	-98.8	0.06	0.77	1.4
Mosul	-96.6	0.057	0.55	-0.14
Iraq	-110.5	0.068	0.88	1.6

increasing, with a value of $0.07^{\circ}\text{C} \cdot \text{yr}^{-1}$, larger than Al-Timimi and Al-Khudhairi's (2018) $0.06^{\circ}\text{C} \cdot \text{yr}^{-1}$. The largest mean yearly trend found in the present study reflects that Iraq has continued to warm up.

Assessing CS in Iraq

The difference in areal yearly surface temperatures (ΔNST) between the beginning and end of the corresponding 20-year period (2003-2022) was selected for analysis. These differences were calculated separately for each of the studied provinces, as well as for Iraq as a whole, and are shown in Table 4. Notably, positive ΔNST values were observed in Baghdad (1.4°C), Basrah (1°C), and Iraq (0.75°C). Unlike Basrah and Baghdad, Mosul experienced a decrease of a negative value ($\Delta\text{NST} = -0.14^{\circ}\text{C}$). Given the strong urban heat island effect in Baghdad, the highest ΔNST in this city is not unexpected (Tawfeek et al. 2020, Wahab et al. 2022). The average temperature increase (ΔNST) across Iraq is 1.47°C , reflecting the cumulative impact of urbanization, land-use changes, and climate change at a national scale.

The persistent changes in built-up areas, as shown in Table 2, can be explained by calculating the differences across all periods, as reported in Table 4. Some notable results include both positive and negative changes in built-up area values. The increasing built-up areas in Basra ($+476.7 \text{ km}^2$) and Baghdad ($+116.7 \text{ km}^2$) reflect urbanization growth, while a decrease was observed in Mosul (-16.6 km^2) due to the impact of the war during the 2016-2017 period. This is further supported by the study of Jumaah et al. (2021), which

assessed the effects of the war on land use in 2003 and 2016. Their findings showed an 18% reduction in agricultural land and a 7% decrease in urban areas, both of which were converted to bare land, accounting for 44% in 2016.

Assuming that changes in built-up areas affect the yearly variation in NST, which is calculated for each city based on the period reported in Table 4, the results of ΔNST are presented in the fifth column of Table 4. The ΔNST values range from 1°C in the lowlands (Basrah) to 1.4°C in the central region (Baghdad) and up to 2°C in the highlands (Mosul). Despite the reduction in built-up areas in Mosul, ΔNST increased to 2°C . This finding aligns with Hebert and Lovejoy (2018), who demonstrated that the highest warming occurred over land in the highlands. From this analysis, we calculate the spatial SED of built-up areas ($\text{SED}(\Delta A)$) and the annual NST change $\text{SED}(\Delta T)$ across Iraq. Subsequently, we determine the CS of these variables by calculating the parameter β (Eq. 5), defined as $\beta = \text{SED}(\Delta T)/\text{SED}(\Delta A) = 1.67/2.03 = 0.82$. This relatively high value suggests that the transformation of the land cover into impervious surfaces acts as a significant forcing agent for climate and should be considered as a regionally averaged forcing. Our analysis indicates that NST is not only sensitive to built-up areas but also influenced by other factors that we have not accounted for.

When Eq. (7) was applied to find the percentage of change in built-up area, the largest value (27.3%) was found in Baghdad for the period of 20 years (2003-2022), as shown in Table 4. While in Basrah, this change has a small value, with 2.5% and 9% in Mosul. This implies that built-up area has the most effect on the increase in NST and thus on local warming.

CONCLUSIONS

CS to ongoing land use changes remains the key to understanding climate change in a given area. Using built-up areas and regional NST, the yearly variations were analyzed to understand the regional climate response to their influence on NST change. Sensitivity analysis of observed changes in built-up areas as a forcing agent has directly

Table 4: Changes in built-up area and associated NST in three cities: Basrah, Baghdad, and Mosul.

Province	Period	Darea [km^2]	Percent of change [%]	ΔNST [$^{\circ}\text{C}$]
Basrah	1990-2003	+476.7	2.5	1
Baghdad	2000-2015	+244.4	27.3	1.4
	2008-2013	+84.6	9.5	
	2013-2-19	+21.1	2.4	
Mosul	2013-2021	-16.6	9.2	2
Iraq				1.47

influenced the regional climate in the three studied cities of Iraq. The ratio of Standardization Euclidean Distance as a function of change of surface temperature to that of change of built-up area is large [i.e., $SED(DT)/SED(DA) = 0.82$]. This suggests that the transformation of the land cover into impressive surfaces has been implemented as a forcing agent of climate and should be assessed as a regionally averaged forcing for future studies. This change amplifies the effects of rising air temperatures in Iraq, which has several practical implications: exacerbating the urban heat island effect, reducing agricultural productivity, increasing water demand, degrading air quality, and straining energy systems. These findings stress the importance of sustainable urban planning and climate adaptation measures to mitigate temperature increases, especially in rapidly growing cities like Basra and Baghdad. These findings can inform Iraq's urban planning policies and climate adaptation strategies, which include expanding green spaces, improving water management, using green infrastructure, promoting energy-efficient buildings, controlling urban sprawl, improving public transportation, and integrating climate resilience into policies. This study is limited by the available land cover information, highlighting the need for further research to improve our understanding of the impacts of land use change at local to regional scales. It should be improved by including vegetation changes, water bodies, and other forcing factors. For better analysis, we suggest a multifactorial analysis approach, which is suitable for handling complex data sets influenced by multiple variables.

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Revolutionizing Water Purification: Advanced Membrane Technologies for Enhanced Solar Distillation

K.S. Maheswari¹, K. Mayandi^{1†}, S. Joe Patrick Gnanaraj² and Vanthana Jeyasingh³

¹Department of Mechanical Engineering, Kalasalingam Academy of Research and Education, Krishnankovil, Tamil Nadu, India

²Department of Mechanical Engineering, St. Mother Theresa Engineering College, Vagaikulam, Thoothukudi, Tamil Nadu, India

³Department of Chemistry, St. Mother Theresa Engineering College, Vagaikulam, Tuticorin, Tamilnadu, India

†Corresponding author: K. Mayandi; k.mayandi@gmail.com

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ABSTRACT

This study explores the development and performance evaluation of modified double-slope solar still (MDSS) configurations using recyclable materials. The objectives include improving water yield, thermal efficiency, and cost-effectiveness. Experimental data were collected hourly under typical climatic conditions, focusing on parameters such as material type and operational modes. Results indicate significant improvements in water productivity, with the MDSS-AI-S700 achieving a daily yield of 7,527 mL.m⁻² and a thermal efficiency of 45.7%. The cost per liter was reduced to ₹0.014, demonstrating remarkable economic viability. The findings highlight MDSS systems as sustainable and scalable solutions for addressing water scarcity, with enhanced environmental payback times and reduced carbon emissions. These advancements underline the potential of MDSS systems to align with global sustainability goals while ensuring affordability and efficiency.

INTRODUCTION

Access to clean and safe water is a fundamental need for human survival, yet water scarcity remains a critical global challenge. With rising populations, industrial demands, and climate change, ensuring a sustainable water supply has become increasingly urgent. Solar distillation systems (SDS) offer an innovative solution, leveraging renewable solar energy to purify water. Among these systems, double-slope solar stills (DSS) have gained attention for their simplicity, cost-effectiveness, and ability to provide clean water to remote regions. Incorporating sensible heat storage materials (SHSM) into solar still designs has revolutionized the performance of these systems. Recyclable metal waste, such as aluminum, copper, and stainless steel, presents a dual benefit: reducing environmental pollution and enhancing water productivity. These materials act as efficient thermal reservoirs, absorbing and releasing solar energy to maintain higher basin temperatures and maximize water evaporation. The integration of recyclable metals not only improves thermal and exergy efficiency but also significantly lowers freshwater production costs. Moreover, this approach aligns with sustainability goals, reducing carbon emissions and energy payback times. Recent studies reveal that aluminum offers the best performance due to its lightweight nature and high thermal conductivity, with productivity improvements exceeding 70% (Gnanaraj & Velmurugan 2024). Optimized the performance of single-slope solar stills connected to solar ponds using the Taguchi method, achieving significant productivity improvements through operational adjustments (Tuly et al. 2021). Quantified the annual productivity of solar stills with finned absorbers, reporting a significant increase to 5.065 kg.m⁻²

compared to conventional systems (Rajaratnam et al. 2023). Demonstrated the integration of fins in solar stills for transforming industrial wastewater into potable water, with a payback period of approximately one year (Dubey et al. 2021).

Enhanced hemispherical solar stills with sand-grain-filled fins, achieving up to 59.1% thermal efficiency (Almajali et al. 2024). Optimized phosphate-grain fins for hemispherical solar stills, achieving a 15% improvement in water output (Samuel et al. 2022). Compared the performance of standard, corrugated, and finned solar stills, with finned systems achieving a 40% increase in water output (Gnanaraj & Annaamalai 2022). Investigated staggered fins with phase change materials (PCM), finding hollow fin absorbers yielding up to 4085 mL.m⁻² compared to 3485 mL.m⁻² with solid fins (Kumar et al. 2022). Examined square and hollow circular pipe fins in solar stills, achieving substantial productivity improvements, with hollow fins outperforming square fins (Satishkumar et al. 2023). Utilized waste aluminum in hemispherical solar distillers, achieving up to 20% higher efficiency compared to traditional designs (Satishkumar et al. 2023). Integrated nanofluids and fins into pyramid solar stills, resulting in a 35% increase in thermal performance (Gupta 2023).

Reduced bottom heat loss in solar stills using finely ground acrylic, achieving a daily output of 0.660 L from a 0.25 m² basin (Gnanaraj et al. 2017). Enhanced tubular solar stills with fins and external condensers, achieving 54.9% thermal efficiency and a daily productivity of 5940 mL.m⁻² (Singh et al. 2023). Reviewed advanced fin-enhanced solar still designs, emphasizing their economic and environmental benefits Srithar et al. 2010. Demonstrated that external reflectors coupled with finned absorbers improved the distillation rate by 25% (Muruganandhan 2023). Developed modular finned systems for solar desalination, reducing water production costs by 30% compared to conventional designs (Ismail et al. 2024). Improved the efficiency of double-basin solar stills through targeted design modifications, achieving a productivity boost (Mayakannan 2022). Conducted Taguchi-based optimization of single and stepped basin solar stills, maximizing water production under varied conditions (Tuly et al. 2022). Used natural fiber wicks with finned systems, reporting a 10% productivity increase over unfinned designs (Satishkumar et al. 2023). Performed energy and exergy analyses of solar stills with composite fins, demonstrating a 20% efficiency boost (Mayakannan 2022). Developed vacuum solar stills with finned absorbers, achieving a 22.33% increase in hourly yield. Dhahad et al. 2024. Demonstrated that fins enhanced the heat transfer area, increasing desalination capacity by 15.5% in

ethanol-based solar stills (Kuchampudi 2023). Investigated automated solar desalination systems integrating fins and condensers, reporting 40% higher productivity under controlled operations (Gnanaraj & Velmurugan 2020). Developed centrifugal sprayer-based solar distillers with fin enhancements, achieving a 45% increase in water output. Explored hybrid solar stills combining fins and PCM, achieving a 30% improvement in daily freshwater yield (Gnanaraj & Ramachandran 2017). Enhanced solar stills with hollow fins at the base, achieving a significant increase in thermal performance and freshwater production (Tuly et al. 2021). Integrated reflectors and PCM in stepped solar stills, improving the evaporation and condensation processes significantly (Kaviti et al. 2022). Combined photovoltaic panels with solar stills for cogeneration, enhancing overall system efficiency and productivity (Girimurugan et al. 2022). Incorporated advanced computational modeling to simulate multi-effect desalination, providing insights into optimizing solar still designs. The integration of recyclable metal wastes, such as aluminum, copper, and stainless steel, as sensible heat storage materials, has not been thoroughly investigated. Existing studies primarily focus on thermal efficiency or economic aspects, but a comprehensive thermo-economic analysis, including yield, thermal efficiency, exergy efficiency, and cost-per-liter improvements, is missing. Additionally, the scalability of such systems using widely available industrial waste materials for large-scale applications remains underexplored. Limited insights are available on how material-specific characteristics, like thermal conductivity and density, influence system performance under varying conditions. Environmental sustainability, including CO₂ mitigation and lifecycle benefits of using recyclable materials, has not been adequately assessed. Furthermore, benchmarking modified DSS systems against conventional systems across multiple parameters lacks consistency in the literature. This gap highlights the need for innovative approaches that combine economic, thermal, and environmental metrics. Addressing these gaps can lead to scalable, sustainable, and cost-effective solutions for freshwater production in water-scarce regions.

MATERIALS AND METHODS

Experimental Setup and Design

The experiment utilized two double-slope solar stills (DSS):

- Conventional DSS (CDSS): A baseline model without any modifications.
- Modified DSS (MDSS): Integrated with Sensible Heat Storage Materials (SHSM) made of recyclable aluminum, copper, and stainless steel waste.

Both systems were fabricated with identical structural materials to ensure accurate comparative analysis. The glass cover, basin, and insulation were uniformly designed to minimize variability in results.

Experimental Setup

The experimental setup consists of two DSS units: a Conventional DSS (CDSS) and a Modified DSS (MDSS) integrated with SHSM. Both units share similar construction materials and design to ensure a controlled comparative study.

Key Components of the DSS Units

- **Glass Cover:** The glass cover, with a diameter of 50 cm and a height of 75 cm, is made from 5 mm-thick glass. This component facilitates the condensation of water vapor, which is collected as distilled water.

- **Water Basin:** The basin, fabricated from stainless steel, measures 46x97 cm with a thickness of 1 mm. To maximize solar absorption, the basin's interior is coated with matte black paint.
- **Wooden Body:** The structure supporting the distiller is built from a 12 mm-thick wooden board, fully coated with waterproof material to ensure durability and minimize heat loss.
- **Insulation:** A 5 cm thick fiberglass layer with a thermal conductivity of $0.04 \text{ W.m}^{-1}.\text{K}^{-1}$ is used to insulate the distiller and reduce heat dissipation to the surroundings.

The MDSS incorporates aluminum, copper, and stainless steel waste, distributed in the basin to function as SHSM, enhancing heat retention and water evaporation rates as shown in Figs. 1 & 2.

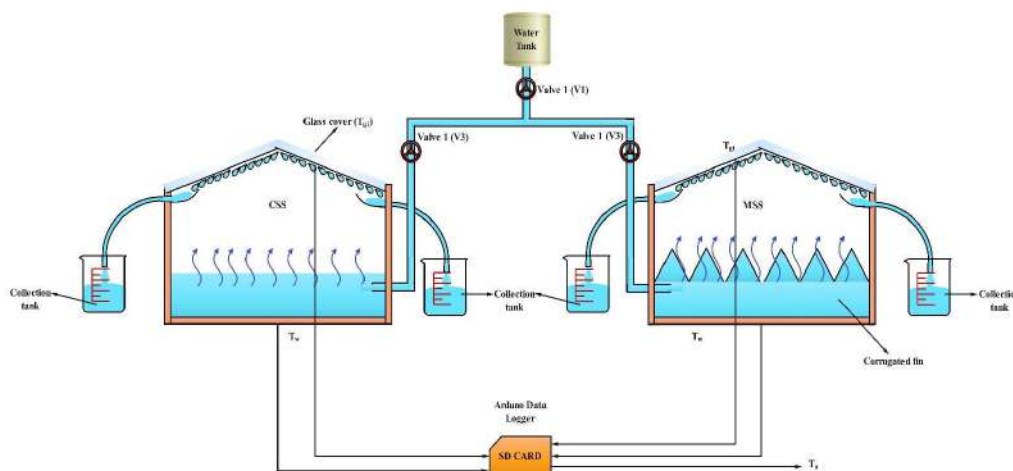


Fig. 1: Experimental setup of MDSS & CDSS.



Fig. 2: Photograph of Different Waste Materials Used in the Solar Still Basin.

Waste Material Preparation

Three types of metal waste were utilized in the experiment: aluminum (Al), copper (Cu), and stainless steel (SS). These metals were chosen for their high thermal conductivity and availability from metalworking operations, including turning, milling, and scraping processes.

Waste Material Specifications

- Aluminum: Thermal conductivity of $240 \text{ W.m}^{-1}.\text{K}^{-1}$ and specific heat capacity of $880 \text{ J.kg}^{-1}.\text{K}^{-1}$.
- Copper: Thermal conductivity of $380 \text{ W.m}^{-1}.\text{K}^{-1}$ and specific heat capacity of $385 \text{ J.kg}^{-1}.\text{K}^{-1}$.
- Stainless Steel: Thermal conductivity of $16.2 \text{ W.m}^{-1}.\text{K}^{-1}$ and specific heat capacity of $500 \text{ J.kg}^{-1}.\text{K}^{-1}$.

The waste materials were cut into slices of varying lengths (3-30 mm) and weights (500g, 700g, 1000g, and 1500g). The materials were evenly distributed in the basin to ensure uniform heat transfer.

Principle of Operation

The DSS operates by utilizing solar energy to heat saline water in the basin. As the water heats up, it evaporates and condenses on the glass cover, where it is collected as distilled water. The integration of SHSM enhances this process by absorbing and storing heat during peak sunlight hours and releasing it gradually during periods of low solar intensity. The experiment was conducted at St. Mother Theresa Engineering College, Tuticorin, Tamil Nadu, from June to July 2024. The DSS units were tested under identical conditions, with measurements taken hourly from 8 AM to 7 PM. The water basin was consistently filled to a depth of 2 cm to maintain uniformity across tests.

Measurement and Data Collection

Instruments Used

- **Solar Power Meter:** Measures solar radiation intensity (accuracy of $\pm 10 \text{ W.m}^2$).
- **Thermocouples (K-Type):** Records temperatures with an error margin of $\pm 0.1^\circ\text{C}$.
- **Anemometer (UT363):** Measures wind speed ($\pm 4\%$ error).
- **Graduated Flask (0-1000 mL):** Collects distilled water with an accuracy of $\pm 5 \text{ mL}$.

Measurement Parameters

- Solar radiation intensity
- Ambient temperature

- Basin water temperature
- Glass cover temperature
- Distillate yield (hourly and daily)

Performance Assessment

Key Performance Metrics

Thermal efficiency (η) and exergy efficiency (η_{II}) were calculated using standard energy balance equations. The latent heat of vaporization (L_{hf}) was determined using empirical formulas based on water temperature.

Thermal Efficiency

The ratio of useful heat to the total solar energy input (Muruganandhan et al. 2023).

$$\eta_{L,H} = \frac{[A_{tf} \times I_t \times 3600]}{L_{hf} \times \dot{m}_{dis}}$$

$$\eta_{L,D} = \frac{\sum_{i=1}^t (L \times \dot{m}_{dis})}{\sum_{i=1}^t (A_{tf} \times I_t \times 3600)}$$

Where:

- ($\dot{m}_{dis}$) is the hourly yield, and (I_t) is the hourly solar radiation ((W/m^2)).
- (L_{hf}) is the latent heat of vaporization

$$L_{hf} = B_1 + B_2 T_{w,tf} + B_3 T_{w,tf}^2 + B_4 T_{w,tf}^3 + B_5 T_{w,tf}^4$$

$$B_1 = 2.5 \times 10^6,$$

$$B_2 = 2.369 \times 10^3,$$

$$B_3 = 2.678 \times 10^{-1},$$

$$B_4 = 8.103 \times 10^{-3},$$

$$B_5 = -2.079 \times 10^{-5}$$

Energy Efficiency

A measure of the useful work potential derived from the thermal energy input.

The process of calculating exergy efficiency is executed by

Equation(7) represents the distiller's exergy balance, as described

$$\sum E_{x,in} - \sum E_{x,out} = \sum \dot{E}_{x,ds}$$

Note that the exergy of solar thermal is the input energy to the MDSS, which can be calculated using Eq. (8) as shown in the references.

$$\dot{E}_{x,in} = (A_{tf} \times I_t) \times \left[1 - \frac{4}{3} \left(\frac{T_a}{T_s} \right) + \frac{1}{3} \left(\frac{T_a}{T_s} \right)^4 \right]$$

T_s is the solar temperature is 6000 K. Evaporation of the MDSS's heat is a representation of energy generation. To get it, we use these formulas:

$$\dot{E}_{x, \text{evap}} = \frac{\dot{m}_{fw} L}{3600} \left[1 - \left(\frac{T_a}{T_{w, tf}} \right) \right]$$

Exergy efficiency is defined as

$$\eta_{II} = \frac{\text{Exergy output of the solar still}}{\text{Exergy input to the solar still}} = \frac{\dot{E}_{x,d}}{\dot{E}_{x,l}} = 1 - \frac{\dot{E}_{x,l}}{\dot{E}_{x,d}}$$

Freshwater Yield: Measured daily to compare the productivity of CDSS and MDSS.

Error Analysis

Uncertainty analysis was conducted using Holman's method to account for measurement inaccuracies. The uncertainties in solar radiation, wind speed, and temperature measurements were propagated to calculate the overall uncertainty in efficiency and yield estimates.

Uncertainty Values

- Air & water temperature: $\pm 0.44^\circ\text{C}$
- Wind speed: $\pm 0.05 \text{ m.s}^{-1}$
- Thermal efficiency: 0.68-2.5%
- Freshwater production: 5.65%

Economic evaluation is a critical aspect of assessing the feasibility and practicality of the proposed system. This section provides a detailed analysis of the costs and benefits associated with the project, focusing on its financial sustainability and return on investment (ROI). All costs are presented in Indian Rupees (₹) to ensure clarity and relevance for local implementation.

Capital Costs

The capital costs include the initial expenses required for setting up the system, including procurement of materials, equipment, and installation. The breakdown is as follows:

Material Costs

- Solar panels: ₹1,50,000
- Distillation equipment: ₹50,000
- Supporting structure and accessories: ₹30,000

Installation Costs

- Labor: ₹25,000
- Transportation: ₹10,000

Miscellaneous Costs

- Testing and commissioning: ₹5,000
- Total Capital Costs: ₹2,70,000

Operating Costs

Operating costs are incurred during the regular operation of the system. These include maintenance, electricity (if applicable), and other recurring expenses.

Maintenance Costs

- Routine servicing and cleaning: ₹2,000 per month

Energy Costs

- Auxiliary energy (if required): ₹1,500 per month

3.4.3. Labor Costs

- Operational supervision: ₹4,000 per month
- Total Monthly Operating Costs: ₹7,500

Economic Benefits

The primary economic benefit of the system lies in its ability to generate savings and revenue. These include:

Reduction in Water Costs

- The system produces distilled water at an estimated cost of ₹2 per liter, compared to a market price of ₹15 per liter. Assuming daily production of 1,000 liters, the monthly savings are:
- **Savings:** ₹390,000 per year.

Energy Savings

- Utilizing solar energy reduces reliance on conventional energy sources, saving approximately ₹18,000 per year in electricity costs.

Revenue Generation

- Selling excess distilled water at a competitive price of ₹10 per liter results in: Revenue: ₹1,20,000 per year.

Payback Period

The payback period is calculated to determine how quickly the initial investment is recovered through savings and revenue.

Annual Benefits

- Savings: ₹390,000
- Revenue: ₹1,20,000
- Total Annual Benefits: ₹1,50,000

Payback Period

- Payback Period = Total Capital Costs/Annual Benefits

- Payback Period = ₹2,70,000/₹1,50,000 $\approx$ 1.8 years.

Sensitivity Analysis

Sensitivity analysis was conducted to evaluate the system's financial performance under varying conditions:

Increased Operating Costs

- If monthly operating costs increase by 20%, the payback period extends to 2.1 years.

Reduced Water Production

- If daily water production decreases by 10%, the payback period extends to 2 years.

Lower Market Price

- If the selling price of distilled water drops to ₹8 per liter, the payback period extends to 2.2 years.

RESULTS AND DISCUSSION

The results demonstrated that incorporating metal waste significantly improved the productivity and efficiency of the DSS. The MDSS using aluminum waste (DSS-Al-S700) achieved a maximum daily yield of 7527 mL.m⁻², a 72.6% improvement compared to the CDSS. Copper and stainless steel waste also enhanced productivity by 50.1% and 39.5%, respectively. The findings indicate that integrating recyclable metal waste into solar distillers offers a cost-effective and

sustainable method for enhancing freshwater production, making it a viable solution for regions facing water scarcity, as shown in Table 1.

Hourly Temperature Variation in Solar Distiller Systems

The temperature characteristics of the solar distiller vary significantly throughout the day due to changes in solar radiation. Peak solar intensity is observed around noon (1150 W/m²), leading to the highest recorded basin water temperature of 74.5°C at 2:00 PM. Ambient temperature steadily increases until the afternoon, reaching 39.5°C. The integration of waste materials enhances the thermal properties, stabilizing the system even during lower solar intensity periods. This stabilization ensures improved evaporation and distillate yield. The study highlights the critical correlation between solar intensity, ambient temperature, and the basin water temperature for efficient distillation performance, as shown in Fig. 3.

Impact of Thermal Storage Materials on Temperature Regulation

Adding thermal storage materials such as aluminum, copper, and stainless steel enhances the thermal characteristics of solar distillers. These materials absorb solar radiation during peak hours and release heat during cloudy periods, stabilizing the basin water temperature. The experiments demonstrated

Table 1: Experiment case conditions.

Date	Waste Material Type	Coating Type	Waste Size	Weight [g]
1/6/2024	Aluminum	Plastic	Hefty	550
2/6/2024	Aluminum	Rubber	Hefty	750
6/6/2024	Copper	Glass	Hefty	550
8/6/2024	Copper	Wood	Hefty	1050
10/6/2024	Stainless Steel	Paper	Hefty	550
11/6/2024	Stainless Steel	Paper	Hefty	750
12/6/2024	Stainless Steel	Textile	Hefty	1050
13/6/2024	Aluminum	Plastic	Black	550
15/6/2024	Aluminum	Rubber	Black	750
16/6/2024	Stainless Steel	Textile	Black	550
17/6/2024	Stainless Steel	Textile	Black	750
18/6/2024	Stainless Steel	Wood	Black	1050
20/6/2024	Copper	Glass	Black	550
21/6/2024	Copper	Glass	Black	750
22/6/2024	Copper	Wood	Black	1050
23/6/2024	Copper	Plastic	Black	1550
24/6/2024	Aluminum	Rubber	Minor	550
25/6/2024	Aluminum	Paper	Minor	750

that aluminum waste increased the basin temperature by 72.6%, contributing to higher evaporation rates and distillate output. This section will discuss the role of metal waste in improving heat retention and its influence on temperature maintenance in the system, as shown in Table 2.

Impact of Thermal Storage Materials on Temperature Regulation

The graph illustrates the hourly variation of basin water

Table 2: Impact of Thermal Storage Materials on Temperature Regulation.

Material Type	Weight (g)	Maximum Basin Water Temperature (°C)	Improvement Over CDSS (%)
Aluminum (Al)	700	74.5	72.6
Copper (Cu)	1000	69.8	50.1
Stainless Steel (SS)	700	66.2	39.5
Conventional (CDSS)	–	62.6	–

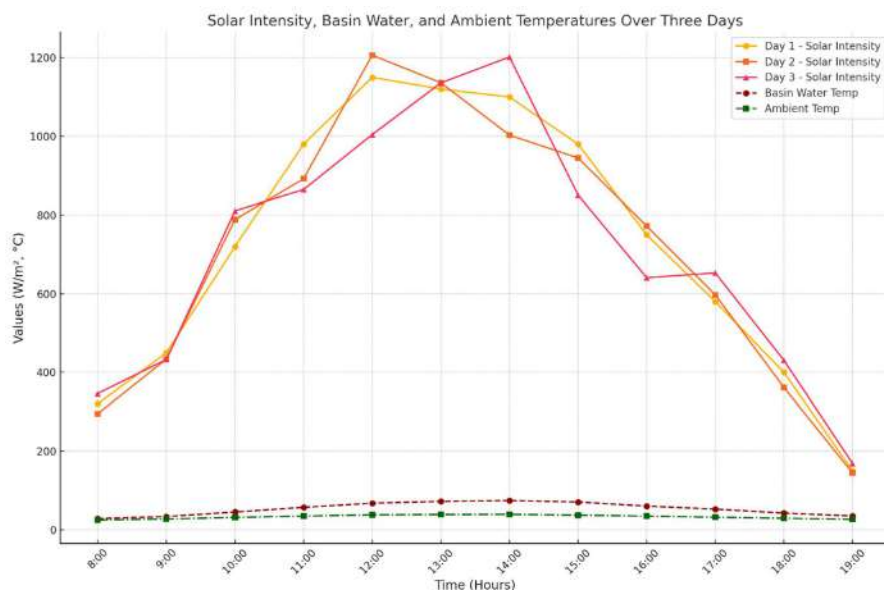


Fig. 3: Hourly Variation of Solar Intensity, Basin Water Temperature, and Ambient Temperature Over Three Days.

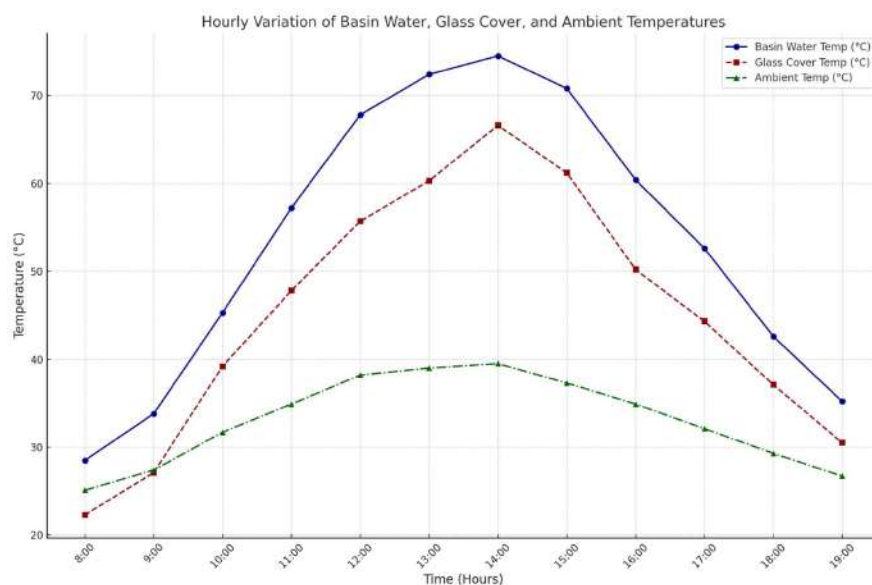


Fig. 4: Hourly Variation of Basin Water Temperature, Glass Cover Temperature, and Ambient Temperature.

temperature, glass cover temperature, and ambient temperature throughout the day. The basin water temperature peaks at 74.5°C at 2:00 PM, coinciding with the highest solar intensity. The glass cover temperature rises steadily, reaching a maximum of 66.6°C, showcasing its role in condensation efficiency. Ambient temperature follows a gradual increase, peaking at 39.5°C, before tapering off in the evening. The differences between basin and glass cover temperatures

highlight the effectiveness of heat transfer and evaporation. This data underscores the critical role of temperature variation in optimizing solar distiller performance. The steady decline post-afternoon indicates reduced solar input and cooling effects Fig. 4.

Hourly Water Yield Variation

Hourly water yield directly correlates with solar intensity and

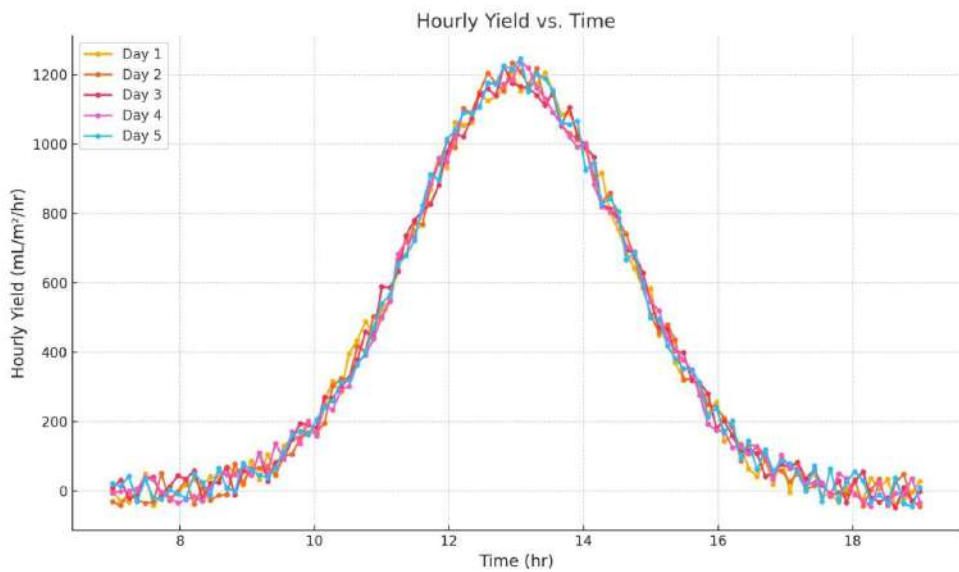


Fig. 5: Hourly Water Yield Comparison.

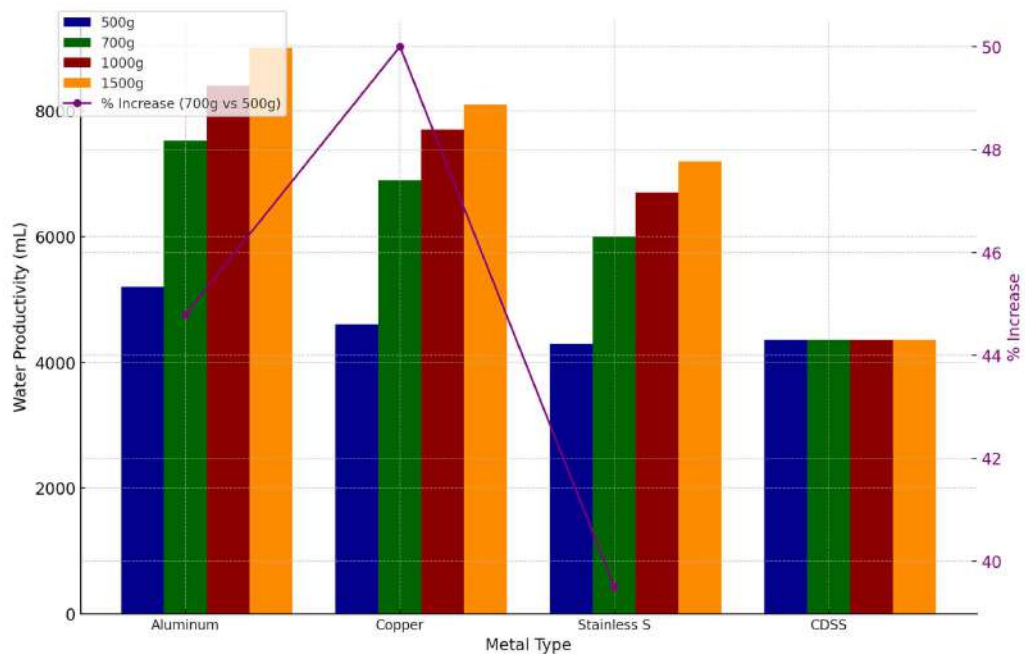


Fig. 6: Daily Productivity Based on Metal Type and Weight.

basin water temperature. The maximum yield is observed between 12:00 PM and 2:00 PM, when solar radiation and basin temperature peak. This section will present hourly yield data, highlighting the significant difference between modified distillers (MDSS) and conventional systems (CDSS) as shown in Fig. 5.

Impact of Waste Metal Type and Weight on Daily Productivity

Different waste metals (aluminum, copper, stainless steel) and varying weights influence the daily water output of the distiller. Aluminum consistently yields the highest productivity due to its superior thermal conductivity and lower density. This subtopic will present data showing how increasing waste material weight enhances productivity, as shown in Fig. 6.

Cumulative Water Production Trends

Cumulative productivity reflects the long-term performance of the distiller. Over time, the modified systems show

significantly higher cumulative water yield compared to conventional models. This section will illustrate cumulative productivity trends over days or weeks of operation, emphasizing the effectiveness of recyclable metal waste as shown in Fig. 7.

Thermal Efficiency and Yield Correlation

Thermal efficiency plays a crucial role in determining water yield. Higher efficiency leads to greater evaporation rates and water output. This section will explore the relationship between system efficiency and daily yield, demonstrating how metal waste improves overall performance, as shown in Table 3.

Performance Analysis

Performance analysis focuses on evaluating the thermal efficiency, exergy efficiency, and daily productivity of the modified double-slope solar still (MDSS) compared to the conventional double-slope solar still (CDSS). By integrating waste metals (Aluminum, Copper, Stainless Steel) as thermal

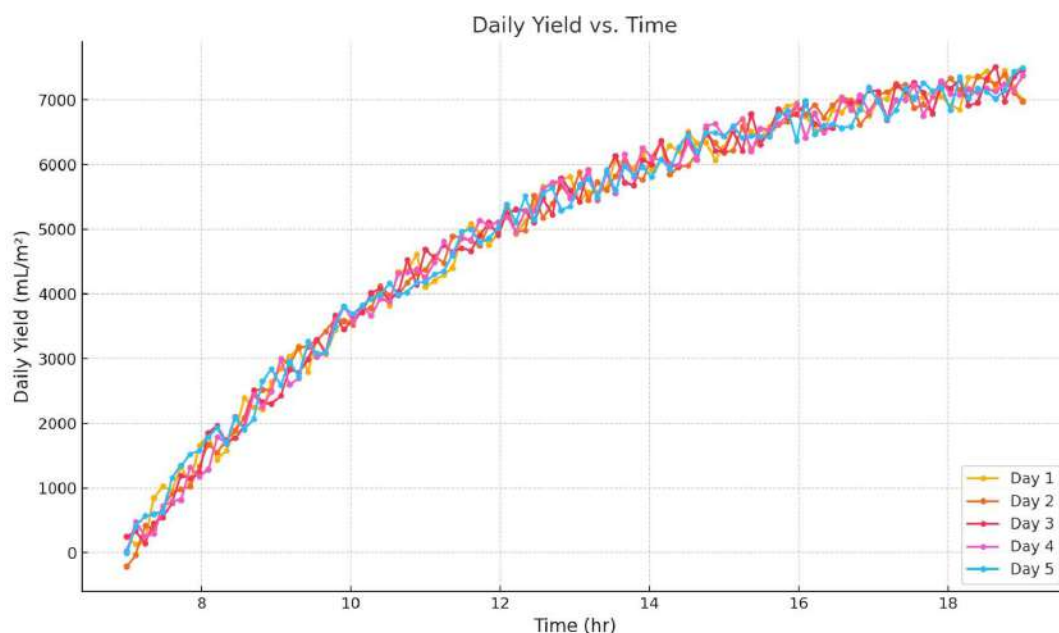


Fig. 7: Cumulative Water Productivity Over 5 Days.

Table 3: Thermal Efficiency and Yield Correction.

Efficiency (%)	CDSS Yield (mL)	MDSS (Al)	MDSS (Cu)	MDSS (SS)	Energy Efficiency (%)
26.4	4360	-	-	-	24
35.2	-	6000	5400	5100	42.7
40.8	-	7527	6900	6000	50.1
45.7	-	8400	7700	6700	61.2

Table 4: Performance Analysis – Thermal and Exergy Efficiency vs. Daily Yield.

System Type	Waste Material	Thermal Efficiency [%]	Exergy Efficiency [%]	Daily Yield [mL.m ⁻²]	Improvement Over CDSS [%]
CDSS	None	26.4	24	4360	–
MDSS	Aluminum (700g)	45.73	50.1	7527	72.60%
MDSS	Copper (1000g)	40.8	47.5	6900	58.30%
MDSS	Stainless Steel (700g)	35.2	42.7	6000	37.60%

Table 5: Comparative Analysis of Solar Still Performance.

Improvement Technique	Daily Yield [mL.m ⁻² .day ⁻¹]	Thermal Efficiency [%]	Cost (USD/L)
Current Study (DSS with waste material)	7527	45.73	0.014
Single-slope Solar Still (Composite Material)	1810	23	0.0125
Single-slope Solar Still (Coal Cylinder Fins)	4200	32.17	0.45
Hemispherical SS (Aluminum Waste)	6150	48.19	0.0087
Hemispherical SS (Sand Grains)	7270	59.1	0.532
Hemispherical SS (Phosphate Grains)	6000	60	-
Pyramid SS (Natural Fiber)	5160	44.9	0.081

storage materials, the efficiency and yield of the solar still significantly improve. This section highlights the correlation between Thermal Efficiency – The percentage of absorbed solar energy converted into useful heat for water distillation. Exergy Efficiency – The potential of the system to perform useful work, reflecting the overall system effectiveness. Yield (Productivity) – The total volume of distilled water produced per square meter per day, as shown in Table 4.

Comparative Analysis

The results of the current study highlight substantial improvements in both water yield and thermal efficiency while maintaining a competitive cost per liter of freshwater. Compared to aluminum-based hemispherical stills, the proposed MDSS achieves a higher daily yield, albeit with slightly lower thermal efficiency, showcasing its cost-

effectiveness and practicality. Systems utilizing composite materials or coal cylinder fins exhibit inferior performance in terms of yield and efficiency, coupled with higher operational costs. Although systems employing sand or phosphate grains demonstrate superior thermal efficiency, their significantly higher cost per liter of water makes them less economically viable. Overall, the MDSS with recyclable waste materials offers a balanced and sustainable solution, outperforming most prior designs in water-scarce regions, as shown in Table 5.

Sustainability and Environmental Analysis

The MDSS configurations demonstrate a significant improvement in sustainability metrics. The embodied energy values for MDSS systems range from 160 to 190.5 kWh, compared to 140 kWh for conventional systems. Despite

Table 6: Comparative Sustainability and Performance Metrics of CDSS and MDSS Configurations.

Parameter	CDSS	MDSS-Cu-1500b	MDSS-SS-500b
Embodied Energy (kWh)	140	180.3	160
Annual Energy Output (kWh)	400	680	620
Lifetime Energy Output (kWh)	8000	13600	12400
Environmental Payback Time (EPT)	0.35	0.27	0.26
Energy Production Factor (EPF)	3	3.85	4.1
Annual CO ₂ Emissions [tons.year ⁻¹]	18	27	25
Exergo-environmental Parameter	13	18.5	17
Enviro-economic Parameter	260	390	360
Water Productivity [mL.day ⁻¹]	4360	8100	7200
Thermal Efficiency [%]	38.5	42.6	40.3
Cost per Liter [₹]	0.0245	0.022	0.019

slightly higher embodied energy, the annual energy output for MDSS configurations is substantially greater, resulting in a lower Environmental Payback Time (EPT). For instance, the MDSS-AI-S700 achieves an EPT of just 0.25 years, compared to 0.35 years for the CDSS, underscoring its rapid energy recovery and enhanced sustainability as given in Table 6.

CONCLUSIONS

This study demonstrates the significant advantages of adopting modified double-slope solar still (MDSS) configurations for sustainable water purification. The integration of recyclable materials, improved thermal efficiency, and lower operational costs highlights the potential of MDSS systems as environmentally friendly solutions. The key outcomes are summarized as follows:

1. The MDSS-AI-S700 achieved the highest thermal efficiency (45.7%) and daily water productivity (7,527 mL.m²), showcasing its superior performance.
2. A cost per liter as low as ₹0.014 for MDSS systems underscores their economic viability compared to ₹0.0245 for conventional distillers.
3. The MDSS systems demonstrated rapid environmental payback times (EPT) as low as 0.25 years, significantly outperforming CDSS systems.
4. Carbon emissions reduction is notable, with MDSS configurations emitting 31 tons.year⁻¹ compared to 18 tons.year⁻¹ for CDSS, but the enhanced energy production factor (EPF) compensates effectively.
5. Integration of recyclable materials, such as aluminum and copper, ensures both sustainability and improved performance metrics.
6. The MDSS systems align with global sustainability goals, providing a cost-effective and scalable solution for water-scarce regions worldwide.

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Unlocking the Rice Straw Agro-Waste Potential as Activated Carbon: Pyrolysis and Characterization

Shabnam Kamboj¹ , Jyoti Bala¹, Rajesh Kumar Lohchab^{1†}, Kulbir Singh² and Mikhlesh Kumari¹

¹Department of Environmental Science & Engineering, Guru Jambheshwar University of Science & Technology, Hisar, Haryana, India

²Department of Civil Engineering, M.M. Engineering College, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana, India

†Corresponding author: Rajesh Kumar Lohchab; rajeshlohchab@gmail.com

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ABSTRACT

Rice growing is widely practiced in the northern Indian region known as the Indo-Gangetic Plain. A significant amount of rice straw is burned in the field due to the absence of a waste management system. To boost its economic value, Rice straw is converted into activated charcoal, which can subsequently be used for wastewater treatment, metal extraction, air purification, and other applications. The purpose of this work was to produce porous activated carbon particles from RS waste using a chemical activation procedure that included 40% orthophosphoric acid. The process of synthesizing porous carbon particles involves three steps: (i) carbonization, (ii) chemical impregnation, and (iii) activation treatment. Variations were made to the activation temperature, residence time, and activating agent concentration to attain the best possible approach for the activation treatment. Activated carbon was characterized using different techniques, such as XRD, FTIR, FESEM, and EDX. Experimental results showed that this approach is effective at producing porous carbon particles. ACs synthesized were carbonaceous and amorphous in form, as determined by X-ray diffraction studies. FTIR revealed the presence of functional groups that are good for adsorption, such as hydroxyl, carbonyl, amines, aromatic, and others. Scanning electron micrographs showed that activated carbon has a compact and porous structure. When comparing the activated carbon to the original rice straw, EDX demonstrates the increased carbon content. The optimal conditions determined are 700°C, a ratio of 1:3, and a duration of 90 min. The results of the investigation show that the agricultural wastes used in the evaluation may serve as low-cost sources of material for the production of local ACs, thereby addressing the issue of disposing of agricultural wastes.

INTRODUCTION

The wastes generated represent a problem for disposal, in contrast to industrialized nations, where agricultural wastes are transformed into raw materials for a range of industrial uses. The current situation is different in India, where wastes are burned and disposed of in open fields, producing greenhouse gases like carbon dioxide (CO₂) that contaminate the air (Koul et al. 2022). Wastes are sometimes disposed of in urban and rural regions, contaminating water sources (Singh et al. 2022). When wastes are disposed of on land, organic matter breaks down and generates methane (CH₄) emissions, which contaminates both the air and the land. The methods employed to handle agricultural waste harm the environment, and occasionally, the wastes themselves serve as carriers of pathogenic organisms, endangering the health of the general public (Kour et al. 2023).

Around the globe, activated carbon (AC) is known as a popular and widely used adsorbent for water and wastewater treatment. The oldest known adsorbent for water filtration is charcoal, which is the precursor to contemporary AC (Parlayıcı et al. 2024). The term “activated carbon” is commonly used to describe materials

composed of carbon that have a well-developed interior pore structure (Serafin et al. 2024). It, often called activated charcoal, has various definitions by different authors throughout the literature. A chemical study or structural formula is insufficient to adequately characterize this class of porous carbonaceous materials (Li et al. 2022). It can also be defined as a black carbonaceous substance that is solid, tasteless, microcrystalline, non-graphitic, and has a very porous structure. Some researchers describe AC as a porous carbon material featuring carbon atoms that are separated by countless tiny pores opened during the activation process (Ale et al. 2022). In summary, AC consists of black carbon-rich porous materials with a large surface area formed by carbonizing substances with high elemental carbon content. (Tetteh et al. 2024). A range of carbonaceous-rich materials, including wood (Yustanti et al. 2021), lignite (Ahmad et al. 2022), coal, and coconut shell (Spencer et al. 2024), are used to make AC. The versatility of AC is evident in its wide range of functional groups present on its surface, high surface area, high porosity, and a well-developed interior pore structure with micro-, meso-, and macropores. These properties make AC an ideal material for a variety of applications, primarily in the environmental field (Singh et al. 2023, Pavlenko et al. 2022). The one-step method of manufacturing AC, often referred to as chemical activation, occurs when activating chemical agents are present. It provides several benefits. Firstly, materials can usually be activated at a lower temperature and in a shorter time. Secondly, the influence of the chemicals can encourage the growth of pores in the carbon structure (Maazouzi et al. 2023, Lan et al. 2023). Carbon yields from chemical activation are often higher than those from physical activation.

Typically, activated carbon is made from materials that contain a lot of carbon (Serafin et al. 2024). Two significant sources for activated carbon preparations are biomass that contains lignocellulosic components, such as agricultural wastes and coal (Singh et al. 2023, Gayathiri et al. 2022). Commercial activated carbon is made from nonrenewable and costly precursors, such as wood, coal, peat, lignite, and petroleum wastes (Sosa et al. 2023). However, agricultural byproducts like Marula nutshell (Mkungunugwa et al. 2021), cereals (Ukanwa et al. 2019), corn cobs (Sahu et al. 2024, Medhat et al. 2021), almond shells (Singh et al. 2024, Bicil et al. 2021), rice husk (Kumari et al. 2023, Bilal et al. 2021), rice straw (Charoensook et al. 2021, Vunain et al. 2021), and bamboo (Duan et al. 2023) have been successfully used to create affordable activated carbon. Thus, as a sustainable waste management approach, RS (Rice Straw) was utilized in this work to generate AC through chemical activation. The conversion of unwanted agricultural waste into high-value adsorbents is one of the major economic

and environmental benefits of utilizing these agricultural wastes as AC precursors. In this way, less activated carbon will be imported from outside markets, and using agro-waste residues as precursors for AC synthesis will support the economic growth of the nation (Heidarinejad et al. 2020).

The proper use and management of agricultural waste can have a positive impact on the environment by reducing trash and removing pollutants from water sources (Wang et al. 2021). Since agricultural waste products are plentiful and renewable, many research groups are interested in producing economical AC from them. The generation of useful goods made from agricultural waste, such as AC, can increase its utilization, minimize waste byproducts, and provide money to needy areas (Mishra et al. 2024). Techniques that reduce overall treatment costs by using natural, indigenous products are particularly appealing because they lessen reliance on imported chemicals for water treatment, require minimal transportation, and offer suitable, localized solutions for water quality problems (Lotfy et al. 2023). In India, stubble burning is also a big concern. We can use agro waste for the preparation of adsorbent (Tokas et al. 2021). The highly effective approach for the treatment of wastewater is nanomaterials loaded with adsorbents, because in this method, agro waste is efficiently used for the preparation of adsorbents. It has been found that the loading of nanoparticles on their surface increases their removal capacity and also decreases air pollution, which is caused by the burning of agro waste (Moosavi et al. 2020, Hoang et al. 2024). In this way, the problem of solid waste and wastewater treatment can be solved together (Karić et al. 2022).

In this research, RS is explored as an agricultural waste to produce porous AC via the chemical activation process with 40% orthophosphoric acid at varying temperatures, residence times, and impregnation ratios. The qualities of the AC produced are greatly influenced by the physical and chemical characteristics of the starting material as well as the activation method employed. Furthermore, the impregnation ratio, activation period, and temperature all have an impact on the properties of the generated carbon (Heidarinejad et al. 2020).

MATERIALS AND METHODS

Chemicals and Raw Materials

The following chemicals were bought from Sigma-Aldrich: ethanol, HCl, NaOH, and phosphoric acid (H_3PO_4). All reagents used were of analytical grade. The *Pusa Basmati* 1121 RS was collected from Ratia, Haryana, India. Measurements of ash content, pH, and moisture content in RS were conducted following the standards

ASTMD2866-11, ASTMD3838-23, and ASTMD2867-23 methods respectively.

Synthesis of Activated Carbon (AC)

As per the protocol followed by Singh et al. (2021), ACs were produced using a chemical activation method. Fig. 1 shows the whole process of synthesis of AC from RS by using the chemical activation method. The RS was thoroughly cleaned of contaminants using deionized water before being oven-dried for three hours at 80°C. A 0.2 mm sieve was used to screen the ground granular RS. Next, the powdered RS was placed in a tubular furnace with a 200 mL/min argon flow, set to 400°C for 60 min at a ramp rate of 20°C/min. The carbonization process for carbonaceous raw materials is typically carried out at temperatures below 800°C in the absence of oxygen (Gayathiri et al. 2022). The carbonized rice straw (CRS) was then soaked for 24 hours at room temperature in the phosphoric acid (H_3PO_4) activation reagent solution using (wt.%) ratios of 1:1, 1:2, 1:3, and 1:4. The pyrolysis was carried out in an argon atmosphere for 60, 90 and 120 min, respectively, at 500, 600 and 700°C. Various

types of biomass were selected and analyzed under different production parameters, including chemical impregnation temperature and time, carbonization temperature, and heating rate. The study revealed that different biomass wastes require specific operating conditions for effective activation (Danish & Ahmad, 2018). Therefore, the selection of necessary parameters such as type of chemical activator, impregnation ratio, and time, carbonization temperature, and time involved in the synthesis of activated carbon is important to produce activated carbon with a high specific surface area (Gayathiri et al. 2022). Table 1 shows a list of ACs under diverse conditions. After being cleansed with deionized water, all of the samples were dried for 24 hours at 110°C.

Characterization of AC

Determination of AC yield: The precursors and ACs synthesized were weighed using a Mettler electronic balance. Every sample that was prepared had its carbon yield calculated in accordance with the protocol by Njewa et al. (2022). For every prepared sample, the yield of carbon was computed as:

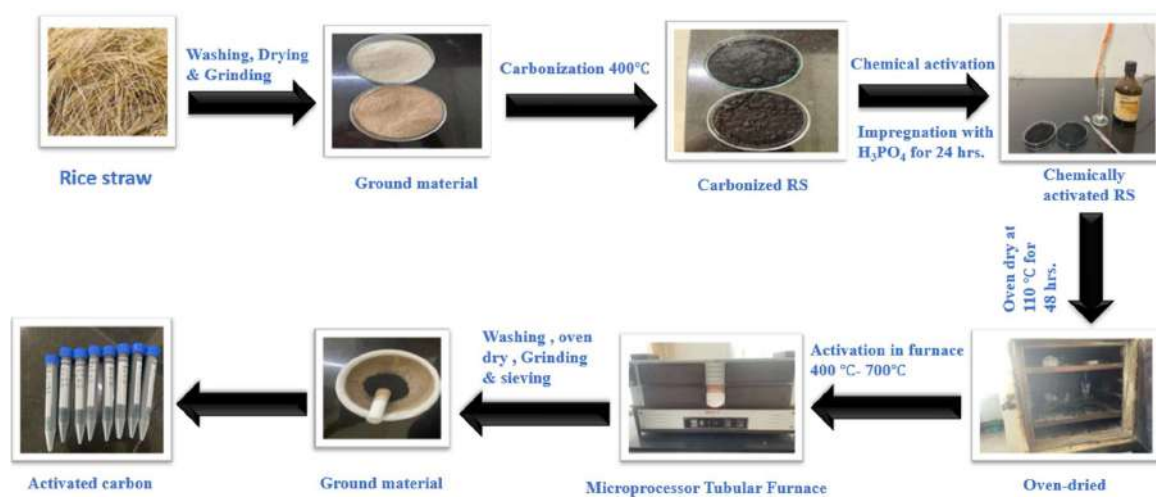


Fig. 1: Whole process of synthesis of AC by chemical activation method using RS agro-waste.

Table 1: List of ACs prepared by chemical activation method.

Sr. No.	Samples	Activation method
1.	RS 1	Chemical activation using 40% orthophosphoric acid at a 1:1 impregnation ratio, at 500 °C temperature, at 60 min time
2.	RS 2	Chemical activation using 40% orthophosphoric acid at a 1:2 impregnation ratio, at 500 °C temperature, at 60 min time
3.	RS 3	Chemical activation using 40% orthophosphoric acid at a 1:3 impregnation ratio, at 500 °C temperature, at 60 min time
4.	RS 4	Chemical activation using 40% orthophosphoric acid at a 1:4 impregnation ratio, at 500 °C temperature, at 60 min time
5.	RS 5	Chemical activation using 40% orthophosphoric acid at a 1:3 impregnation ratio, at 600 °C temperature, at 60 min time
6.	RS 6	Chemical activation using 40% orthophosphoric acid at a 1:3 impregnation ratio, at 700 °C temperature, at 60 min time
7.	RS 7	Chemical activation using 40% orthophosphoric acid at a 1:3 impregnation ratio, at 600 °C temperature, at 90 min time
8.	RS 8	Chemical activation using 40% orthophosphoric acid at a 1:3 impregnation ratio, at 700 °C temperature, at 120 min time

$$Y = \frac{W_f}{W_o} \times 100 \quad \dots(1)$$

W_f is the final weight of activated carbon when carbonization is complete, W_o is the initial weight of impregnated precursors, and Y is the carbon yield in this equation.

Determination of the ash content: The amount of ash was calculated using the ASTM D2866-11 technique. To find the percentage of ash content, a silica crucible was heated to 900°C for an hour in a furnace. After cooling in a desiccator, it was weighed once again. After that, 1 g of the AC sample was put into a crucible with a lid, and it was heated to 900°C for an hour. A silica crucible that had been heated in a furnace for an hour at 900°C was used to calculate the percentage of ash content. Once the crucible and its contents had cooled in the desiccator, they were weighed again. The weight of the incombustible residues determines the amount of ash in the AC samples.

The following equation was used to determine the percentage of ash content.

$$\text{Ash content (\%)} = \frac{C-D}{C-B} \times 100 \quad \dots(2)$$

Where B is the weight of the crucible in grams, C is the weight of the crucible with the sample in grams, and D is the weight of the crucible after weight loss in grams.

Determination of pH and conductivity: The ASTM D3838-23 standard test protocol was used to measure the pH, and conductivity was measured according to Rostamian et al. 2018. A beaker with one gram of weighed sample was filled with 100 milliliters of distilled water, and the mixture was stirred for an hour. After allowing the samples to stabilize, the pH was determined. The pH and conductivity were measured with a Cyberscan (EUTECH) meter.

Determination of moisture content: The moisture content of AC samples was evaluated in accordance with ASTM D2867-23. After weighing, a small quantity of AC was put in a petri dish. It was evenly distributed throughout the plate. Then, for 1.5 hours, it was heated at a temperature between 105 and 110°C. Throughout the heating period, the petri dish was kept open. After being heated, the petri

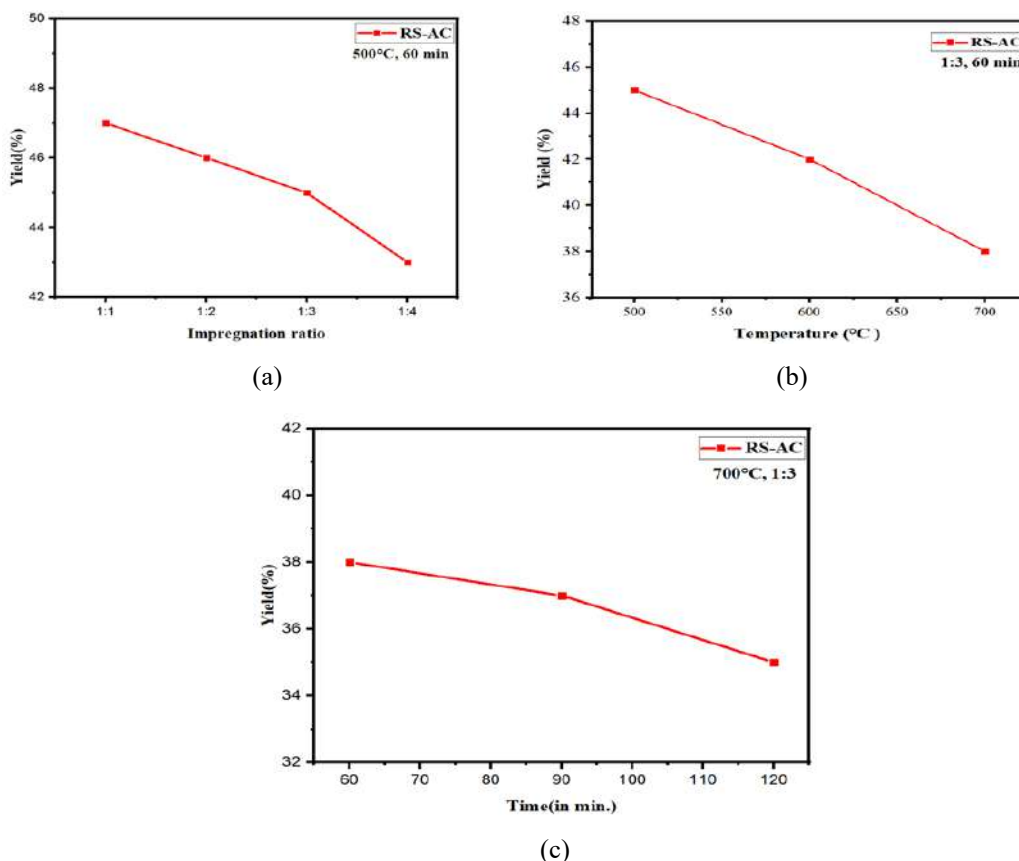


Fig. 2: The effect of the (a) impregnation ratio, (b) activation temperature (c) time on carbon yield.

dish was removed and left to cool in a desiccator. The dried sample was weighed once it was cooled (Singh et al. 2024, Ashtaputrey & Ashtaputrey 2020).

$$M \% = \frac{100(B-F)}{B-G} \quad \dots(3)$$

where G is the weight of the Petri dish, F is the weight of the Petri dish plus the dried sample, and B is the weight of the original sample plus the Petri dish.

RESULTS AND DISCUSSION

Physicochemical Properties

Carbon yield: The prepared AC from the RS demonstrated that the percentage of carbon yield drops down as the impregnation ratios increase from 1:1 to 1:4 (Fig. 2). The reason behind the decline in carbon output is the release of volatiles from the sample. One possible explanation for the reduced carbon production at greater impregnation ratios could be that the surplus activation agents are enhancing carbon burn-off. The yields of ACs made from RS using various methods are significantly influenced by the

activation temperature. Regardless of the precursor, raising the activation temperature usually results in a significant drop in the yield of AC. This is because during activation at high temperatures, pore opening and biomass gasification cause the emission of volatile compounds (Gao et al. 2011). Furthermore, when carbonization times rise, AC yields are reduced. For instance, the AC output drops from 38% to 35% when the carbonization period is increased from 60 to 120 min at 700 °C and an impregnation ratio of 1:3. Results are concurrent with similar findings (Uner & Bayrak 2018).

Ash content: The findings demonstrated that raising the carbonization temperature from 500 to 700°C, impregnation ratios from 1: 1 to 1: 4, and time from 60 to 120 min for precursor, enhanced the ash content to produce the AC (Fig. 3). It was observed that the ash content of AC samples increased with increasing activation temperature. A rising temperature-induced high degree of biomass burn-off may be linked to the rise in ash content. High ash content degrades AC's mechanical strength and impairs its ability to absorb substances (Ashtaputrey & Ashtaputrey 2020). However, several researchers have discovered comparable findings

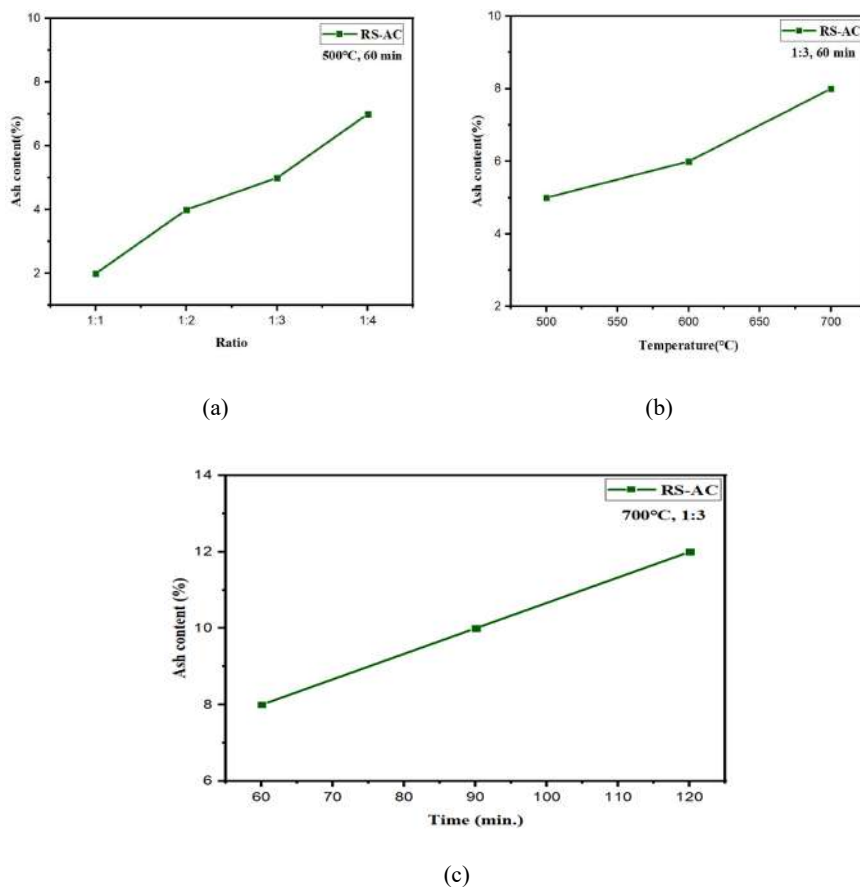


Fig. 3: The effect of the (a) impregnation ratio, (b) activation temperature (c) time on ash content.

in the literature on ACs obtained from agricultural waste (Njewa et al. 2022).

pH and conductivity: All of the synthesized ACs had somewhat acidic pH, ranging from 6 to 6.5. ACs having pH values ranging from 6 to 8 are found suitable for the majority of applications (Ashtaputrey & Ashtaputrey 2020). As a result, the investigated ACs may be suitable for the majority of aqueous solution-adsorption applications.

The ACs had EC values ranging from 0.09 to 0.29 dS m⁻¹. The EC of all the ACs was low, indicating a low concentration of ions that are soluble in water in the produced samples (Rostamian et al. 2018).

Moisture content: To evaluate the prepared AC's appropriateness for adsorption research, its moisture content was measured. The findings show that when the impregnation ratios were increased from 1:1 to 1:4 and the carbonization temperature rose from 500°C to 700°C, the moisture content of the generated AC samples dropped. Additionally, as the activation time increases, the moisture content drops. This result could be linked to the elimination of volatile

components, which reduces the carbon yield and moisture content of the produced AC samples (Njewa et al. 2022).

Analytical Characterization

Fourier transform infrared (FTIR): FTIR spectroscopy was used to investigate samples of raw and activated RS to look for functional groups, as indicated in Fig. 5. Using infrared spectra, the cellulose, hemicellulose, and lignin structures of RS were assessed. The region of 4000 – 400 cm⁻¹ was detected in an FTIR spectrum. The fingerprint region, indicative of the substance, was found to be below 1500 cm⁻¹ (Malik et al. 2022). The FTIR spectrum's broadband at 3000–3500 cm⁻¹ was typically associated with cellulose's hydroxyl group (O–H) stretching (Dhull et al. 2024) (Fig. 4a, b, c). Additionally, it was noted that the absorbance intensity dropped in the activated RS scenario. As the temperature rose from 500 to 700 °C during the pyrolysis process, the intensity of the peak at approximately 3427 cm⁻¹ rapidly reduced from the biomass of RS, indicating that the organic O–H group broke down at higher temperatures. The band around 2920, 2854 and 1384 cm⁻¹ were caused

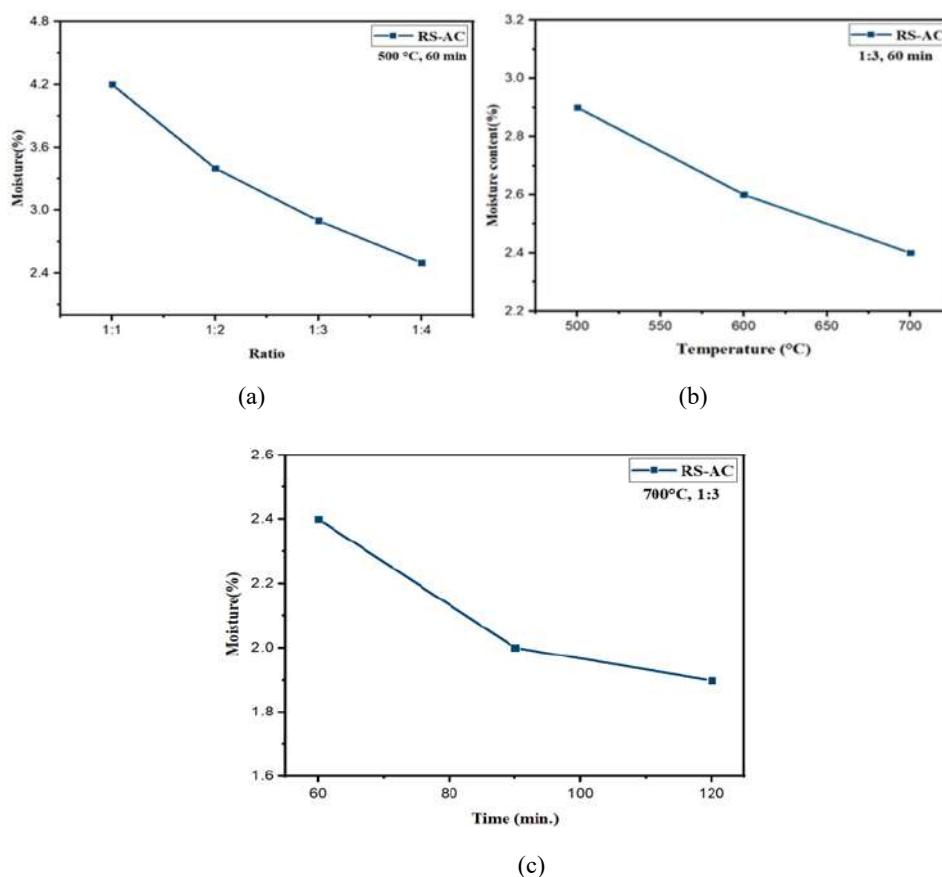


Fig. 4: The effect of (a) impregnation ratio, (b) activation temperature (c) time on moisture content.

by the axial deformation of C–H bonds, which are present in lignin, hemicellulose, and cellulose, the C=C group has been seen at 1633 to 1638 cm^{-1} , C–O stretching caused the region of hemicellulose and cellulose between 1200 and 1100 cm^{-1} to attain its maximum value at 1170 cm^{-1} , peak appears in activated RS samples but is absent in raw RS samples (Kumari & Singh 2022). It intensifies from 1:1 to 1:4. The absorption band at 1038 to 1055 cm^{-1} is C–O–C stretching of lignin, cellulose, and hemicellulose, bending vibrations of O–P–O are responsible for the tiny peaks at 672 to 676 cm^{-1} in activated RS. Bands at 460 – 520 cm^{-1} are allocated to Si–O–Si and Si–O–C cross-linking bonds. In raw RS samples, the peak is located at 471 cm^{-1} and is

less intense. In activated RS samples, the peak was found at 493 – 494 cm^{-1} and becomes more intense from 1:1 to 1:4 (Borges et al. 2023, ElGawad et al. 2023, Sakhiya et al. 2023, Singh et al. 2022, Phoung & Loc 2022).

X-ray diffraction (XRD): The X-ray diffraction method is a useful technique for comprehending the structure and crystallinity of activated RS and RS biomass. The raw biomass and activated RS results of the XRD spectra are shown in Fig. 6. The sharp, fine peak at 2θ value around 22° proved the presence of a crystalline structure in the biomass caused by the cellulose. The broad peaks appeared in between 24° and 30° express the amorphous nature of the adsorbents (Mullick et. al. 2018). The raw RS exhibits a single, less

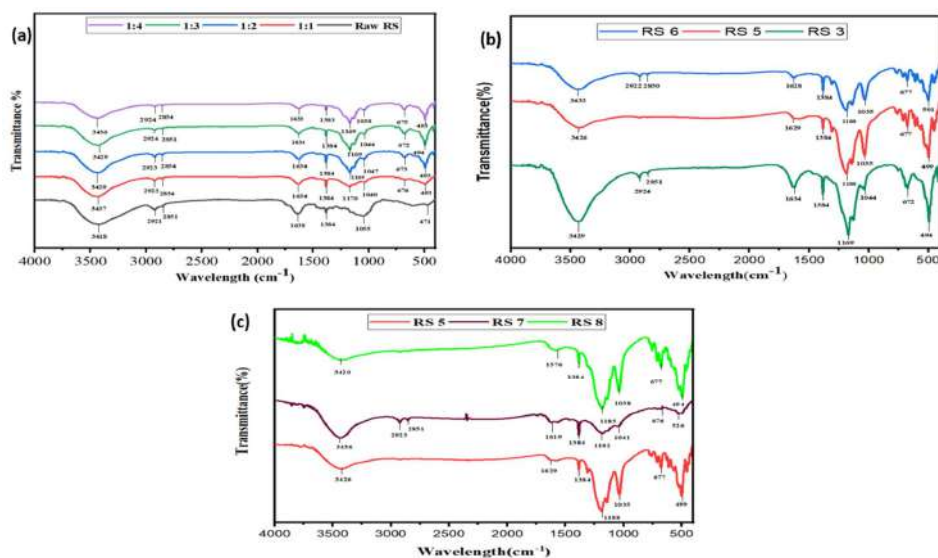


Fig. 5: FTIR analysis at different conditions (a) impregnation ratio, (b) activation temperature, (c) time.

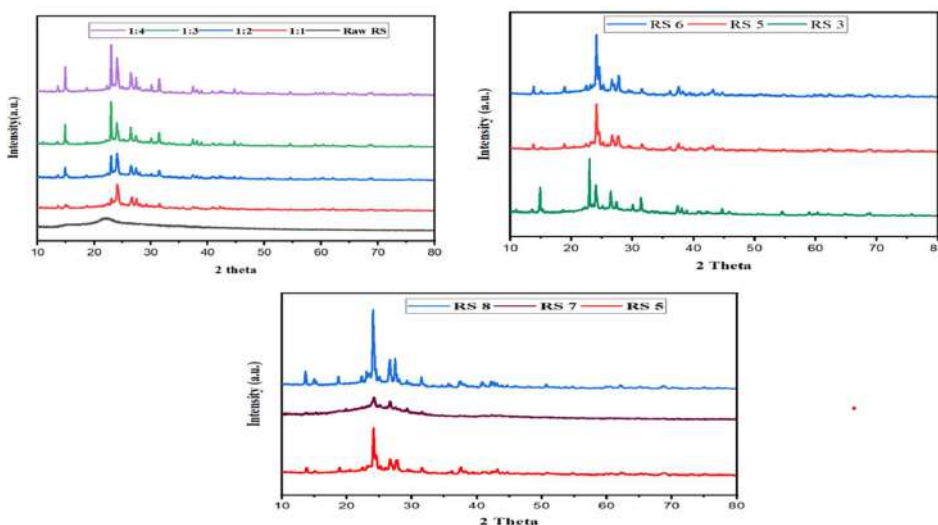


Fig. 6: XRD pattern at diverse conditions (a) impregnation ratio, (b) activation temperature, (c) time.

intense peak at 22° , while RS1, RS2, RS3, and RS4 show distinct peaks at 15° , 23° , 24° , 26° , and 27° . In contrast, RS5, RS6, RS7, and RS8 display peaks at 13° , 14° , 18° , 22° , 24° , 26° and 27° . Pyrolysis of the RS biomass resulted in the breaking of the crystallinity structure's distinct, narrow peaks, and the pyrolysis-produced biochars have a larger, less intense peak. The cellulose crystalline structure of the biomass persisted in the biochar despite partial degradation

at low temperatures. These peaks, however, were not stable and entirely disappeared as the pyrolysis temperature increased. So, it may be concluded that the biochar was found amorphous based on the XRD spectra (Biswas et al. 2024).

SEM-EDX: SEM was used to analyze the surface morphology of raw RS and activated RS at different magnifications, as illustrated in Fig. 7. The surface of the

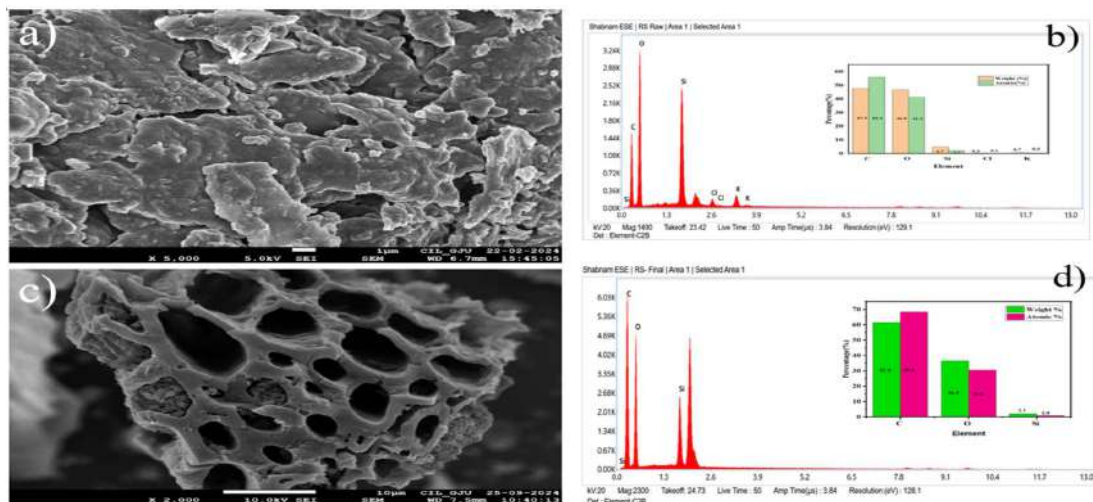


Fig. 7: SEM micrograph of different adsorbents (a) Raw RS, (b) EDX spectrum of Raw RS, (c) Activated carbon, (d) EDX spectrum of activated carbon.

Table 2: Comparison of the characteristics of the activated carbons with reported values in the literature.

Precursor	Activator	Activation Temp. [°C]	Time [min.]	Carbon Yield [%]	Ash content [%]	Moisture content [%]	XRD	FTIR	FESEM EDX	Reference
Coffee Grounds	H ₃ PO ₄	500	60	40 ± 3.4	35 ± 8	---	---	C-H, C-C, C=O, C=C,	mesoporous	Ferraz et al. 2019
Almond shell	H ₃ PO ₄	600	---	---	---	---	---	COO-, C=O, C-H,	Microporous surface	Rai et al. 2018
Rice husk	NaOH	650	---	---	---	---	Amorphous	---	Mesoporous surface	Mullick et al. 2018
Wood Apple Fruit Shell	ZnCl ₂	500	60	---	3.82	7.75	---	---	Honeycomb-like structure.	Ashtaputrey & Ashtaputrey, 2020
Rice Husk	H ₃ PO ₄	500	90	41	30.8	4:1 ± 0:18	Amorphous	C=O, OH, C-O, C=C	Microporous	Njewa et al. 2022
Potato Peels	H ₃ PO ₄	500	90	47	33.3	4:4 ± 0:24	Amorphous	C≡C, C=O, C-O, OH	Microporous	Njewa et al. 2022
Rice Straw	H ₃ PO ₄	700	90	37	10	2	Amorphous	C≡C, C-O-C, C-O, OH, Si-O-C	Cylindrical and porous structure	This study

raw material (RS) was rougher, less porous, and had some cracks (Sakhiya et al. 2023). The morphological structure of the activated RS differs significantly as a result of the activation process (Abd-Elhamid et al. 2020). Rice straw activation releases volatile materials, creating a compact and porous structure (Sakhiya et al. 2023).

Fig. 7 displays EDX (energy dispersive X-rays) spectra of Raw and Activated RS at 700°C, 1:3, and 90 min, revealing chemical contents. The composition of raw RS is primarily organic as evidenced by its high C (47.5%) and O (46.8%) content, with varying amounts of inorganic (K (0.7%) and Cl (0.3%)) material. Carbonization of rice straw in an inert atmosphere resulted in activated RS, which was validated by increasing the carbon content (61.4%) (Abd-Elhamid et al. 2020). A decrease in oxygen concentration signifies the elimination of carboxylic functional groups and the development of aromatic ring structures with activation (Qin et al. 2020). This is caused by the dissipation of hydrogen or oxygen functional groups (Park et al. 2019). Table 2 shows a comparison of characteristics of the activated carbons with reported values in the literature.

CONCLUSIONS

From a waste agricultural product, rice straw, an affordable, effective, and environment-friendly activated adsorbent was synthesized. The experimental results demonstrate that the present method is very effective in producing porous carbon particles. To determine the ideal conditions for the greatest surface area and micropores, an H_3PO_4 chemical activation was done at various activation temperatures and times. It has been noted that the physicochemical and structural properties of the biochar vary with variations in the impregnation ratio, pyrolysis temperature, and time. The oxygen and hydrogen content dropped, and a greater degree of carbonization took place at high temperatures. It is also feasible to control the porosity by adjusting the temperature, time, and amount of activation agent, orthophosphoric acid. Changes in porosity directly impact the product's capacity for adsorption. The optimal conditions of activated RS were 700°C temperature, a ratio of 1:3, and a time duration of 90 min. Overall, activated RS is a carbon-rich substance, including various applications that are the treatment of wastewater, the manufacturing of super-capacitors, the fertilization of soil, and catalytic support. The current research shows the potential of rice straw utilization in the synthesis of good-quality activated carbon for application, in addition to a decrease in rice straw burning, which leads to a decrease in air pollution and improved soil health and farmers' economic conditions.

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Oxidative Stress and Associated Neurotoxicological Impact in *Cirrhinus reba* from the River Mahananda, Malda: An Ecotoxicological Assessment

Mayukh Hore, Shubham Bhattacharyya, Samir Barai and Subhrajyoti Roy†

Immunopharmacology and Molecular Cell Biology Laboratory, Department of Zoology, University of Gour Banga, Malda-732103, West Bengal, India

†Corresponding author: Subhrajyoti Roy, subhrajyoti_roy@rediffmail.com

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ABSTRACT

The water quality of the River Mahananda has continuously deteriorated due to increased exposure of untreated wastewater from the urban areas, increasing the concentration of anthropogenic toxicants in aquatic environments that might enhance the cellular oxidative stress-induced physiological imbalance on the aquatic biota. In the present study, we have assessed the water quality of the River Mahananda and evaluated its detrimental effects on the oxidative stress parameters and neurotoxic biomarker of *Cirrhinus reba*. The principal component analysis revealed a significant impact of zinc, copper, fluoride, and ammonia on the pollution status of the River Mahananda. A significant decrease in the activity of superoxide dismutase, catalase, and glutathione reductase was observed in the liver, while significantly increased ($p < 0.001$) concentrations of TBARS in the liver, kidney, brain, and gill of *C. reba* were found at the polluted sites. An organ-specific significant decrease ($p < 0.001$) in the acetylcholinesterase activity was noted in the brain tissue of *C. reba* at the polluted sites (S2<S3<S4) compared to the control. The result of our study indicates the noxious impact of anthropogenic pollutants on the physiological metabolisms of *Cirrhinus reba*, an alternative model for ecotoxicological study.

INTRODUCTION

Freshwater pollution is a major global concern, contributed by rapid industrialization, agricultural activities, population growth, and urbanization (Qin et al. 2020). Anthropogenic pollution significantly contributes to the degradation of river water quality, particularly in urban regions where water quality monitoring is severely hindered by inadequate testing facilities and capabilities (Chen et al. 2022). Nowadays, the issue of anthropogenic pollution and its effects on the aquatic ecosystem has gained immense attention. Anthropogenic pollutants, viz., heavy metals, pesticides, microplastics, etc., affect aquatic animals by generating oxidative stress mediated by reactive oxygen species (ROS) like superoxide radicals ($O_2^{\cdot-}$) in their cells and hamper cellular metabolism (Melegari et al. 2013, Rahman et al. 2024). The gradual accumulation of these ROS collapses the antioxidant machinery, which in turn, generates many free radicals, viz. $NO_2^{\cdot}$, $OH^{\cdot}$, $ONOO^-$ and others (Tharmalingam et al. 2017). The over accumulation of these free radicals causes lipid peroxidation, protein denaturation, DNA damage, alteration in the tissue architectures, and neurotoxicity via modulation of the enzyme acetylcholinesterase (AChE) (Hore et al. 2023, Sharma et al. 2023). The alteration in AChE activity is responsible for several essential physiological functions in fishes, including locomotion, prey detection and consumption, social communication, and others. The modulation in AChE activity may be attributed to several anthropogenic activities, viz., agricultural runoff, gardening, and biomedical waste, etc. (Massei et al. 2023).

The River Mahananda, a transboundary Himalayan River flowing through the Malda district of West Bengal, India, is presently suffering from anthropogenic crisis,

and its water quality, along with its biota, is continuously deteriorating (Hore et al. 2023). As the population along the river bed continues to grow, untreated wastewater as well as agricultural runoff are increasingly discharged into the river (Mondal & Sinha 2020). Some regions of the river bed are utilized as dumping sites, where all the sewages from urban areas and farms cause the water flow to cease. These anthropogenic disturbances not only deteriorate the physico-chemical qualities of the river water but also have deleterious effects on its aquatic biota, and due to which numerous indigenous fish species have declined in this river, causing a major economical setback to the local fishermen.

To assess the effects of anthropogenic toxicants, fish have been considered as potential bio-indicators (Ali et al. 2020). *Cirrhinus reba* is one of the most popular Gangetic edible fish species in this region, with high nutritional values that plays a significant role in the local economy. In recent years, a notable decline has been observed in the *C. reba* population in the River Mahananda, which may be attributed to overfishing, habitat degradation, and other man-made ecological disturbances. Therefore, the present study was aimed to investigate the effects of the anthropogenic

pollutants on (a) the water physico-chemical properties of the River Mahananda, and (b) to evaluate its detrimental effects on the enzymatic- (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and non-enzymatic (reduced and oxidized glutathione) antioxidants, cellular lipid peroxidation, and acetylcholinesterase activity.

MATERIALS AND METHODS

Description of the Sampling Sites

The study area was divided into four sampling sites, S1, S2, S3, and S4 (Fig. 1). S1, the upstream sampling site ($25^{\circ}03'39.1''\text{N}$, $88^{\circ}07'50.5''\text{E}$ to $25^{\circ}03'00.2''\text{N}$, $88^{\circ}08'01.6''\text{E}$) exhibits low population density and minimal anthropogenic activities, followed by the S2 ($25^{\circ}01'01.8''\text{N}$, $88^{\circ}08'24.6''\text{E}$ to $25^{\circ}00'28.7''\text{N}$, $88^{\circ}09'12.9''\text{E}$) and S3 ($24^{\circ}59'43.2''\text{N}$, $88^{\circ}08'59.3''\text{E}$ to $24^{\circ}58'57.1''\text{N}$, $88^{\circ}09'05.4''\text{E}$) sampling sites, characterized by high population density and potential anthropogenic activities. The S4 sampling site of the river is the downstream region ($24^{\circ}58'21.7''\text{N}$, $88^{\circ}09'46.7''\text{E}$ to $24^{\circ}57'51.0''\text{N}$,

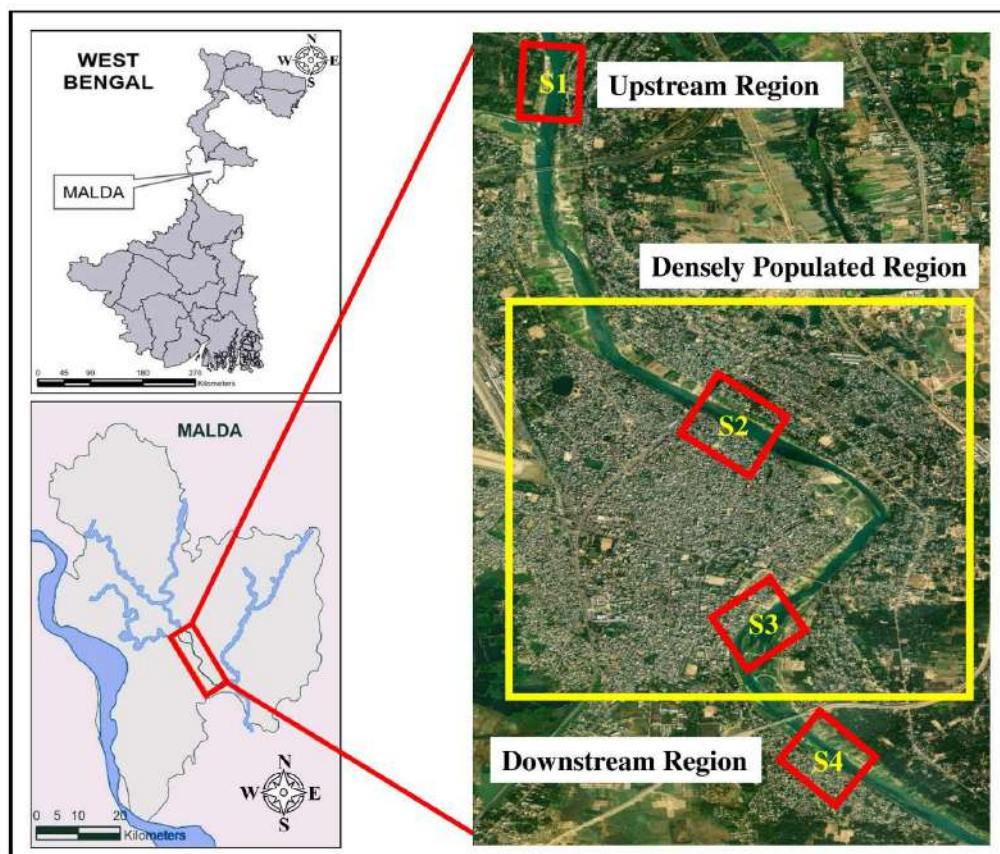


Fig. 1: Location map of the study area in the River Mahananda, Malda.

88°10'28.1"E), which exhibited lower anthropogenic activity compared to the S2 and S3 sampling sites.

Collection of Samples (Water and Fish)

Samples (water and *Cirrhinus reba*) were collected from different sampling sites twice a month in March, April, May, and June 2024, as per the standard method (Central Pollution Control Board 2007). *Cirrhinus reba* (six in number) (body weight: 80±15 g, each) were sampled from each site using fish nets and brought alive into the laboratory for further evaluation.

Experimental Methods

Estimation of physico-chemical parameters and evaluation of the water pollution load: To assess the physico-chemical quality of the River Mahananda, physical parameters, including temperature, total dissolved solids (TDS) and electrical conductivity (EC), and chemical parameters such as pH, dissolved carbon dioxide, dissolved oxygen, total alkalinity, total hardness, calcium hardness, ammonia, free chlorine, chloride, nitrate, nitrite and fluoride were measured. Fe (total), Zn²⁺, and Cu²⁺ were also estimated to determine the load of heavy metals in the water. Quantification of all the physico-chemical parameters was performed using “Hanna Instruments” and “Himedia Aqua CheckTM water analysis system”.

To evaluate the pollution load in the river, the Water Pollution Index (WPI) was computed as per the standard method developed by Hossain & Patra (2020), considering the combined effect of all estimated water physico-chemical parameters, including heavy metals, based on their upper permissible limits (Bureau of Indian Standards 2012). According to the authors, it is an alternative approach that disregards the issues of opacity, weighting, and aggregation related to the other conventional methods, and is easy to compute, flexible, and allows incorporation of several parameters as per requirement. WPI is considered a precise, comprehensive method for illustrating surface water quality in relation to pollution. The result of WPI scores was categorized into “excellent” (WPI <0.5), “good” (WPI 0.5–0.75), “moderately polluted” (WPI = 0.75–1), and “highly polluted” (WPI >1) to classify the water quality or pollution load.

Preparation of tissue homogenate and biochemical analysis of oxidative stress parameters: Liver, kidney, brain, and gill tissues were isolated from *C. reba* immediately after sampling to prepare tissue homogenate. Tissue homogenates were prepared using 0.1M Na/K phosphate buffer, pH 8, 0.1% Triton X-100, and 0.6% sulfosalicylic acid in potassium phosphate buffer and 1% Triton X-100 (1:9,

w/v) and centrifuged at 3500g at 4°C for 30 min. Following centrifugation, the supernatant was collected and stored at -20°C for further assessment.

Estimation of intracellular enzymatic and non-enzymatic antioxidants: The relative enzymatic activity of superoxide dismutase (SOD) has been measured using a standard protocol with slight modifications and defined as U.mg⁻¹ protein, where a single unit (U) signifies 50% inhibition of the formation of the tetrazolium salt (Kumari et al. 2014). Briefly, 0.025 mL of liver homogenate was mixed subsequently with 0.975 mL phosphate buffer (0.1 M, pH 7.5), followed by 24 µM riboflavin, 840 µM nitro blue tetrazolium (NBT), 1.2 mM Na₂-EDTA, and 150 mM methionine. Following an incubation period of half an hour, the absorbance change in the reaction mixture was measured at 560 nm. The CAT activity was determined according to the conventional method, which measures the decreasing concentration rate of H₂O₂, spectrophotometrically at 240 nm, and expressed by $k \cdot \text{mL}^{-1}$, where k is the rate constant of the first-order reaction (Aebi, 1984). GPx activity was measured based on the Box-Behnken design (BBD) (Ahmed et al. 2021). The enzyme activity was determined by spectrophotometrically measuring the rate of reduction of Cu (II)-neocuproine complex (Cu (Nc)₂²⁺) to strongly colored Cu(I)-neocuproine complex (Cu (Nc)₂⁺) at 450 nm. GR activity was measured according to a standard method, which involved spectrophotometric analysis of NADPH oxidation at 340 nm, coupled with the reduction of GSSG (Mannervik 1999). Briefly, 0.2 M EDTA-Phosphate buffer (pH 7.0) was mixed with 20 mM oxidized glutathione, followed by 2 mM NADPH solution and tissue homogenate in a ratio of 10:1:1:0.2, well mixed, and then measured at 340 nm. Intracellular GSH and GSSG concentration was determined spectrophotometrically, where the absorbance of yellow-colored 5'-thio-2-nitrobenzoic acid was measured at 412 nm (Rahman et al. 2006).

Estimation of cellular lipid peroxidation: Cellular lipid peroxidation level was determined spectrophotometrically by quantifying the level of thiobarbituric acid reactive substances (TBARS) at 535 nm and expressed as nmoles per mg protein (extinction coefficient, $1.56 \times 10^{-5} \text{ M}^{-1} \text{ cm}^{-1}$) (Dubovskiy et al. 2008). Briefly, the liver, kidney, gill, and brain tissue homogenates were mixed with 20% trichloroacetic acid (TCA) and 0.8% thiobarbituric acid (TBA), followed by an incubation period of 1 hour in a boiling water bath. After the incubation, the reaction mixtures were cooled and centrifuged at 15000g for 10 min, and the supernatant was separated to measure the concentration of TBARS.

Estimation of acetylcholinesterase activity: The activity of acetylcholinesterase (AChE) was determined according

to a standard method (Ellman et al. 1961). Briefly, the reaction mixtures of tissue homogenate (liver and brain) and phosphate buffer (pH 8, 0.1M) were mixed with 25 μ L DTNB (0.01M), and the absorbance was measured at 412nm till the OD was stabilized. After that, acetylthiocholine iodide was added to the reaction mixture. The rate of change of absorbance was measured at 412 nm in a plate reader (Bio-Rad, Hercules, USA).

Statistical Analysis

The Shapiro-Wilk test was performed to determine the normality of the dataset. Differences in mean $\pm$ SD ($n = 6$) between the groups [comparing S2, S3, S4 with S1 (as control)] were evaluated employing one-way ANOVA, followed by Dunnett's test. $p < 0.05$ was considered the level of significance. Standardized Principal Component Analysis (PCA) was performed to identify the predominant contributors to the water quality deterioration in the river. Pearson's correlation (r) test was also performed to assess the correlations between the contributors of water pollution and oxidative stress biomarkers. All statistical analyses were performed using XLSTAT 2016 and KyPlot 6.0.

RESULTS AND DISCUSSION

Determination of Water Pollution Level and Identification of the Major Contributors of Pollution

The general physico-chemical nature and quality of the river water are depicted in Fig. 2, which demonstrates that the sampling sites S2, S3, and S4 of the River Mahananda possess a varying degree of pollution load. In our present

study. It was noted that, among the physical parameters, TDS was significantly increased ($p < 0.01$) at the S2, S3, and S4 sampling sites of the River Mahananda, when compared to the control site S1. The highest value of dissolved oxygen was recorded at S1, while an average value of 6.61 ± 0.17 was recorded at the downstream sites, very close to the lower permissible limit (Bureau of Indian Standards 2012). Total hardness, calcium hardness, and total alkalinity were found to be significantly higher at S2 sampling sites, followed by $S3 > S4 > S1$ (control site), respectively. The highest concentration of ammonia was recorded at the S2 and S3 (0.35 ± 0.11 mg.L⁻¹) sampling sites, approximately three times higher than the S1 (0.13 ± 0.04 mg.L⁻¹) sampling site, along with the measured anionic concentrations sequenced in order of $Cl^- > NO_3^- > NO_2^-$ at the polluted sites compared to the control. Zn^{2+} and Cu^{2+} were both found to be significantly higher at the S2 site, with the highest value of 11.44 ± 1.10 mg.L⁻¹ and 0.95 ± 0.62 mg.L⁻¹, respectively, exceeding the value of the upper permissible limit (Bureau of Indian Standards 2012).

The sampling site S1 showed "Excellent" water quality as indicated by WPI (0.309), maybe due to low population density and fewer anthropogenic disturbances. In contrast, the WPIs of the S2 and S3 were found to be 1.3 and 1.5, respectively, representing "highly polluted" water quality, mainly attributed to the exposure and overaccumulation of anthropogenic toxicants in these regions of the River Mahananda. The S4 sampling site exhibited a WPI of 0.76. Although the population load and anthropogenic disturbances around the S4 sampling site were negligible, the pollutants from the upstream region (S2 and S3)

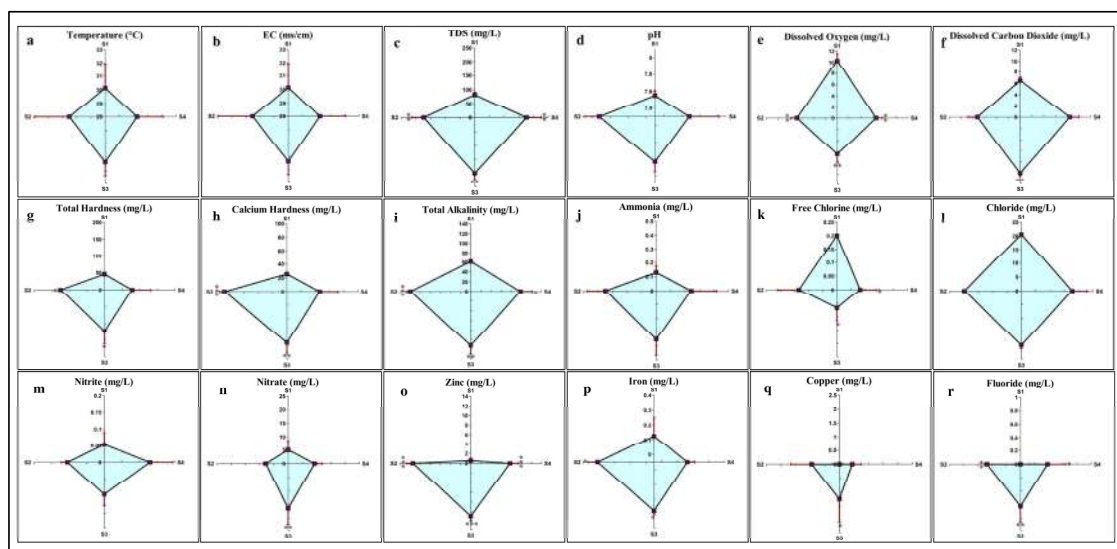


Fig. 2: Water physico-chemical parameters of different sampling sites in the River Mahananda, Malda.

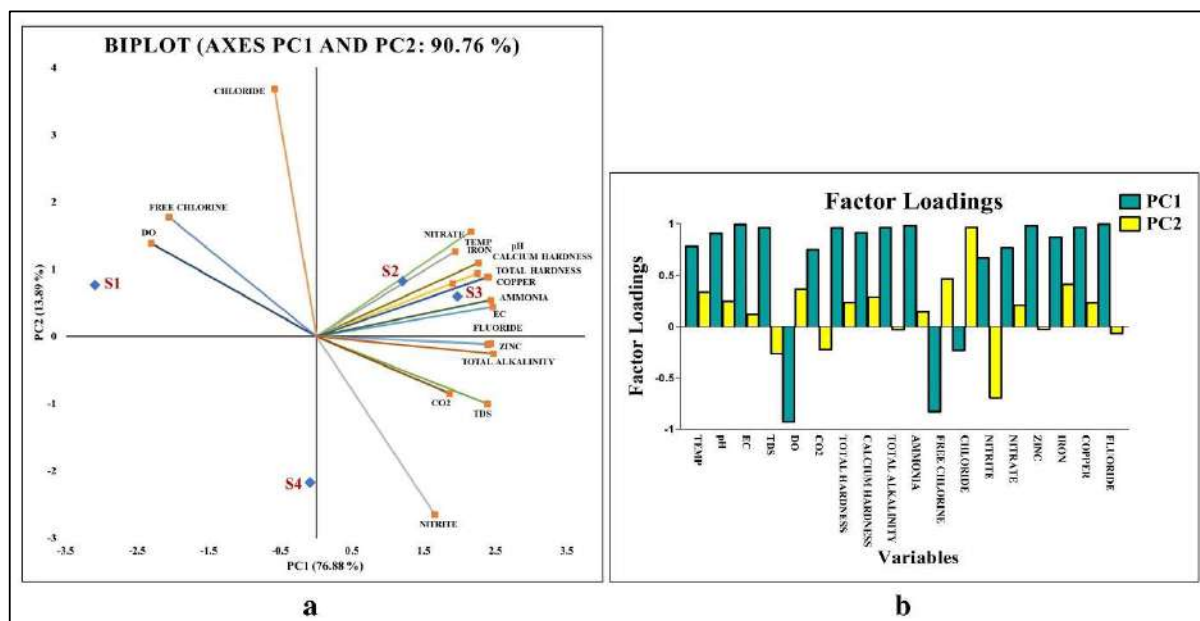


Fig. 3: (a) The PCA biplot and (b) factor loading of water physico-chemical parameters of the River Mahananda, Malda.

tend to accumulate in this region, making it “moderately polluted”.

To reduce the dimensionality of the dataset and define the principal contributors to water pollution, principal component analysis (PCA) was performed using the physico-chemical parameters of the water. In the principal component analysis of the physico-chemical properties of water, the principal components PC1, PC2, and PC3 can account for 100% of the variation in the data set, of these, PC1 and PC2 explain 76.87% and 13.88% of the total variance (Fig. 3). From the outcomes of PCA, it was confirmed that heavy metals like

zinc and copper, fluoride, and ammonia, were the principal contributors to polluted water quality, in association with pH, electric conductivity, TDS, total hardness, calcium hardness, total alkalinity and chloride were the principal descriptors that had a great impact on the water quality of the River Mahananda, determining the pollution levels.

Estimation of Oxidative Stress Biomarkers

Oxidative stress biomarkers, including intracellular antioxidant enzymes (SOD, CAT, GPx, and GR), non-enzymatic antioxidants (GSH and GSSG), lipid peroxidation,

Table 1: Oxidative stress biomarkers of *Cirrhinus reba* collected from the River Mahananda.

Biomarkers	S1	S2	S3	S4
SOD [U.mg ⁻¹ protein]	0.085±0.01	0.048±0.008***	0.045±0.01***	0.053±0.01***
CAT [k.ml ⁻¹]	8.017±1.19	1.678±0.55***	1.783±0.73***	2.378±0.32***
GPx [U.mg ⁻¹ protein]	0.352±0.09	0.275±0.09	0.33±0.09	0.328±0.10
GR [U.mg ⁻¹ protein]	0.187±0.03	0.118±0.05*	0.127±0.05	0.125±0.04
GSH [nM.mg ⁻¹ protein]	16.778±1.56	11.208±2.27***	9.623±2.04***	12.665±2.26**
GSSG (nM.mg ⁻¹ protein)	9.377±1.31	11.482±0.91*	12.285±1.96**	10.647±0.72
TBARS (L) [nmoles/L/min/mg protein ×10 ⁻⁶]	94.552±9.48	374.643±112.71***	414.173±107.57***	215.6±60.55
TBARS (K) [nmoles/L/min/mg protein ×10 ⁻⁶]	76.240±14.30	360.258±44.33***	374.502±80.15***	204.460±55.77**
TBARS (B) [nmoles/L/min/mg protein ×10 ⁻⁶]	67.038±4.22	377.9±74.08***	313.973±57.33***	233.347±68.27***
TBARS (G) [nmoles/L/min/mg protein ×10 ⁻⁶]	89.6±10.74	443.555±130.28***	374.928±37.70***	278.017±89.82**

¶ All values are mean ± SD of six observations.

**p* < 0.05 when compared with S1 (control) (significantly different).

***p* < 0.01 when compared with S1 (control) (significantly different).

****p* < 0.001 when compared with S1 (control) (significantly different).

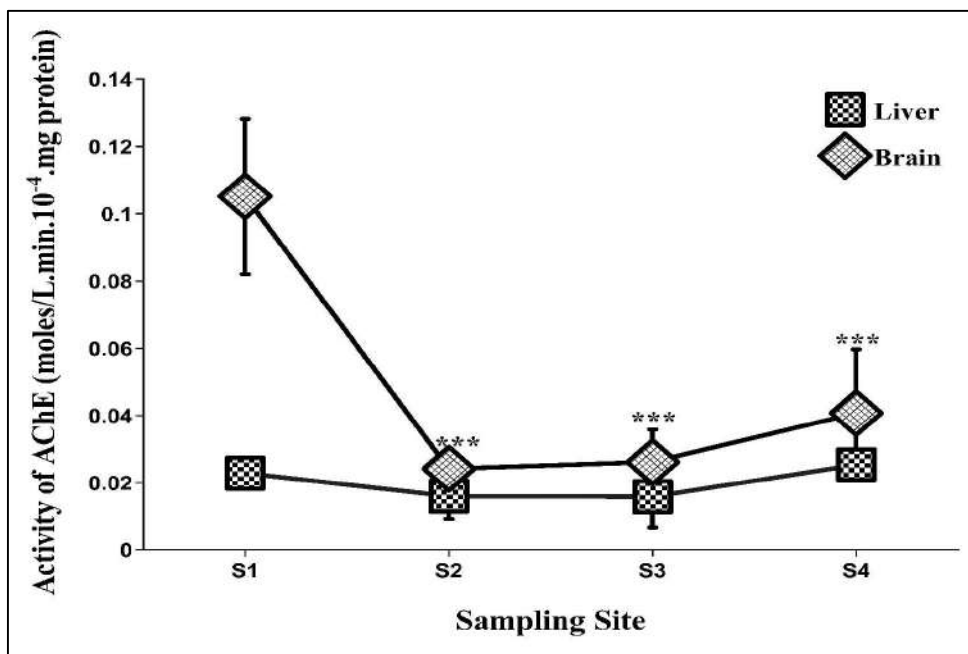


Fig. 4: The activity of acetylcholinesterase of *Cirrhinus reba* collected from the River Mahananda, Malda.

and acetylcholinesterase activity, are widely utilized biomarkers to assess the vulnerability and adverse effects of aquatic pollution on fish (Varó et al. 2002). The results of estimated oxidative stress biomarkers in *C. reba* are shown in Table 1, which demonstrates the significant alterations in antioxidant enzymes and other oxidative parameters attributed to anthropogenic stresses.

In our present study, it was observed that among the intracellular antioxidant enzymes, only the activity of SOD and CAT was significantly decreased ($p < 0.001$) in the liver tissue of *C. reba* collected from the polluted sites (S2 and S3), and S4 of the river Mahananda, compared to the control S1 site. A significant decrease ($p < 0.001$) in the AChE activity was noted only in the brain tissue of *C. reba* at the polluted sites (S2 < S3 < S4) compared to the control (Fig. 4). On the other hand, the concentration of reduced glutathione (GSH) was found to be significantly lower ($p < 0.001$ and $p < 0.01$), while higher ($p < 0.01$ and $p < 0.05$) concentration of GSSG was found in the liver of *C. reba* in the polluted sites, compared to control. Notable alterations in the amount of TBARS concentrations were observed in the case of all organs (liver, kidney, brain, and gill) of *C. reba*, which depicted increased levels of cellular lipid peroxidation in the organism.

DISCUSSION

The health of aquatic ecosystems is significantly influenced by water quality, which is determined by various physico-chemical and biochemical parameters. Consistent evaluation

of these water quality parameters is becoming a more crucial component in managing and protecting aquatic ecosystems (Boussaha et al. 2024). In our present study, we have observed a higher degree of pollution load of the River Mahananda in the regions that are in proximity to the densely populated area, attributed to the deteriorating water quality. The anthropogenic interferences in and around these areas influence the amount, duration of exposure, and chemical composition of the contaminants that may hamper the water resources, along with the sedimentation process. Hence, it is reasonable to assume that the deterioration in water quality is a major issue that is influenced by urbanization and its noxious intervention with the water resources (Umwali et al. 2021). Dey (2022) found that the deterioration of water quality is mainly influenced by the increased population burden around the riverbank of the River Mahananda, Malda. As per our findings, notable alterations in DO, EC, TDS, hardness of the water, alkalinity, ammonia, and presence of the heavy metals viz., zinc, copper, and iron may be responsible for deteriorating water quality. The concentration of dissolved oxygen at the polluted sites was found to be decreased by approximately 40% compared to the control, while the concentration of dissolved CO₂ was increased by 50% (approx.) at the S3 sampling site. Decreased levels of dissolved oxygen are most frequent in surface water due to the deposition of untreated organic Wastewater and biogenic and humus fecal materials (Aristarkhova et al. 2021). Decreased concentrations of DO in river water

can profoundly affect aquatic ecosystems, water quality, and environmental health (Rajesh & Rehana 2022). Total hardness, calcium hardness, and total alkalinity were found to be highest at S2 sampling sites in the order of S3>S4>S1, respectively. Calcium-based compounds can be found in a variety of solid wastes, including building and demolition waste, lime dewatering sludge, household garbage, and municipal solid waste incineration residue. The disposal of these untreated pollutant sources may increase water hardness and alkalinity (Li et al. 2021). Increased concentration of TDS by several folds in the river water is also impacted by over-urbanization, as the surface impermeability of urban areas allows several anthropogenic dissolved solids from buildings, construction sites, roads, and other urban infrastructures to flow into rivers (Adjovu et al. 2023). Similar kind of results were also recorded in previous studies conducting the water quality of River Mahananda, Siliguri, West Bengal and it was found that electric conductivity, TDS, dissolved oxygen, and chloride were highly influenced by the dense population and associated anthropogenic activities in this area, altering the water quality (Parween et al. 2022, Shil et al. 2019). It was also found that the river flow was ceased in some downstream regions due to the accumulation and dumping of urban waste. According to our previous study, the urban anthropogenic sources can alter the water physico-chemical parameters, affecting the water quality of the river (Hore et al. 2023). Similarly, the current pollution status of the Upper Awash River Basin, Ethiopia, is an example of anthropogenic stressors induced riverine pollution, attributed to the presence of excessive amounts of heavy metals coming from various industrial sources (Dessie et al. 2022). In this present study, we have also detected heavy metals, *i.e.*, zinc, iron, copper, and fluoride, exceeding the upper permissible limits, indicating metallic contamination in the river water. According to Shil and Singh (2019), this metalloid contamination in the River Mahananda may arise from household and municipal wastes, not industrialization, an indication of anthropogenic pollution. This increased concentration of Zn^{2+} , Cu^{2+} , Fe (total), and F^- at the downstream sites of the River Mahananda may be due to the improper disposal of urban solid wastes, *viz.*, rubber garbage, battery trash, discarded electronic gadgets, and other e-wastes (Ishchenko 2019).

Pollutants may act as an exogenous factor that elevates the physiological and cellular oxidative stress via dysregulating ROS generation and the cellular protection mechanism against it (Song et al. 2023). An imbalance between the production and neutralization of reactive oxygen species (ROS) leads to oxidative stress. Excessive oxidative stress can hamper cells and tissues via DNA hydroxylation, protein denaturation, lipid peroxidation, and cell death. In the present

study, it was noted that among the oxidative stress parameters, the highest fall in the enzymatic activity from the control was observed in the case of catalase (75.72%) followed by SOD (41.04%) in the liver, while, acetylcholinesterase (71.24%) in the brain of *C. reba* collected from the polluted sites. An excessive amount of $O_2^{\cdot -}$ may lower SOD activity, which could hasten the influx of $O_2^{\cdot -}$ and cause the catalase enzyme to become inactive, which may activate certain redox-sensitive pathways (Kono & Fridovich 1982). The level of cellular lipid peroxidation was found to be increased almost six times (compared to the control) in the brain tissue, followed by the gill, kidney, and liver of *C. reba* collected from polluted sites of the River Mahananda. In our previous study, anthropogenic stress-induced oxidative damage was noted in *Puntius sarana* collected from the River Mahananda, West Bengal (Hore et al. 2023). Other scientific evidence also indicated that excessive cellular ROS generation can cease the activity of antioxidant enzymes like SOD and CAT in fishes (Azevedo et al. 2021, Carvalho et al. 2012, Naz et al. 2023). It was reported that heavy metals decrease enzymatic antioxidant activity while inducing lipid peroxidation in several organs of the *Labio rohita*, collected from the River Yamuna (Mahamood et al. 2021). A somewhat similar organ-specificity of AChE has been seen in *Anabas testudineus*, exposed to naphthalene (Nayak & Patnaik 2021). Inhibition of acetylcholinesterase activity in the brain tissue of fish exerts severe necrotic changes, such as condensed chromatin and neuronal shrinkage, along with altered biochemical parameters (Jindal & Sharma 2019). The Pearson's correlation matrix revealed that zinc was positively correlated with TBARS ($p<0.05$), while negatively correlated with SOD ($p<0.05$, $r=-0.979$), CAT ($p<0.05$, $r=0.947$), GR ($p<0.05$, $r=0.957$), GSH ($p<0.05$, $r=0.965$) and AChE ($p<0.01$, $r=0.991$) (Fig. 5). A strong positive correlation ($p<0.01$) was found between concentration of copper and lipid peroxidation level. Similarly, a positive correlation was also recorded among copper, zinc, and cellular lipid peroxidation levels of *Leporinus obtusidens*, previously studied by Gioda et al. (2007). Heavy metals are non-biodegradable and can harm aquatic biota at low concentrations. Prolonged exposure to these hazardous substances can cause oxidative stress in fish and alter cellular metabolism (Rani et al. 2022). According to Abdel-Tawwab et al. (2015), exposure to heavy metals can deteriorate the health status of fish. It has been demonstrated that elevated amounts of copper may seriously harm cells by generating an excessive amount of reactive oxygen species (Pourahmad & O'Brien 2000). The bioaccumulation of copper and zinc in *Pavlova viridis* to varying concentrations resulted in increased cellular lipid peroxidation, indicating oxidative damage (Li et al. 2006). Exposure to $120 \mu g.L^{-1}$ Cu for 48 h significantly increased

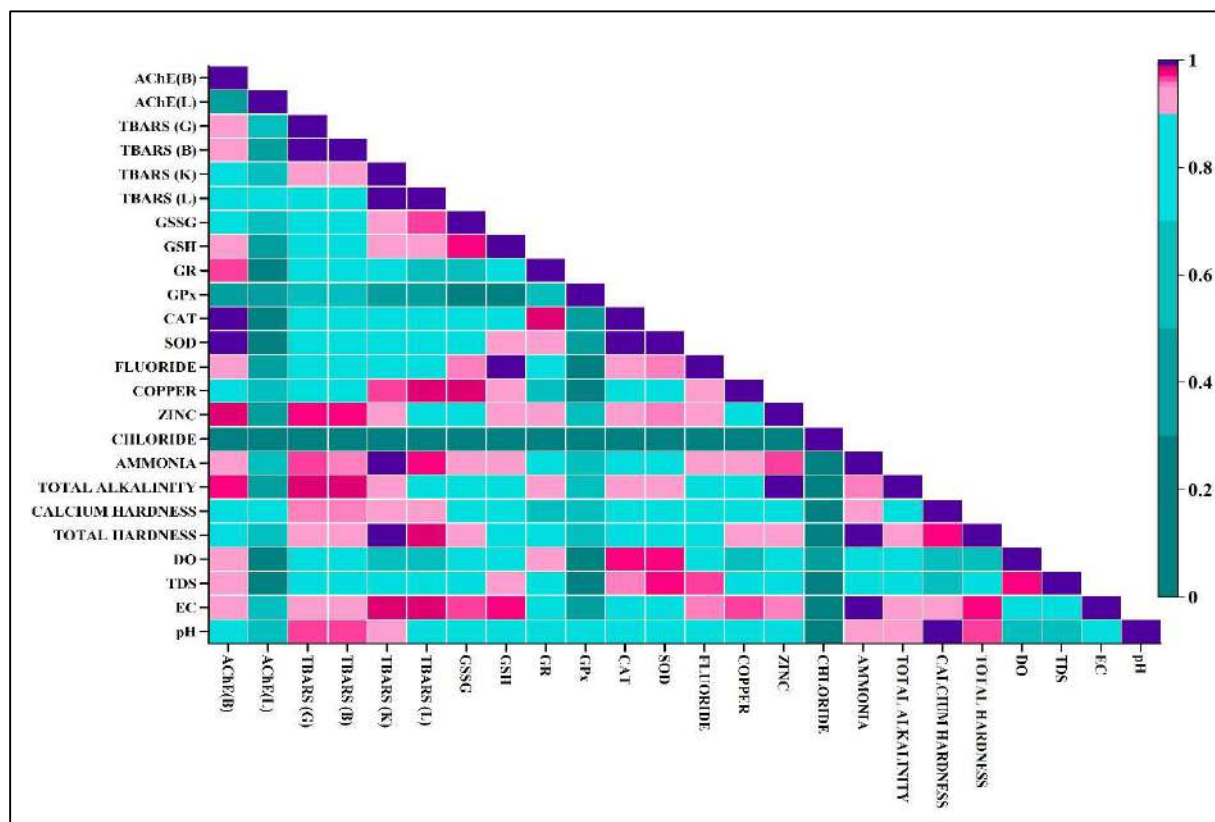


Fig. 5: Heat map of the Pearson's correlation matrix between water physicochemical parameters and oxidative stress biomarkers of *Cirrhinus reba*.

the lipid peroxidation in the liver, whereas it significantly decreased the catalase activity in the large yellow croaker (Pan et al. 2020). Waterborne Zinc at a dose of 8 mg.L⁻¹ for a week also considerably reduced the GSH levels in the liver and gill of *Paralichthys olivaceus* (Kim et al. 2019). Fluoride in urban waste comes from a variety of places, including industrial effluents, agricultural runoff, domestic garbage, and sloppy trash disposal (Bahukhandi et al. 2020). Because of its high electronegativity, fluoride may inhibit enzyme activity through the generation of ions (Barbier et al. 2010). In our study, it seemed that fluoride was negatively correlated with SOD ($p < 0.05$, $r = -0.978$), CAT ($p < 0.05$, $r = -0.953$), GSH ($p < 0.01$, $r = -0.998$) and AChE ($p < 0.05$, $r = -0.965$). Chronic exposure to sodium fluoride in zebrafish can significantly decrease the activity of acetylcholinesterase, superoxide dismutase, and catalase (Dondossola et al. 2022). On the other hand, increased lipid peroxidation was recorded in the liver of *Heteropneustis fossilis* exposed to fluoride at 35 and 70 mg.L⁻¹ (Yadav et al. 2015). Although there was no statistically significant change in SOD levels, fluoride at the dose of dosages of 10-50 mg.L⁻¹ was able to fortify TBARS associated with lipid peroxidation while decreasing CAT activity in blood. Moreover, due to

alterations in enzyme metabolism and disruption of redox equilibrium, high fluoride exposure is linked to harm to cellular functions in several tissue types (Miranda et al. 2018). In aquaculture environments, ammonia is the most prevalent stressor and pollutant, and it has the potential to result in significant fish mortality through the production of oxidative stress (Parvathy et al. 2023). The oxidative stress biomarkers, such as reduced glutathione and TBARS, were found to be significantly correlated ($p < 0.05$ & $p < 0.01$) with the ammonia concentration of the River Mahananda. Similar findings were observed in various studies, where ammonia exposure at different concentrations can increase cellular lipid peroxidation, whereas GSH, as well as some other antioxidants, were decreased in freshwater fishes (Elshopakey et al. 2023, Long et al. 2023, Zhang et al. 2023).

CONCLUSIONS

The degradation of surface water in developing nations is mostly attributed to pollution associated with urban populations, along with agricultural and industrial sources. The water quality of the River Mahananda is deteriorating due to anthropogenic contamination. This condition can be made worse by the high intensity of the exposure of anthropogenic

toxicants into the river bed without proper environmental management. As per our knowledge, this is the first study to evaluate the toxic effects of anthropogenic pollutants on the oxidative stress level and associated neurotoxicity in *Cirrhinus reba*. Our findings showed that fluoride, copper, zinc, and ammonia were the principal toxicants, which not only influenced the quality of river water but also induced cellular ROS generation that caused multiple physiological impacts in the fish. This may be due to the over-accumulation of heavy metals and other toxicants. Consequently, the activity of intracellular enzymes, including SOD, CAT, and GR, was significantly decreased at polluted sites of the river may also be due to the presence of heavy metals such as zinc and copper. The level of lipid peroxidation in the organs of *C. reba* collected from the polluted sites was several-fold higher than that of the control. The deteriorating water quality and the presence of heavy metals also modulate the acetylcholinesterase activity via inhibition of its active catalytic site. Additionally, in this investigation, we tried to identify potential pathways within a single chain that may explain the mechanism of toxicant-induced oxidative stress in fish.

Future research should focus on utilizing analytical techniques such as atomic absorption spectrophotometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) to accurately measure the concentration of heavy metals in the river water and confirm their bioaccumulation within the fish species. High-performance liquid chromatography (HPLC), Raman Spectroscopy, etc., should also be utilized to quantify the potential presence of other toxic substances, viz., microplastics, pesticides, organic pollutants, etc., that could negatively impact the health of aquatic animals, particularly fish. In conclusion, monitoring of water quality is an essential metric for achieving the “Sustainable Development Goals” and “Namami Gange Programme” pertaining to clean river water and sanitation. Our study aims to offer critical insights for policy formulation and wastewater management development by monitoring and analyzing water quality data, thereby mitigating anthropogenic activities to address this issue and protect natural resources, ensuring universal access to safe drinking water for the local people, and enhancing sanitation. In order to address the growing problem of urban run-off and its impact on water quality, our research suggests that local administration should implement new strategies for wastewater collection and treatment. One possible approach should be the use of adsorption and phytoremediation technologies to recycle wastewater and effectively remove heavy metals. Other strategies include aeration, biofilm, microbial preparation, and microorganism dosing technique. Moreover, our research will facilitate the cultivation and

propagation of this commercially significant fish species, as well as educate the local population regarding the hazards and impact of contaminants in the water.

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Potential of Microalgal Growth in Laundry Effluents for Phosphate Phytoremediation

Nuraini Aulia^{1†}, Fahrurddin² and Muhammad Farid Samawi³

¹Department of Environmental Health, Makassar, Indonesia

²Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia

³Department of Marine Science, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia

†Corresponding author: Nuraini Aulia; aulia.1614317@gmail.com

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ABSTRACT

The objective of this study is to develop a large-scale technology for phosphate wastewater management. Laundry activities are one of the largest producing phosphate contaminants by the use of detergent. Various contaminants, such as nutrients of phosphate, chemicals, and pathogens, can pollute the environment and endanger human health. The experiment was conducted by batch method by using water in a stationary or non-flowing state. The results showed that combining phytoremediation technology and monitoring the microalgae growth phase could reduce TSS, pH, BOD₅, COD, and phosphate values in wastewater. The treatment in this study was to combine two species of microalgae. Studies have shown that the optimal pH for microalgae is in the range of 7.5. Providing moderate amounts of aeration and CO₂ promoted algal growth. The decrease in phosphate levels was 27.86%, with the best phase observation at the fourth hour of exponential time. Water quality evaluation of BOD, COD, and TSS parameters had a decrease of 51.87%, 51.06%, and 52%, respectively. Thus, it can be concluded that the combining of two species of microalgae in the exponential growth phase has been proven to affect and improve the quality of wastewater from laundry waste and meet the quality standards.

INTRODUCTION

Laundry activities require the use of a lot of water and detergent, so it is linear with the waste that will be generated (Lade & Gbagba, 2018, Lu & Leung 2003, Pakula & Stamminger 2010). One of the largest waste compositions in detergents is the builder with the compound sodium tripolyphosphate (Bajpai & Tyagi 2007, Morse et al. 1995). Waste phosphate compounds entering water bodies will result in over-nutrient events that trigger increased aquatic activity and stimulate the logarithmic growth of certain plant species (Cunningham & Cunningham 2018, Fetahi 2019, Nadia et al. 2007, Nyenje et al. 2010). Many systems have been used for removing phosphate in wastewater, such as a coagulant neutralization mechanism (Owodunni et al. 2023, Patel et al. 2022), a combination of electrocoagulation and photocatalyst process (Wibowo et al. 2023), electrocoagulation method (Fayad et al. 2017), constructed wetlands and struvite precipitation (Reddy & Angelo, 1997). Environmental considerations also underlie the development of biological wastewater treatment, where the adverse environmental impact is much smaller than chemical treatment (Dutta & Bhattacharjee, 2022, Liu et al. 2020). However, compliance with regulatory standards for effluent management often requires considerable cost and sophisticated technology. Therefore, there is an urgent need for practical and cost-effective wastewater treatment methods (Aslam et al. 2017, Saraswati et al. 2021, Waqas et al. 2023). It requires effective and efficient wastewater treatment technologies to overcome this problem.

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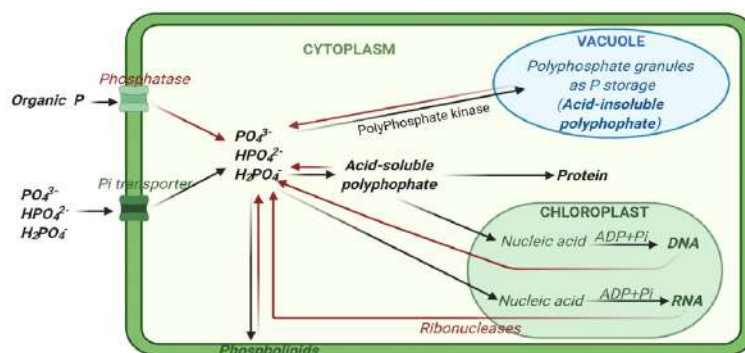


Fig. 1: Biochemical Pathway of P in cells of microalgae.

One other method that has been reported to reduce surfactant and phosphate levels is bioremediation (Scott & Jones 2000). Bioremediation is responsible for degrading, removing, transforming, suppressing, or detoxifying various physical and chemical wastes (Kulshreshtha et al. 2014). The chosen bioremediation technique is the utilization of microalgae as a bioremediator, better known as the phytoremediation technique. Microalgae is a phytoremediator of laundry liquid waste with a focus on phosphate compounds because it considers the ability of microalgae to grow by utilizing phosphate compounds as an energy source so that phosphate waste produced in the laundry industry can be minimized by adding microalgae before the waste is released into the waters (Chai et al. 2020, Touliabah et al. 2022).

The utilization of microalgae as a phytoremediator is based on several advantages that make it a potential choice for management on a small scale, including the few raw materials are required, processes can be more easily monitored, natural and renewable material sources, less secondary waste, low carbon footprint, wastewater reclamation, and nutrient recovery, allows using plants that accumulate contaminants to be utilized. It is easy for small industries to use applications at a low cost (Ahmad & Abdullah 2017). In line with this, some research has been conducted on the effectiveness of microalgae in decreasing organic compounds (Molinuevo-salces et al. 2019, Plöhn et al. 2021).

Fig. 1 shows the biochemical pathway of phosphate in cells. The ultimate goal of utilizing microalgae as bioremediator agents of phosphate compounds in laundry waste is to obtain the results of the suitability of the percentage of phosphate waste contained in wastewater with the provisions of phosphate wastewater quality standards, which later, when discharged into water bodies will not damage aquatic ecosystems and are safe for use.

MATERIALS AND METHODS

The research is an experimental study of a wastewater treatment system combining bioremediation techniques and microalgae growth phase monitoring. The parameters observed are physical parameters, including temperature and total suspension solid, and chemical parameters, including pH, BOD₅, COD, and phosphate levels. Effluent samples were collected from wastewater discharged by a laundry station in Makassar. Sampling of laundry liquid waste directly on the liquid waste disposal hose, which will then be collected in a plastic container and brought to the laboratory to be put into a closed container with a capacity of 10 L (420 mm × 270 mm × 150 mm), followed by the preparation of microalgae taken from the breeding center each put into a 1000 mL volume glass bottle that has been sterilized first.

Samples taken at the specified service are analyzed at the Marine and Fisheries Laboratory with proper handling following applicable standards. Then, the samples are tested using the test methods and standards shown in Table 1.

The phytoremediation process is given four types of treatment: the first treatment with the addition of microalgae *Chlorella vulgaris*, the second treatment with the addition of microalgae *Skeletonema costatum*, the third treatment by

Table 1: Sample analysis methods.

No	Parameters	Source	Methods
1	Phosphate	mg.L ⁻¹	Spectrophotometer UV-VIS
2	TSS (Total Suspended Solids)	Mg.L ⁻¹	Gravimetry
3	Temperature	°C	Thermometer
4	pH (Degree of Acidity)	-	Potentiometry
5	BOD (Biological Oxygen Demand)	mg.L ⁻¹	Titrimetric/ Potentiometry
6	COD (Chemical Oxygen Demand)	mg.L ⁻¹	Spectrophotometer UV-VIS

mixing the two species of microalgae *Chlorella vulgaris* and *Skeletonema costatum* and the last is the control experiment without the addition of microalgae. The batch method is used in the phytoremediation process by using water in a stationary or non-flowing state. The treatment was carried out by providing four containers with a capacity of 10 L, which had previously been filled with 5 L of laundry wastewater each, given aeration and temperature setting at 25°C. Marked with the numbering P1, P2, P3 and P4. Water sampling was carried out every 2 hours for 10 hours with variations of 2, 4, 6, 8, and 10.

By the process of phytoremediation, microalgae growth populations are monitored. Monitoring of microalgae was carried out every time variation by taking several 1mL of samples and observing by microscope with 400x magnification, cell counting by using a hand tally counter with Haemocytometer method.

Microalgae cell density monitoring was calculated using the formula:

$$N = \frac{\Sigma cell \times f \times p \times 10^4}{4}$$

RESULTS AND DISCUSSION

Wastewater Characteristics

The pre-treatment wastewater exhibited physical characteristics, including a physical checking test for odor and a brownish turbid appearance. Laboratory analysis showed that temperature and pH values were within acceptable limits based on regulatory standards, while parameters such as BOD, TSS, COD, and Phosphate exceeded quality standards. The initial characteristics of the wastewater effluent can be seen in Table 2.

Table 2: Characteristics of laundry wastewater.

No.	Parameters	Contents	Unit	Maximum rate*
1	Temperature	28	°C	<30
2	pH	8,17	-	6 – 9
3	BOD ₅	187	mg.L ⁻¹	75
4	COD	315	mg.L ⁻¹	160
5	TSS	150	mg.L ⁻¹	60
6	Phosphate	15,29	mg.L ⁻¹	10

*Wastewater quality standard; Source: primary data

Wastewater Parameters

1. pH

All tests have reached the quality standard value of laundry wastewater of 6-9, but there is a decrease in pH in the *Chlorella vulgaris* microalgae test, and the mixed test of *Chlorella vulgaris* and *Skeletonema costatum* significantly the pH of wastewater is 8.01 and 8.13, respectively, while the recommended pH based on regulation for hygienic sanitation is 6.5-8.5 (Fig. 2).

In the test containing microalgae, *Chlorella vulgaris* has a lower pH than the control pH due to the amount of dissolved carbon dioxide decreasing due to the photosynthesis process by microalgae cells. Microalgae *Chlorella vulgaris* is more capable of growing rapidly in wastewater than other species so to reduce the concentration of carbon dioxide in water rapidly (Molinuevo-salces et al. 2019).

BOD Concentration (Biochemical Oxygen Demand)

BOD concentrations in all treatments dropped significantly; the initial control concentration was 187, and there was a decrease of 24%, the largest decrease in BOD concentration in the test with a mixture of microalgae *Chlorella vulgaris*

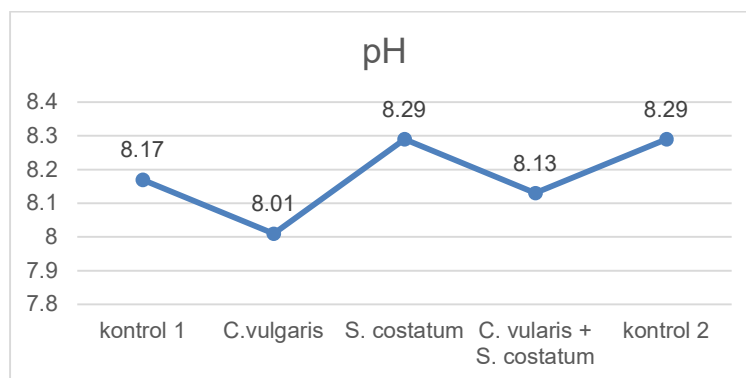


Fig. 2: Graphic of pH concentration.

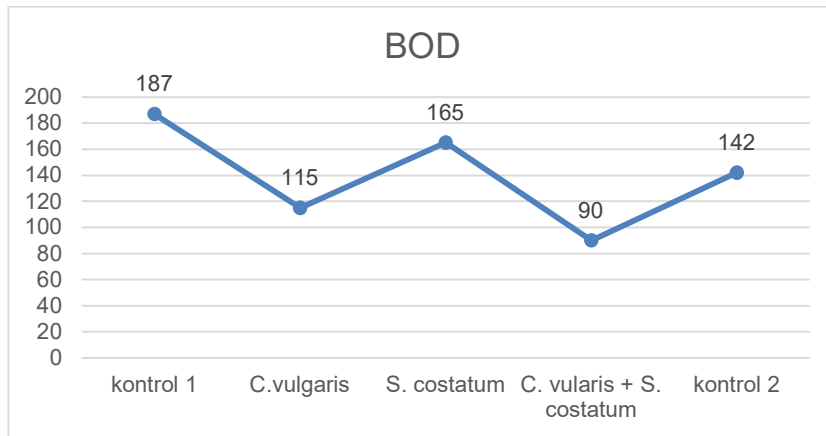


Fig. 3: Graphic of BOD.

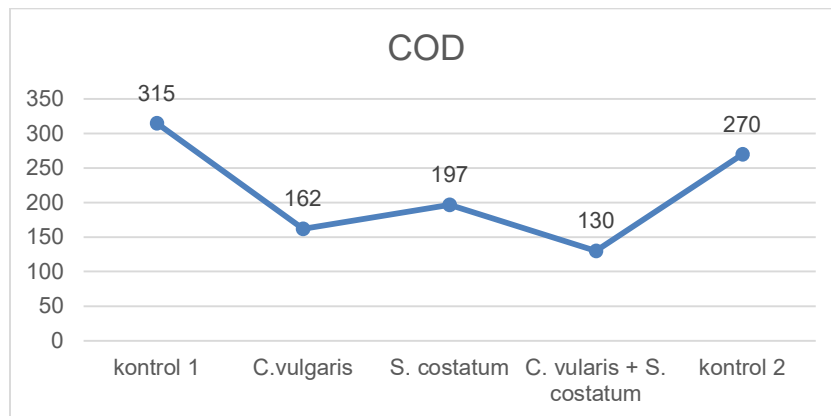


Fig. 4: Graphic of COD.

and *Skeletonema costatum* by 51.87% from the initial control without the addition of microalgae (Fig. 3). The decrease in BOD levels in the microalgae addition treatment was related to the growth in the amount of microalgae biomass, linearly with this, the total organic substances that must be degraded also increased. The decrease in BOD concentration was also shown in the control treatment without the addition of microalgae, and this is due to the presence of potential bacterial species that also can degrade liquid waste, namely *Staphylococcus aureus*, *Pseudomonas pseudomallei*, and *Actinobacillus* sp.

COD Concentration (Chemical Oxygen Demand)

The percentage reduction in COD levels in all treatments was 25.53% (control), 48.09% (*Chlorella vulgaris*), 46.91% (*Skeletonema costatum*), and 51.06% (*Chlorella vulgaris* and *Skeletonema costatum*), respectively (Fig. 4). According to Reddy & D'Angelo (1997) and Ghaly et al. (2005), several things cause a decrease in COD concentration in the treatment

with the addition of microalgae, among others: (i) microalgae can degrade nutrients and other organic materials (ii) oxygen present in wastewater is utilized by microorganisms thus increasing the removal of organic materials and (iii) the nutrient content contained in wastewater is consumed by microorganisms (Ghaly et al. 2005, Reddy & Angelo 1997). The content of organic compounds contained in wastewater acts as nutrients for microorganisms that are eventually degraded into simpler compounds.

TSS (Total Suspended Solids)

The addition of microalgae can significantly reduce the TSS value by 46.66% (*Chlorella vulgaris*), 43.33% (*Skeletonema costatum*), and 52% (*Chlorella vulgaris* and *Skeletonema costatum*), respectively (Fig. 5). High values of suspended solids, although not directly toxic, but excessive suspended material can increase the value of turbidity, which will further inhibit the penetration of light into the water and will ultimately affect the photosynthesis process. However,

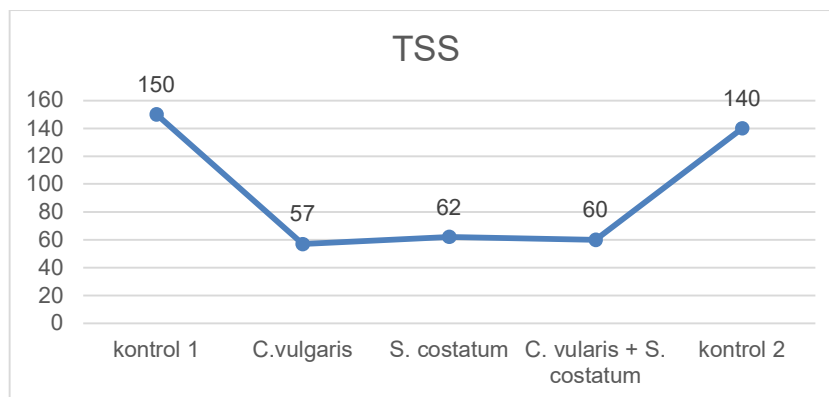


Fig. 5: Graphic of TSS.

the amount of suspended solids can also be generated by the amount of dead microalgal biomass that has blocked light penetration, so it must pay attention to the life phase of the microalgae (Terry & Raymond 1985).

Phytoremediation Treatment

Observations on each treatment were carried out with time variations at the 2nd, 4th, 6th, 8th, and 10th hours. Observations were made by looking at physical changes and the amount of microalgae density during time variations. Time variations were determined in accordance with the theory of microalgae growth phases, namely the adaptation phase, logarithmic phase, growth rate decline phase, stationary phase, and death phase (Borowitzka 1997).

In Table 3, the density of the two microalgae seedlings was first calculated before being introduced into the test wastewater for the phytoremediation process. The determination of microalgae seedling density is calculated as hour 0. At the 2nd hour, calculated as the microalgae adaptation phase, microalgae P1 experienced an increase in cell density by 8% from the initial cell density, while microalgae P2 experienced a decrease in the number of cells in the adaptation stage by 13.60% and microalgae P3 also experienced an increase in cell density by 13.58%. The density of microalgae P1, *Chlorella vulgaris* in the 2nd-hour adaptation stage immediately increased due to the

characteristics of this species; microalgae *Chlorella vulgaris* tends to be able to live in extreme conditions (Sabeti et al. 2018), morphologically has mitochondria formed from double membranes, proteins, and phospholipids that control all growth processes and maintenance processes (Coronado-Reyes et al. 2022). While in P2 microalgae, *Skeletonema costatum* has a physical character in the form of strands of chains or filaments.

At the 4th hour, the growth of microalgae P1 increased exponentially to almost double by 46.40%, while in microalgae P2, there was an increase with a percentage of 22.12% from previous growth, microalgae P3 also experienced an increase in growth of 36.36%, but the increase in the amount of growth was still lower than the initial control value. A significant decrease in microalgae cell density concentration began to occur at the 6th hour in all variations of microalgae species; maximum biomass productivity has been achieved in this phase. Microalgae P1 decreased by 41.76%, microalgae P2 by 49.78%, and microalgae P3 also decreased by 29.73%.

In this phase, all types of treatments of microalgae species experience competition between cells for total nutrients in wastewater so that with a maximum density at the 4th hour, there is an extreme decrease in the number of cells due to reduced nutrient availability, decreased phosphate levels in wastewater also affect carbon fixation by microalgae (Lee et al. 2014). Until entering the death phase, the number of cell densities continues to decline (Table 4). One other factor

Table 3: Cell density in time.

Time	Cell density ($\times 10^4$ sel.mL ⁻¹)		
	P1	P2	P3
2	14,15	9,15	13,475
4	26,40	11,75	21,175
6	15,375	5,925	14,880
8	7,55	2,975	8,37
10	4,45	2,075	4,425

Table 4: The percentage of cells decrease.

Microalga	Cell density percentage of death
P1	65.50 %
P2	80.03 %
P1 + P2	62 %

P1: *Chlorella vulgaris*; P2: *Skeletonema costatum*

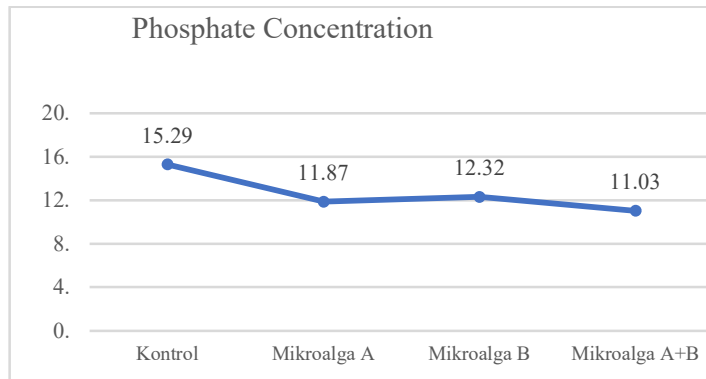


Fig. 6: Phosphate concentration.

causing low light penetration is covered by the amount of cell biomass that has died (Prayitno 2016).

Effect of Microalgae in Phosphate Phytoremediation

Phosphate is used by microalgae for growth and metabolic processes in cells, so phosphate concentration is a limiting factor for growth, lipid production, fatty acid yield, energy transfer, signal transduction, and photosynthesis (Chu et al. 2013, Solovchenko et al. 2022). Microalgae can absorb phosphorus in the form of polyphosphate or orthophosphate to enhance growth and nutrient content in the cell (Procházková & Brányiková 2013, Radin et al. 2017).

After the phytoremediation process with the addition of microalgae *Chlorella vulgaris* and *Skeletonema costatum*, there was a decrease in all variations of phytoremediation treatments. A significant decrease in phosphate concentration occurred in wastewater with the addition of *Chlorella vulgaris* microalgae and in the variation of mixing two types of microalgae species, respectively 11.87 mg.L⁻¹ and 11.03 mg.L⁻¹ (Fig. 6). The percentage reduction in phosphate levels in phytoremediation by *Chlorella vulgaris* was 22.36% and 27.86% in the mixing of two types of microalgae. It was also seen that there was a decrease in phosphate concentration in phytoremediation by *Skeletonema costatum* microalgae by 12.32 or 19.42%. The loss of phosphate during the phytoremediation process is caused by the use of phosphorus for growth (Rao et al. 2011). The decrease in phosphate levels in the environment is utilized by microalgae during the metabolic process so that in microalgae cells, phosphate is stored as accumulated phosphorus and will be reused by microalgae when in a critical state for survival (Acid & An 2021).

All types of treatments reached the optimum value at time-4, with each being *Chlorella vulgaris* at 26.40%, *Skeletonema costatum* at 11.75%, and a mixture of both types of microalgae at 21.175%. *Skeletonema costatum*

microalgae tend to be more difficult to develop well due to one of them because the morphological structure of this microalgae species is spiral-shaped with inter-cell connecting chains. The existence of this chain will be damaged and cause cell death due to several factors. Some of these are the presence of heavy metal nutrients, bacteria, and parasites (Kshirsagar 2013).

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Biodegradation of Polyethylene Using *Lysinibacillus macroides*: Isolation, Characterization and Evaluation

Shaguftanaz S. Shaikh and G. R. Pathade

Krishna Institute of Science and Technology, Krishna Vishwa Vidyapeeth, Deemed-to-be University, Karad (Formerly Krishna Institute of Medical Sciences), Maharashtra, India

†Corresponding author: Shaguftanaz S. Shaikh; shaikhshaguftanaz9@gmail.com

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ABSTRACT

Urbanization and economic development have led to an increase in the production of plastics. The increased production of plastics has resulted in the accumulation of plastics in the environment, leading to plastic pollution. The plastics are exposed to various weathering processes and undergo decomposition, which leads to the formation of microplastics. Polyethylene is one of the microplastics which contributes to the maximum share of pollution and is very hazardous. The safe degradation of polyethylene can be done by microbial degradation. This study examined the extent of plastic degradation through the use of microbes. The species of bacterium were isolated from Plastic dumping grounds in Karad. The isolated and screened microbes were assessed further in terms of their degradation potential. The evaluation of polyethylene degradation potential was conducted using the weight loss method, FTIR analysis, and scanning electron microscopy. One bacterial isolate showed positive results, and the screening results showed growth, which measured 7mm around the inoculated well. The screened-out isolate degraded 40% of the polyethylene, which was evaluated by weight loss method. Scanning electron microscopy showed the pits and holes which were formed by degradation. The promising isolate was later identified by 16S rRNA gene sequencing as *Lysinibacillus macroides*.

INTRODUCTION

The lengthy carbon chains that make up the backbone of plastic molecules are organic polymers, commonly called plastics, which are developed as a result of polymerization (Koushal et al. 2014). In addition to carbon and hydrogen, nitrogen, sulfur, and other diverse organic and inorganic components produced from fossil fuels make plastics (Kumari et al. 2013). The production of plastics has increased to another limit due to increased demand as a result of urbanization. Plastics are causing detrimental effects by accumulating in the environment and causing pollution. Plastics on the land are exposed to UV light and weathering processes such as photodegradation, thermal oxidation, hydrolysis, biodegradation, and fragmentation, which leads to the formation of fragments and pieces (Bakht et al. 2020). This leads to the formation of microplastics. The microplastics affect various forms of life by their deleterious impact. Microplastics affect the atmosphere as well as various living creatures such as animals, aquatic ecosystems, and humans. There are numerous varieties of microplastics, such as polyethylene, polypropylene, polyethylene terephthalate, and many more. Polyethylene contributes to the maximum share of pollution. The inert synthetic polymer known as polyethylene is made up of a lengthy chain of ethylene monomers.

One of the microplastics, the one present in the highest percentage, which is polyethylene, accumulates in the environment and causes hazardous effects to multiple life forms. There are various methods used for plastic management, like incineration and landfilling (Bakht et al. 2020). However, these techniques have

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several drawbacks which affect the environment as they are not eco-friendly procedures. Thus, we need to adopt some method that is safe, eco-friendly, and useful. Biodegradation is a safe and useful method in which microorganisms are employed for the degradation of plastics. Polyethylene is a hydrophobic polymer with a high molecular weight, making it resistant even to microbial degradation.

Additionally, the microbial enzymatic system is unable to recognize functional groups on polyethylene. These characteristics of polyethylene make it tough to degrade. Hence, polyethylene needs to be targeted for the degradation to manage the pollution. However, various microorganisms are isolated, which have the potential to degrade polyethylene up to a certain extent. There are certain reports that *Pseudomonas* sp., *Bacillus* sp., *Mycobacterium* sp. and *Nocardia* sp. have the potential to degrade PE. It was reported that *Lysinibacillus* sp. is capable of degrading polyethylene and polypropylene by 4% within 26 days of the evaluation period (Jeon et al. 2021). *Lysinibacilli* forms a biofilm on the polyethylene and targets it for degradation (Oliveira et al. 2021). Among the known microplastic-degrading microbes, *Lysinibacilli* shows promising results in the degradation of polyethylene.

In the current investigation, an attempt is made for microorganisms that break down polyethylene, and evaluation of the degradation potential of the isolate and isolate *Lysinibacillus macroides* is promising.

Many reports are proving the potential of *Lysinibacillus* to degrade microplastics. There are reports of degradation of polyethylene and polypropylene degradation by *Lysinibacillus* sp., which was investigated by GC-MS, SEM, XRD, and FTIR. According to a research study by Jeon et al. (2021), *Lysinibacillus* sp. reduced the weight of polyethylene by 9% over 26 days. Similarly, in a research study by Mukherjee et al. (2016), it was found that *Lysinibacillus fusiformis* degraded $2.97 \pm 0.05\%$ within 1 month of incubation. It was also reported that mixed cultures of *Lysinibacillus* and *Aspergillus* sp. have the potential to degrade 29.5% and 15.8% for the UV-irradiated and non-UV-irradiated films, respectively. It is clear from this study that the performance of *Lysinibacillus macroides* to degrade polyethylene is simply astounding, which degraded 40% of polyethylene within 40 days of incubation. *Lysinibacillus* leads to enzymatic degradation by synthesizing enzymes, which results in plastic degradation. There are reports of various enzymes hydrolyzing plastic, which eventually leads to plastic degradation. Enzymes such as cutinase, lipase, esterase, protease, laccase, and peroxidase are involved in the degradation of plastics (Kaushal et al. 2021)

MATERIALS AND METHODS

Collection of Samples (Divyalakshmi & Subhashini 2016)

Microorganisms are known as the most adaptable living beings to environmental changes. Soil, water, and wastes are teeming with diverse groups of microorganisms. When they get exposed to recalcitrant material like plastics, they develop an enzyme system to degrade it. Hence, various samples were collected from diverse sources/sites (contaminated with polyethylene-based plastics and hence possible sources of polyethylene degrading microorganisms) like marine water, mangrove sediment, soil from plastic dumping grounds, soil from coastal regions, and waste treatment plant effluents. Additionally, small pieces of polyethylene were buried in the soil 5-10 cm deep and were allowed to be there for three months, and periodically, soil from that site was sampled after 20, 40, 60, 80, and 90 days surrounding the polyethylene pieces. The collection of samples from such sites was carried out as there is a high chance of the presence of microbes that degrade multiple forms of microplastics, which is a natural process. Later, these microbes are tested for their potential to degrade polyethylene.

Enrichment, Isolation, and Screening of Polyethylene-Degrading Microorganisms (Rani et al. 2021, Patil & Bagde 2015)

Enrichment of polyethylene-degrading microorganisms:

The samples acquired from various sources, such as marine water, mangrove sediment, soil from plastic dumping grounds, and coastal regions, were inoculated in $1\text{g}.100\text{mL}^{-1}$ amount and subjected to enrichment of microplastic degrading microorganisms using Mineral Salt liquid culture medium with polyethylene as the sole carbon source. For increasing the microbes and enriching the microbes that break down plastics from different soil and water samples, the samples were infused separately into different flasks containing liquid culture nutrient medium supplemented with polyethylene at 0.1% W/V concentration and were kept incubating at 30°C for 30 days on incubator shaker with shaking speed of 147 rpm. The samples from enrichment flasks were then kept ready for the isolation of organisms using solid media.

Isolation of microorganisms from enriched samples (Divyalakshmi & Subhashini 2016): The enriched samples were diluted in distilled water (10^{-1} - 10^{-3}). 0.1mL of each dilution from the enrichment culture flask was spread on a solidified medium with mineral salts supplemented with polyethylene and was kept incubating at 30°C for a duration of 30 days. In the control plate, the standard culture degrading polyethylene (*Bacillus megaterium*) was used.

Subculturing of the representative colonies was done on a solidified medium with mineral salts and polyethylene and preserved at 4°C in triplicates. The growth of MSM agar with polyethylene was compared with the control.

Screening of polyethylene degrading microorganisms (Divyalakshmi & Subhashini 2016): The screening of microorganisms that break down polyethylene was performed by following the method. The isolated bacteria were assessed to ascertain their skills to breakdown polyethylene using a medium infused with mineral salts, and the composition of the mineral salt medium is listed as follows: (per liter of D/W) 0.1g each of Dipotassium phosphate, sodium chloride, and ammonium sulfate; potassium dihydrogen phosphate (0.2 g), Calcium chloride dihydrate (0.002 g), Boric acid (0.005 g), magnesium sulfate heptahydrate (0.5 g), copper sulfate (0.001g), zinc sulfate heptahydrate (0.001 g), manganese sulfate (0.001 g) and ferrous sulfate heptahydrate (0.01g). Polyethylene was infused in the mineral salt medium at 0.1% W/V concentration, followed by autoclaving at 121°C for 20 min. The prepared media was then transferred into plates, and after solidification, wells were cut, and a 20 µL culture of separate organisms was infused in the well. Plates were then kept for incubation at 30°C for 4 weeks, and growth around the wells was observed. The experiments were run in triplicates, and the average of triplicates was recorded as a result. In the control, sterile distilled water was used in place of the culture of isolates in the wells as the negative control, and the culture of the standard organism (*Bacillus megaterium*) as the positive control.

Evaluation of Bacterial Degradation of Polyethylene

The following methods will be used for the evaluation of the degradation of plastics:

- Weight Reduction Method. (Divyalakshmi & Subhashini 2016)
- FTIR Analysis (Divyalakshmi & Subhashini 2016)
- Scanning Electron Microscopy (Auta et al. 2017)

Weight Loss Method (Divyalakshmi & Subhashini 2016)

The polyethylene bags were trimmed to size, weighed initially, and then cleaned with distilled water that was sterile. After that, they were allowed to soak in unrefined black phenol for thirty minutes, and then they were allowed to dry for 15 min under laminar airflow. 50 milliliters of sterile mineral salt medium were infused with weighed polyethylene strips. 0.1 mL (at 10⁹ CFU.mL⁻¹) of each screened isolate was infused into separate flasks containing polyethylene film and mineral salt media. One flask was kept as a negative control without any inoculated microbe,

and the other with standard culture as a positive control (polyethylene degrader). For 4 weeks, flasks were incubated at 30°C in an incubator shaker. Following incubation, the polyethylene strips were rinsed with sterile distilled water, sprayed with alcohol, allowed to air dry properly under Laminar airflow, and then weighed to determine their final weight. The following calculation was used to determine the percentage of polyethylene degradation:

$$\text{Percentage(\%) Degradation} =$$

$$\frac{\text{Initial weight of milk cover} - \text{Final weight of milk cover}}{\text{Initial weight of milk cover}} \times 100$$

FTIR Analysis

In FTIR, samples were exposed to infrared radiation. There was some IR transmission and some IR absorption. The obtained spectrum showed the molecular absorption and transmission of the sample. FTIR analysis was performed on samples with a high percentage (%) deterioration, and the findings were noted. When percentage transmission was increased and percentage absorption was decreased in the case of the treated polyethylene sample after exposure to the test organism, it was taken as a distortion of the molecular structure of the polyethylene sheet, and it was taken as degradation and was compared to control sheets.

Scanning Electron Microscopy

A scanning electron microscope was used to examine the structural changes in the plastic surface before and after degradation at various time intervals. Following a brief washing with 2% (V/V) SDS and distilled water and a flush with 70% ethanol to eliminate the inoculated microbial cells, the treated samples were examined using a scanning electron microscope for distortion of structures. When polyethylene sheets, upon exposure to test organisms, are distorted as compared to the control sheet, it was taken as a sign of polyethylene degradation.

Characterization and Identification of Promising Isolates (Divyalakshmi & Subhashini 2016)

The identification of selected potential organisms at the molecular level was done by 16 S rRNA gene sequencing. The obtained genome sequence was then employed for the generation of a phylogenetic tree by BLAST analysis, and similarity was examined to identify the organisms at the species level. The genome sequence was then deposited to NCBI, and an accession number was obtained. This characterization study was carried out at Esonowa Innovations Private Ltd., Nagpur. The methodology

at a glance is depicted in the flow chart form (Fig.1) below:

RESULTS AND DISCUSSION

Collection of Samples

Different soil samples from plastic dumping grounds were collected from the Malkapur plastic dumping ground and Karad plastic dumping ground. The samples were collected in zip-lock bags and immediately processed in the laboratory (Table 1).

A slightly alkaline pH (7.0-8.0) and temperature in the mesophilic range (30-32°C) will allow the flourishing of mesophilic microorganisms, which will be slightly alkalophilic to grow at neutrality.

Enrichment, Isolation, and Screening of Polyethylene Degrading Microorganisms

Enrichment of polyethylene-degrading microorganisms: Enrichment of the polyethylene-degrading microorganisms was obtained in a Mineral Salt medium with polyethylene,

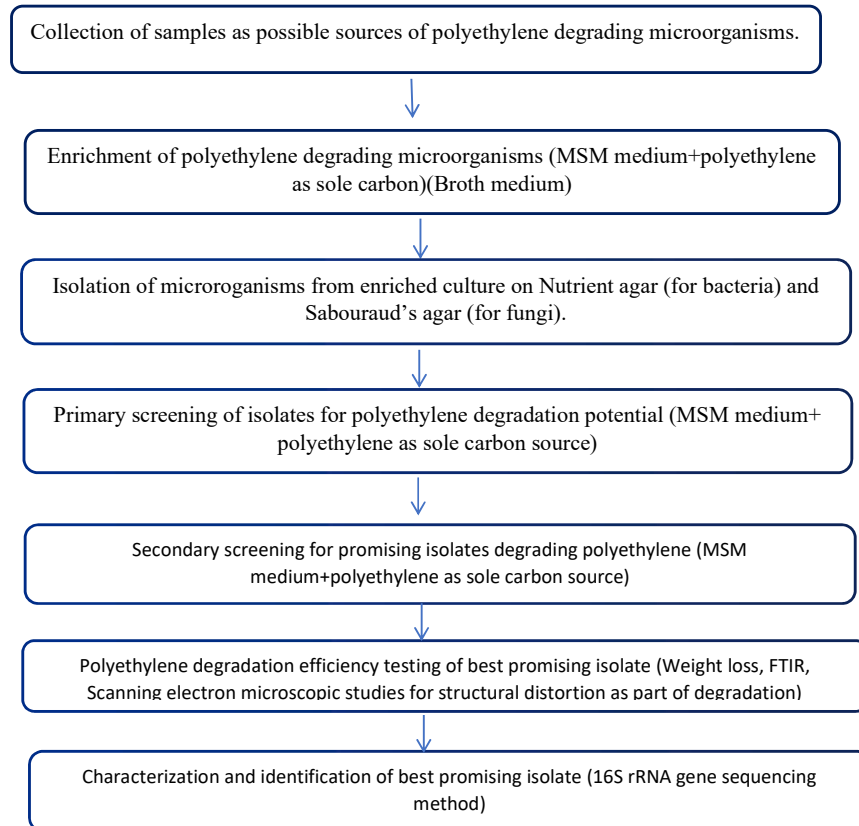


Fig. 1: Methodology at a glance.

Table 1: Source-wise sample collection.

Sr. No.	Types of samples with pH and temperature	Source of samples.	Number of samples collected.
1	Soil sample pH- 7.8; Temperature- 31°C	Soil samples collected from a plastic dumping ground, Karad	1
2	Soil sample pH- 7.8; Temperature- 30°C	Soil samples collected from landfill with plastic waste, Malkapur.	1
3	Marine water pH- 8.0; Temperature- 30°C	Water samples were collected from coastal regions in Mumbai.	1
4	Soil from coastal regions pH- 8.0; Temperature- 32°C	Soil samples were collected from coastal regions in Mumbai.	1



Fig. 2: Tube showing enrichment of polyethylene degrading microorganisms.

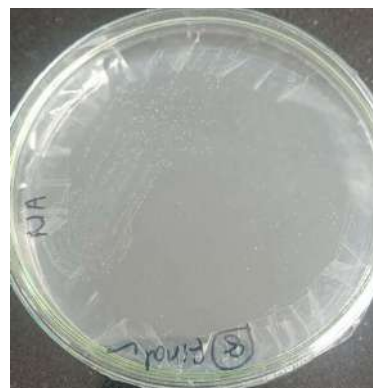


Fig. 3: Isolate from enriched sample on Nutrient Agar medium at 30°C for 48h incubation.

Table 2: Screening of polyethylene degrading microorganisms.

Sr. No.	Isolate No.	Degradation activity	Size of growth
1.	BIS8	++	7 mm

as the sole carbon source and were taken as polyethylene degraders and compared with positive and negative controls. The weight loss evaluation method showed that isolate BIS8 was efficient in polyethylene degradation, and its growth on agar well media is depicted in Table 2 and Fig. 4.

The results of the screening of the isolate, which was named BIS8, are summarized in Table 2:

As shown in Table 2 and Fig. 4, the isolate BIS8 showed growth around the inoculated well in the media containing polyethylene as the sole carbon source.

Evaluation of Polyethylene Degradation by BIS8 Isolate

The screened isolate BIS8 was evaluated for degradation potential. The polyethylene sheets were treated with the isolate, and their degradation was examined by Weight

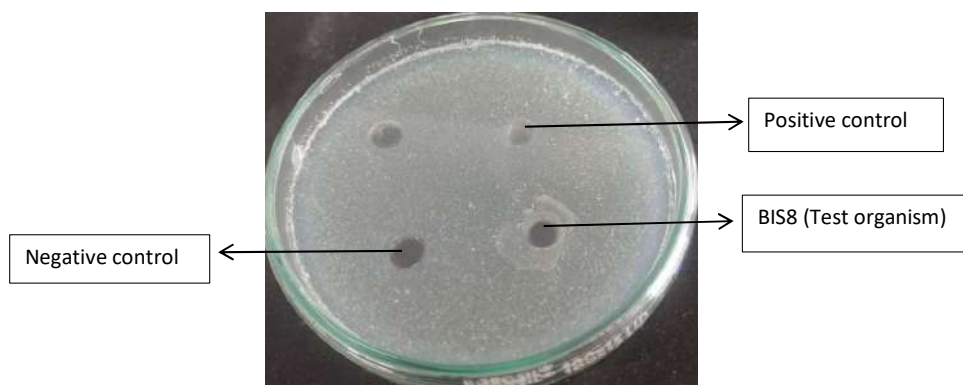


Fig. 4: Growth of isolate BIS8 and positive control wells and no growth in negative control well.

Table 3: Percentage degradation of polyethylene with BIS8 isolate, positive control, and negative control.

Set of experiments in triplicates.	Initial Weight (Average results of triplicate)	Final weight (Results of triplicate)
BIS8 set	0.05	0.03
Positive control set	0.05	0.035
Negative control set	0.05	0.05

loss method, FTIR analysis, and Scanning electron microscopy.

Weight Loss Method

- I) Percentage degradation of polyethylene by BIS8.

Percentage (%) Degradation =

$$\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

$$= \frac{0.05 - 0.03}{0.05} \times 100 = 40\%$$

The percentage degradation of polyethylene by the isolate BIS8 is 40%.

- II) Percentage degradation of polyethylene by positive control = The percentage degradation of polyethylene by positive control is = $\frac{0.05 - 0.035}{0.05} \times 100 = 30\%$

- III) Percentage degradation of polyethylene in negative control set = $\frac{0.05 - 0.05}{0.05} \times 100 = 0\%$

The percentage degradation of polyethylene by negative control set is 0%.

The degradation results (Table 3) depicted that BIS8 isolate showed polyethylene degradation (40%) in comparison with the positive control (30%), which is 10% more than the standard test organism, while in the negative control expected, 0.0% degradation was obtained.

It was found that the control polyethylene sheet didn't cause any change when weighed after the evaluation period.

There are certain reports of biodegradation of polyethylene by *Lysinibacillus* with *Aspergillus*, and it was 29.5% within 126 days of the incubation period (Esmaeili et al. 2013).

FTIR analysis of polyethylene treated with isolate BIS8:

The polyethylene sheet, which was treated with BIS8, was analyzed by FTIR. It is clear from the graph (Fig. 5) that the peaks at 2914, 2848, 1464, and 722 corresponded to CH stretch, CH stretch, CH bend, and CH rock bonds, respectively, and alkanes functional group, which indicated the degradation of polyethylene. In a research study, no extra functional groups were formed; just the intensity in many groups was changed. (Divyalakshmi & Subhashini 2016). Here decrease in absorption and an increase in transmission in FTIR was noted.

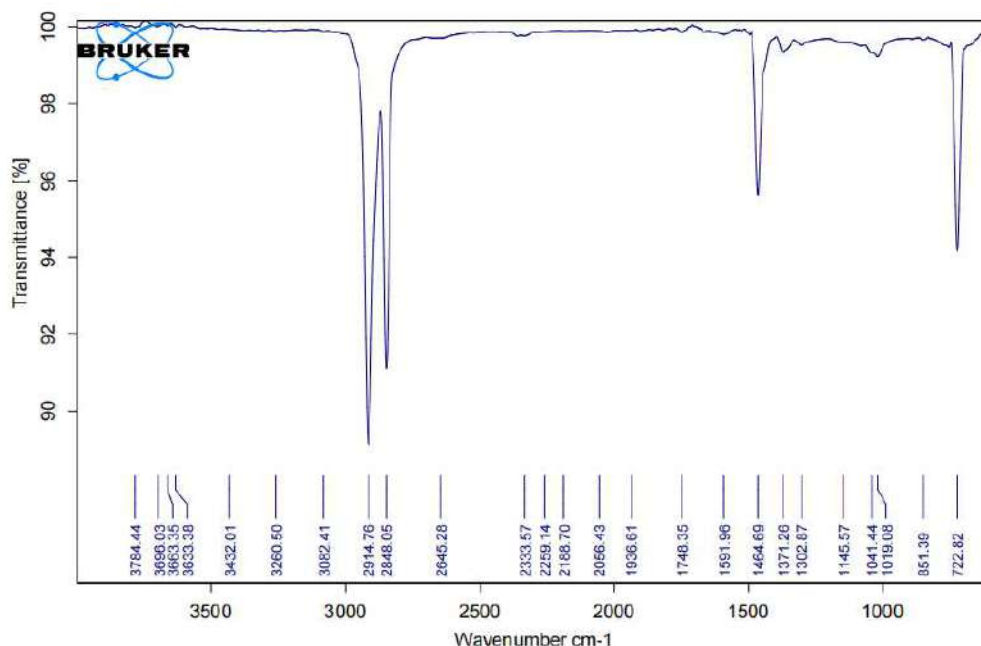


Fig. 5: FTIR analysis of polyethylene sheet treated with isolate BIS8.

Scanning electron microscopy: The promising isolate was used for treating polyethylene. Then, further, the isolate was found promising to degrade polyethylene. The microplastic sheets after the treatment were analyzed by SEM. The change in morphology and structure was examined by SEM (Distortion of polyethylene sheet) (Figs. 6 and 7).

As depicted in Fig. 6 and 7, it is clear that there is a change in the morphology and structure. The control sheet is plain and unaffected, while the treated sheet has pits and holes (distortion) on it, which proves the potential of the isolate to degrade polyethylene. The formation of pits and holes, as shown in Fig.7, depicts the initiation of degradation of the process while the control sheet is plain without any change in morphology. The isolate BIS8 has been found promising to degrade polyethylene, which is evident in Fig. 7.

The isolate BIS8 has carried out a weight loss of 40%. In a research study, polypropylene degradation was analyzed by SEM. The plastic weight, when treated with *A. fumigatus*, degraded up to 9.5% of plastic for 6 months. The SEM of the treated sample resulted in the formation of wrinkles and foldings with the development of streaks and rifts (Oliya et al. 2020). In this study, large pits and holes were formed, which indicated the promising properties of *L. macroides*.

Characterization and Identification of Promising Isolates

The isolate BIS8 was identified by 16S rRNA gene sequencing, and it was found that it was *Lysinibacillus macroides*.

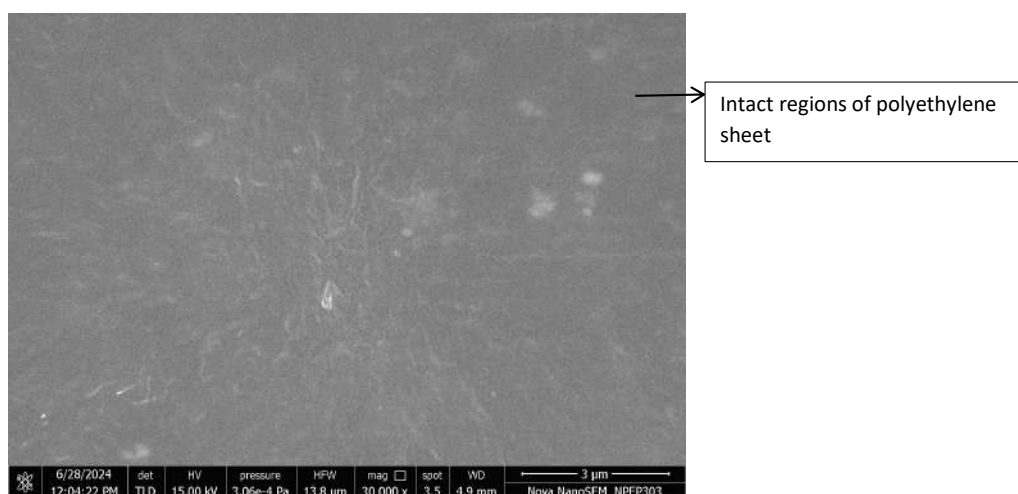


Fig. 6: SEM of Polyethylene control.

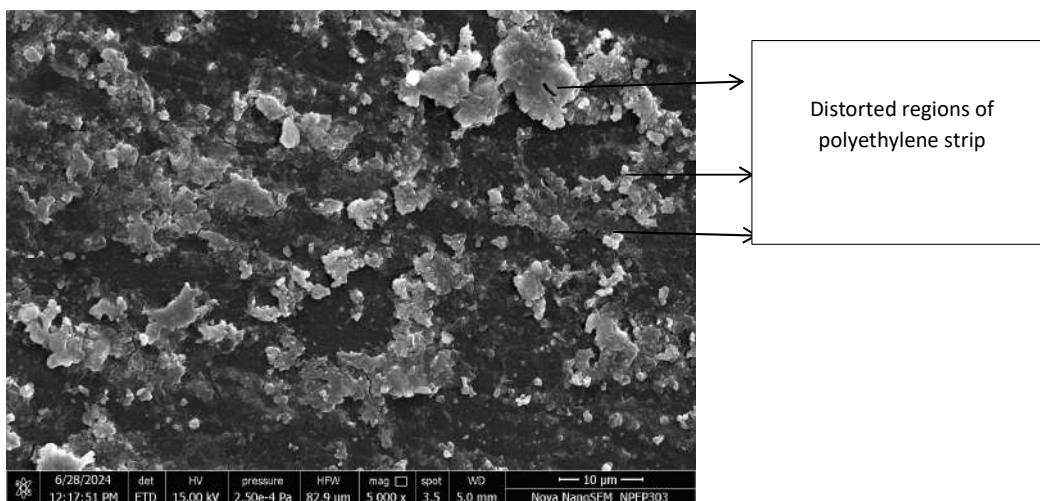


Fig. 7: SEM of Polyethylene sheet treated with BIS8.

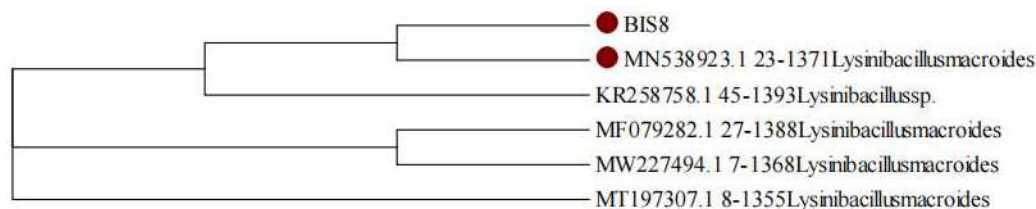


Fig. 7: Phylogram of *Lysinibacillus macroides* based on 16S rRNA gene analysis.

The promising isolate to degrade polyethylene was found to be *Lysinibacillus macroides*. There are reports of *Lysinibacillus* sp, which was isolated from soil, showing promising results in the degradation of polyethylene and polypropylene. There are reports that *Lysinibacillus* when combined with *Aspergillus*, gives promising results in the degradation of microplastics by degradation of 29.5% for UV-irradiated films and 15.8% for non-UV-irradiated films (Esmaeili *et al.* 2013). There are certain reports of *Lysinibacillus* degrading polyethylene by 9% within 26 days of incubation without any pretreatment (Jyoti *et al.* 2021). The isolate *Lysinibacillus macroides* showed better results of degradation, i.e., 40% in 30 days (4 weeks). The positive control *Bacillus megaterium* showed 30% degradation in 30 days. Better results may be owing to proper enrichment and adaptation. The employment of *Lysinibacilli* for degrading polyethylene gives promising results. These bacteria, along with other fungus such as *Aspergillus*, carry out the degradation of polyethylene and give remarkable results. The use of *Lysinibacillus* for biodegradation on a scale gives amazing results, but the use of this bacterium on-field is quite a challenging task and has limitations. The involvement of other microbes and their enzymes can influence the action of *Lysinibacillus*. Thus, research must be done on the application of microbes in the field. The use of consortia of bacteria and molds can be done to carry out onfield degradation. The consortia of compatible organisms give better results than the single isolate (Bardaji *et al.* 2020, Gao & Sun 2021). The present isolate BIS8 (*Lysinibacillus macroides*) can be further used in consortia for better results.

CONCLUSIONS

Natural sources such as plastic dumping areas have microorganisms, which are a natural process contributing to reducing the problem of plastic pollution. The microorganisms on the plastic dumping grounds carry out the biodegradation. The samples were collected, and the microorganisms were isolated and multiplied for employment in the biodegradation process in the laboratory. The isolate BIS8 showed promising results by degrading 40% of the polyethylene in a Mineral Salt medium with polyethylene as the only carbon source and identified as *Lysinibacillus macroides*. This depicts that the

microorganisms have the potential to degrade microplastics. The degradation potential of the bacteria can be employed to degrade polyethylene on a large scale. However, there is a chance of alteration of the activity of the bacteria by other microbes so several measures can be adopted for a successful remediation process. We can use consortia of compatible organisms for fast and promising results. Even the promising isolate can be genetically engineered to withstand various environmental changes and outdoor effects. Even various enzymes produced by the isolate to carry out enzymatic degradation can be extracted and used on-field. The use of microbes on-field is not a good idea as the interference of other microbes and their enzymes may affect the activity of promising microbes. Hence, the isolation of enzymes from promising microbes and the treatment of plastic with the enzyme will give remarkable results. This will give remarkable results without any interference from other factors.

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Bioremediation of Congo Red Using Polyvinyl Alcohol-Chitosan Supported Peroxidase as an Efficient and Reusable Catalyst

Neha Bhatkar¹, Aparna Kulkarni^{1†}, Seema Devasthali¹ and Varsha Ghadyale²

¹Department of Chemistry, Gogate Jogalekar College (Autonomous), Ratnagiri, Maharashtra, India

²Department of Biochemistry, Gogate Jogalekar College (Autonomous), Ratnagiri, Maharashtra, India

†Corresponding author: Aparna Kulkarni; drkulkarni.gjc@gmail.com

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ABSTRACT

The discharge of textile effluents containing azo dyes is critical due to their persistence in wastewater and carcinogenic, mutagenic impacts on aquatic organisms. Considering the toxicity of azo dye, an effective remediation strategy should be applied before disposing into the environment. Among all the advanced techniques like electrochemical degradation, Fenton oxidation, photocatalysis, ozonation, etc., biodegradation using biocatalysts is an eco-friendly and economic process to deal with this problem. Along with advantages, biocatalysts, particularly enzymes, face limitations like instability, single-use restriction, and reduced efficiency under operational conditions. Immobilization addresses these challenges by enhancing enzyme stability, reusability, and catalytic performance. The present study focuses on the development of an efficient bioremediation approach for the removal of Congo red dye from aqueous solutions using peroxidase (HGP) extracted from germinated *Macrotyloma uniflorum* (horse gram) seedlings. The enzyme was immobilized on polyvinyl alcohol-chitosan beads through epichlorohydrin-mediated crosslinking, enabling its application as a reusable biocatalyst for dye degradation. The immobilization method achieved high efficiency with 96% enzyme retention. The immobilized peroxidase exhibited enhanced stability and was evaluated for its efficacy in degrading Congo red dye. Under optimized conditions, 26 units of immobilized peroxidase achieved complete decolorization (100%) of a 160 mg.L⁻¹ Congo red solution within 10 minutes at 28°C and pH 4. Environmental safety of the degradation products was confirmed through phytotoxicity and microbial growth assessments. Additionally, the immobilized enzyme retained its catalytic activity across eight successive cycles, underscoring its reusability and potential for practical applications in bioremediation of dye-contaminated wastewater. A newly developed biocatalyst demonstrates a simple method of preparation, environmental benignity, biocompatibility, high efficiency, enhanced stability, and facile recyclability.

INTRODUCTION

Contamination of soil, water, and air by industrial effluents from various chemical industries is one of the major concerns of the 21st century. Among various chemical industries, azo dye industries are one of the major contributors to environmental pollution (Carliell et al. 1995). Azo dyes are extensively employed in both the textile and food industries due to their appealing, vibrant shades and impressive colorfastness properties (Chen 2006). However, their widespread use comes with a significant environmental challenge. Azo dyes possess high water solubility and resist easy biodegradation, leading to the generation of wastewater during their production and industrial application. This wastewater, if released directly into water bodies, poses a grave threat to aquatic ecosystems and human health (Kaushik & Malik 2009, Somasekhara Reddy et al. 2012, Chatha et al. 2017). It is imperative to address this issue, as the pollution caused by these non-biodegradable dyes can have harmful effects on both our environment and well-being. It is well known that it is responsible for many health conditions like skin, eye, and gastrointestinal

irritations, blood clotting, mutation, reproduction-related diseases, respiratory problems (Mittal et al. 2009, Li et al. 2010), etc. Considering the serious environmental and health impacts associated with the effluents from azo dye industries, the development of protocols for the total removal of azo dyes from the effluents is highly desirable (Hai et al. 2006, Rajkumar & Kim 2006).

Effective strategies for remediation of dye-contaminated wastewater are the use of oxidizing agents, adsorption of pollutants over various supports, and photocatalytic treatments (Chanathaworn et al. 2012, Nidheesh et al. 2013, Fisli et al. 2014, Ojedokun & Bello 2017, Lubbad et al. 2019, Cao et al. 2024, Haroon et al. 2023, Jadon et al. 2022), etc. These conventional procedures suffer from drawbacks like the creation of sludge in significant amounts, high cost, cumbersome operational procedures, and toxic end products (Ahmedi et al. 2015), etc. In recent years, in this context, eco-friendly biological methods involving the use of enzymes for cleaning up pollutants have gained considerable interest (Bhandari et al. 2021). This is because, enzymatic treatments for bioremediation are known to offer advantages like mild reaction conditions, substrate specificity, cost effectiveness (Esposito & Durán 2000, Husain & Jan 2000), etc. At the same time, poor stability of enzymes at higher temperatures, higher cost of their isolation, and difficulties in their recovery from the reaction media limit their use on an industrial scale (Chiong et al. 2016, Long et al. 2023). As enzyme stability and its recovery for possible reuse can be enhanced enormously by immobilizing the enzyme on a suitable solid support, the development of suitably tailored immobilized enzymes for the bioremediation of pollutants has become an interesting area of research.

Peroxidase, an oxidoreductase class of enzyme, is abundantly found in plants like horseradish, white radish, bitter gourd, garlic, tomato, soya bean, turnip (Husain 2010, Osuji et al. 2014), etc. Owing to their wide substrate specificity and excellent bio-catalytic activities, plant peroxidases are the molecules of commercial interest (Akhtar et al. 2005, Ali et al. 2018, Zhang et al. 2010). Especially, the ability of peroxidases to catalyze oxidation at low concentrations makes them the enzyme of choice for bioremediation of a variety of synthetic dyes (Singh & Taranjeet Singh 2019). In light of these considerations, this study aimed to develop a potential bioremediation process for the degradation of Congo red dye using solid-supported peroxidase enzyme.

In the present study, peroxidase enzyme (HGP) was extracted from *Macrotyloma uniflorum* (Horse Gram) and was crosslinked on PVA-Chitosan beads using epichlorohydrin as a crosslinker. The potential of crosslinked

peroxidase enzyme was investigated in the degradation of the model dye Congo red. A simple degradation process of Congo red dye without the use of any mediator (TEMPO, HOBt) was developed using immobilized peroxidase enzyme and hydrogen peroxide.

MATERIALS AND METHODS

Congo red ($C_{32}H_{22}N_6Na_2O_6S_2$), hydrogen peroxide 30% w/v (H_2O_2), ammonium sulphate AR ($(NH_4)_2SO_4$), sodium hydroxide (NaOH), o-Phenylenediamine, glacial acetic acid AR (CH_3COOH), Polyvinyl alcohol (Molecular weight approx. 14,000), epichlorohydrin (C_3H_5ClO) were supplied by MOLYCHEM (Mumbai, India), chitosan ($(C_6H_{11}NO_4)_n$) from shrimp shells was purchased from Himedia, horse gram (*Macrotyloma uniflorum*) was purchased from local market and allowed to germinate for minimum 2 days.

For all the experiments, different concentrations of dye solutions ranging from 20 ppm to 200 ppm were prepared with deionized water, and these solutions were stored protected from light at 4°C, but no special treatment was given to the dye solutions. Extraction of HGP from horse gram was performed using the ammonium sulfate precipitation method. Dye degradation study was evaluated using a UV-Visible spectrophotometer and HPLC.

Extraction and Purification of Peroxidase Enzyme from Horse Gram (HGP)

For the isolation of the peroxidase enzyme, germinating horse gram (*Macrotyloma uniflorum*) seedlings were used. Seeds were soaked on a cotton bed in saline solution for 48 hours. Crude extract was prepared by homogenization using a buffer saline solution (Phosphate buffer saline, pH 7.4, 0.2M). Crude extract was treated with ammonium sulfate for 30% saturation. After centrifugation, the supernatant was subjected to 80% saturation. Precipitate was collected after centrifugation, and the dialyzed and partially purified enzyme was used for further optimization.

Preparation of Polyvinyl (PVA)-Chitosan Beads as Support

Chitosan beads were selected as a support due to their biocompatibility and multiple active binding sites. However, the formed chitosan beads exhibited relative fragility. To enhance their structural stability throughout the process, polyvinyl alcohol (PVA) was added. Chitosan and PVA solutions were prepared by dissolving each in glacial acetic acid and distilled water, respectively. These two homogenous solutions were mixed in a 1:1 proportion, followed by stirring for 1 hour at room temperature. The obtained homogenous

mixture was then added dropwise through a syringe into 1N NaOH solution to get PVA-chitosan beads. These beads were allowed to stand in the same solution for 2 to 3 hours for hardening. The hardened beads were then washed with distilled water till neutral pH.

Immobilization of Extracted Peroxidase Enzyme on PVA-Chitosan Beads

Immobilization of the extracted peroxidase enzyme was performed by the crosslinking method. PVA-chitosan beads were then treated with 2% Epichlorohydrin solution for 2 hours with occasional stirring. After treatment, beads were washed with distilled water twice and suspended in 5 mL phosphate buffer of pH 7.4 containing 0.2 mL (26U) peroxidase enzyme. To ensure immobilization, this system was kept overnight in cold conditions. Beads were then washed twice with 5 mL of distilled water to remove unbound enzyme and stored in phosphate buffer (pH 7.4) at 4°C till further use. The washings were collected to measure residual peroxidase activity. Percent immobilization was then calculated by subtracting the activity lost in the washings from the initial enzyme activity used for immobilization. For comparative evaluation of immobilization techniques, peroxidase from horse gram seedlings (HGP) was also immobilized on sodium alginate beads via the entrapment method.

Assay of Free HGP and Immobilized Peroxidase Enzyme

Peroxidase activity and stability of crosslinked HGP were determined and compared to that of the free enzyme and HGP immobilized on sodium alginate by the entrapment method.

A study of the activity of free HGP and HGP immobilized on PVA-Chitosan support and sodium alginate support was performed using o-Phenylenediamine (OPD) as a substrate at room temperature (Gutema Dinkisa Idesa 2018)

The procedure followed was as outlined below. The assay mixture comprised 0.4 mg.mL⁻¹ o-phenylenediamine (OPD), 0.1 mL of 30% hydrogen peroxide, 0.9 mL of distilled water, and 0.2 mL (26 U) of free enzyme. After dilution with an additional 1.0 mL of distilled water, the absorption was measured at 492 nm following a 1-minute incubation.

The same procedure was performed for assay of immobilized enzyme, where 0.2 mL of free enzyme immobilized on sodium alginate beads and PVA-chitosan beads was used for assay. The activity of free and immobilized enzymes was checked for 7 days to study the stability of the enzyme.

Aqueous Phase Degradation of Congo Red Dye Using HGP

The dye degradation reaction was carried out in a 100 mL beaker containing 10 mL of dye solution, buffer solution, and a fixed amount of enzyme. To this, 0.3% H₂O₂ was added dropwise, and the reaction mixture was stirred continuously. 20 µl of sample was taken at regular intervals and the dye degradation reaction was monitored using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) at 570 nm.

The reaction parameters like pH (2–10), Congo red dye concentration (20–200 mg.L⁻¹), H₂O₂ dose (0.0 mL–1.0mL), dose of free enzyme (0.1 mL–1.0 mL), and reaction time (10 to 90 minutes) were studied. The percentage degradation was calculated using the formula,

$$\text{Dye removal (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

where A₀ and A_t are the absorbance before and after enzymatic treatment, respectively.

The degraded dye was characterized by the HPLC technique and compared with the HPLC spectra of Congo red.

Toxicity Study

A phytotoxicity study of Congo red was performed before and after enzymatic treatment using Vigna aconitifolia seeds. For this, 10 seeds of Vigna aconitifolia were allowed to germinate in Congo red dye solution, and the supernatant was obtained after dye degradation. Both samples were compared with a standard sample germinated in distilled water.

For the microbial growth study of Congo red, two microorganisms were selected to check growth, S.aureus and E.coli. The inoculum was allowed to grow on nutrient agar using standard plating technique, and growth was compared with the dye solution and supernatant obtained after dye degradation.

RESULTS AND DISCUSSIONS

This section can be organized using subheadings. It should present a clear and concise description of the experimental results, their interpretation, and the conclusions derived from them. If the flow of the paper suggests combining the results and discussions in one Section, you can do it here.

Extraction and Immobilization of HGP

Extraction of HGP (Protein estimation): Protein content estimation of extracted HGP was performed at each step of extraction by the Folin Lowry method (Lowry et al.1951). Enzyme activity was quantified by measuring the change in

optical density (OD) over time, with activity calculated using the Beer-Lambert law to determine concentration changes. One enzyme unit was defined as the amount catalyzing the transformation of 1 μmol of substrate per minute under the assay conditions. The highest protein content was observed in the case of dialyzed enzyme than the crude extract. The enzyme obtained after dialysis showed activity of 130 units.mL⁻¹.

HGP immobilization study: Immobilization of peroxidase is essential to enhance its operational stability, reusability, and resistance to environmental fluctuations. This technique not only extends the enzyme's functional lifespan but also facilitates its repeated use, which is crucial for efficient and cost-effective bioremediation processes. With this objective, the immobilization of extracted HGP was studied by two methods. Immobilization of HGP on sodium alginate beads by the entrapment method and immobilization of HGP on PVA-Chitosan beads by the crosslinking method. The results were compared with the assay of free HGP.

It was observed that the synthesized PVA-chitosan beads are effective in the immobilization of peroxidase enzyme and gave 96% (Table 1, entry 2) immobilization, whereas the immobilization of peroxidase enzyme by the entrapment method on sodium alginate was 81% (Table 1, entry 3). Thus, comparison of two methods showed that immobilization of peroxidase enzyme by the crosslinking method has the

advantage of better percent immobilization (96%) compared to immobilization of peroxidase enzyme by the entrapment method (80%). The table shows the comparative study of assay of free HGP, HGP immobilized on PVA-Chitosan beads, and HGP immobilized on sodium alginate beads.

Comparison of Activity and Stability of Free and Immobilized HGP

The prime aim of enzyme immobilization is to enhance stability, yet it can potentially lead to a reduction in enzyme activity. Both enzyme activity and stability hold vital significance, and a robust immobilization technique helps in achieving stability while maintaining activity levels that are comparable. HGP was immobilized using two immobilization techniques, the crosslinking method and the entrapment method. To check the efficacy of the immobilization technique used, the activity and stability of crosslinked HGP and entrapped HGP were checked and compared with free HGP by performing an assay using OPD. An assay was performed for 7 days with 24-hour interval between two screenings. The comparative results of the enzyme assay of free HGP, crosslinked HGP, and entrapped HGP are shown in Fig.1. The results indicated that the activity of crosslinked HGP is comparable to that of free enzyme and showed improved stability.

Table 1: Comparative % immobilization of peroxidase enzyme immobilized on solid support.

Entry	Enzyme	Free enzyme concentration [in mL]	Free enzyme concentration [Units]	Enzyme concentration after immobilization [Units]	% immobilization
1	Free HGP	0.2	26	-	-
2	HGP immobilized on PVA-Chitosan beads	0.2	26	25	96%
3	HGP immobilized on sodium alginate beads	0.2	26	21	81%

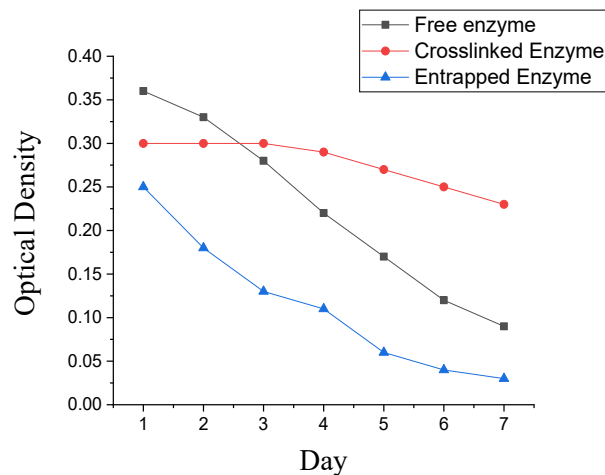


Fig. 1: Activity comparison between free HGP, HGP immobilized using the crosslinking method, and HGP immobilized using the entrapment method.

For free enzymes, a plot of absorbance vs days showed an exponential decrease in activity of HGP with time. After 5 days, free HGP loses more than half of its original activity. In comparison to free HGP, the activity of crosslinked enzyme remained consistent for 72 hours, and it remained stable for almost 7 days (Fig. 1). While in the case of an entrapped enzyme, activity starts decreasing after 24 hours. This indicates that the immobilization technique used is suitable for immobilizing peroxidase and improves the stability of the enzyme.

Aqueous Phase Degradation of Congo Red Dye Using Free HGP

To evaluate the efficacy of the enzyme in degrading Congo red dye, preliminary experiments were conducted using the extracted free enzyme. Key parameters, including contact time, pH, and concentrations of dye, enzyme, and H_2O_2 , were optimized. Once these conditions were established, the performance of cross-linked HGP immobilized on PVA-chitosan beads was assessed under the optimized conditions.

Optimum contact time: The optimal contact time required for the dye removal was evaluated initially. To a sample solution bearing 10 mL of 40 ppm dye solution, 0.2 mL of enzyme and buffer solution of pH 4, 1 mL of 0.3% H_2O_2 was added dropwise and allowed to stand for 10 to 90 minutes. After each interval of 10 minutes, a small amount of sample solution was taken and examined for the residual dye concentration. Maximum dye degradation was observed within the initial 10-minute interval, with no significant change in degradation observed up to 90 minutes. Consequently, for subsequent optimization studies, the contact time was set to 10 minutes.

Optimum pH: Enzymes exhibit optimal activity within a specific pH range. To fix the optimal pH range of extracted HGP for Congo red dye degradation, a pH optimization study was carried out on an aqueous solution of Congo red dye. For this series of buffers, ranging from pH 2 to pH 10

were prepared and fixed amount of each buffer was added to 9 different vials containing 10 mL of 40 ppm Congo red dye and 0.2 mL of free enzyme. To each of the nine vials, 1.0 mL of 0.3% H_2O_2 was added gradually with constant stirring, and the percentage degradation at various pH levels was studied. Fig. 2 illustrates the variation in dye degradation across different pH values. The results indicate that approximately 100% dye removal occurred at pH 4 due to the HGP-catalyzed reaction, decreased at pH levels below 4 and above 5. Since maximum degradation was achieved at pH 4, a buffer of pH 4 was selected for subsequent experiments.

Optimization of H_2O_2 dose: Preliminary studies on Congo red dye degradation indicated that 0.3% H_2O_2 is sufficient to achieve effective degradation. Employing such mild conditions is advantageous, particularly in minimizing environmental impact. To find out the minimum amount of H_2O_2 required for dye degradation, optimization of the amount of H_2O_2 dose was carried out by keeping all the other experimental conditions constant. Thus, a series of doses ranging from 0.0 mL to 1.0 mL was gradually added in 6 different vials containing 10 mL of 160 ppm Congo red dye solution, 0.2 mL (26U) of enzyme, and 4 mL buffer of optimum pH. (Fig. 3) represents a graph of dye degradation as a function of H_2O_2 dose. 0.2 mL of H_2O_2 was found to be adequate for the maximal dye degradation under the experimental conditions defined before.

Optimization of free enzyme dose: The amount of catalyst utilized for dye degradation holds significant importance. Considering the high cost of the enzyme, the enzyme should facilitate dye degradation at lower concentrations. Optimization of the enzyme dose was done by varying the enzyme concentration. A series of doses of 0.1 mL (13U), 0.2 mL (26U), 0.4 mL (52U), 0.6 mL (78U), 0.8 mL (104U) and 1.0 mL (130U) of HGP were gradually added in 6 different vials containing 10mL of 160 ppm Congo red dye solution 0.2 mL of 0.3% H_2O_2 and 4mL buffer of optimum

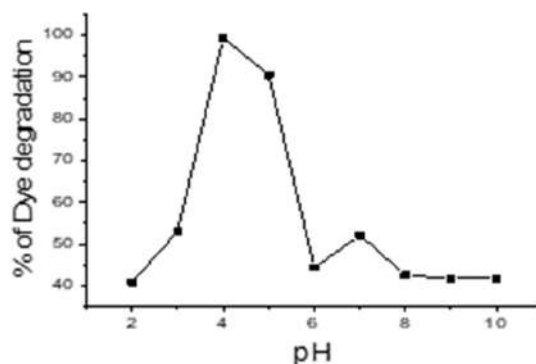


Fig. 2: Effect of pH on dye degradation using HGP.

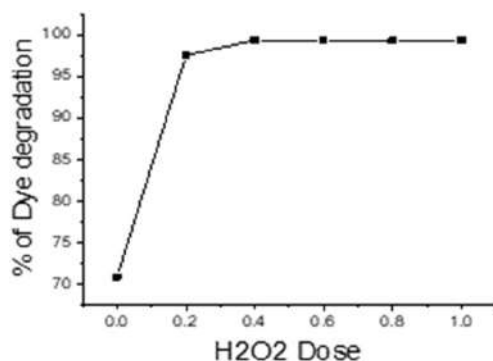


Fig. 3: Effect of H₂O₂ on dye degradation.

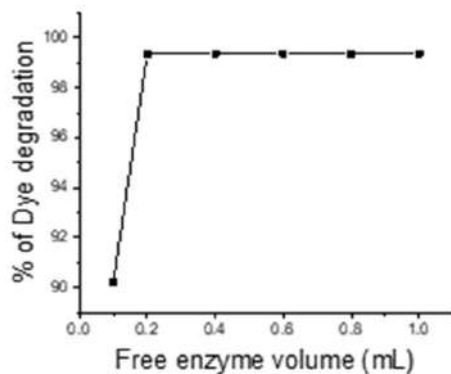


Fig. 4: Concentration of enzyme required for dye degradation.

pH. (Fig. 4) represents percentage dye degradation as a function of the concentration of the enzyme. It was observed that 0.2 mL, i.e., 26 units of enzyme, is sufficient to bring about 100% dye degradation.

Optimization of Congo red dye concentration: To determine the maximum concentration of Congo red dye that can be degraded by free HGP enzyme, a series of Congo red dye solutions was prepared with concentrations ranging from 20 to 200 ppm. The enzyme concentration and H₂O₂ were maintained at constant values. For each concentration,

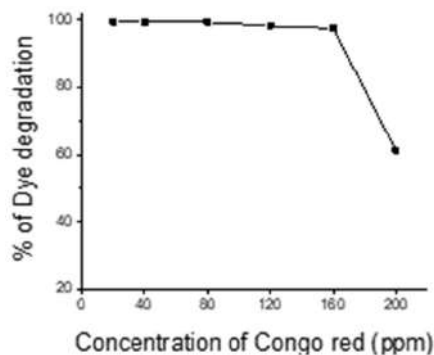


Fig. 5: Effect of dye concentrations on dye degradation using HGP.

a 10 mL aqueous solution of Congo red dye was combined with 4.0 mL of pH 4 buffer, 0.2 mL of free enzyme, and 1.0 mL of H₂O₂. This mixture was stirred for 10 minutes, and the dye degradation was monitored. This study revealed that complete removal of the dye was achieved up to a concentration of 160 ppm (Fig. 5).

Congo Red Degradation Using Immobilized HGP

The efficacy of immobilized HGP for Congo red degradation was studied at optimized conditions, and it was observed that immobilized HGP showed similar results to those shown by free HGP. For 100% removal of dye using immobilized enzyme, the contact time was found to be 10 mins.

Reusability Study

Achieving economic viability in the process remains a central goal in any enzyme-catalyzed reaction. A proficient immobilization technique contributes to enhancing the enzyme's stability and its capacity for multiple uses. In the present study, it was observed that immobilized enzyme can be successfully reused for 8 cycles, achieving almost 100% removal for the first six runs, 98% and 85% for the 7th and 8th runs, respectively (Fig. 6).

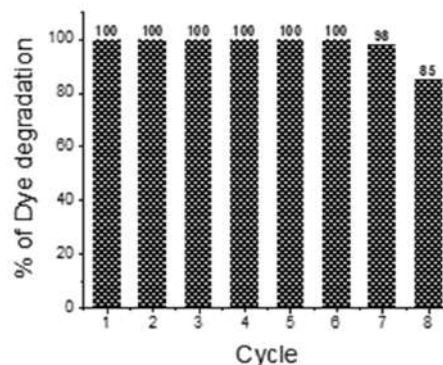


Fig. 6: Reusability study of immobilized peroxidase enzyme.

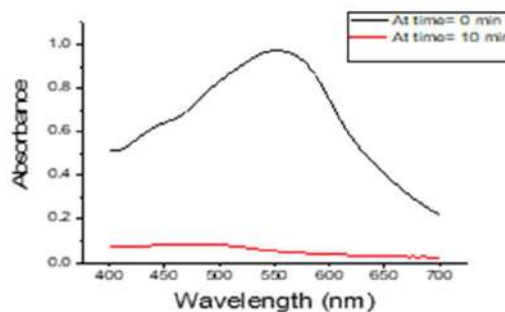
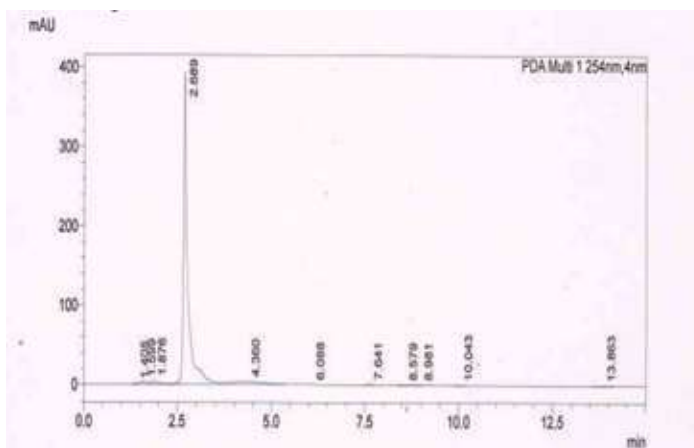
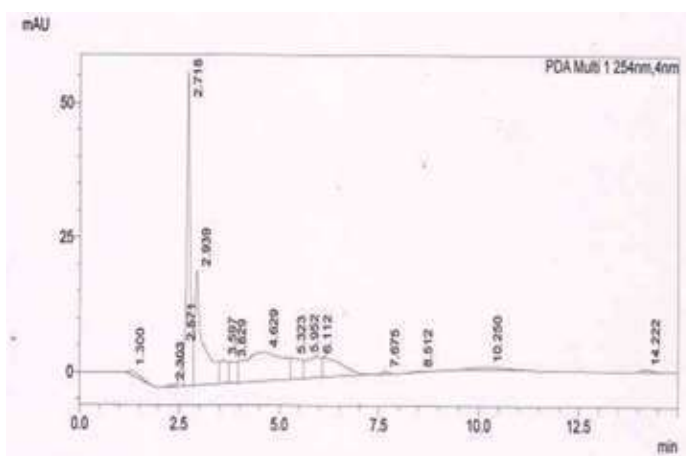


Fig. 7: UV-Visible spectrum of Congo red and reaction mixture.



(a)



(b)

Fig. 8: HPLC Chromatogram of (a) Congo Red dye, (b) degraded dye.

Dye Degradation Study

UV-Visible spectrum of the control and sample was observed at 570 nm, as Congo red has maximum absorption at 570 nm in acid medium. The absence of an absorption peak at 570 nm indicates almost complete removal of Congo red from the reaction mixture (Fig.7).

HPLC Analysis

Being a part of the oxidoreductase class of enzymes, HGP follows the oxidative pathway for degradation of Congo red (Ahmedi et al. 2012). HPLC analysis was performed to confirm Congo red degradation. Chromatogram of control, i.e., Congo red dye and dye solution after HGP treatment, was recorded at retention time 2.5 min. (Fig. 8)

The reduction in peak height, that is reduction in absorption, indicates a decrease in concentration of the model dye. The appearance of extra peaks in the chromatogram of the HGP-treated solution indicates degradation of the parent molecule.

Toxicity Study

For any enzymatic dye degradation process, it is necessary to check the safety of the treated dye solution before discharging it into natural water sources. Therefore, the supernatant solution after the enzymatic degradation of Congo red was tested for phytotoxicity and microbial growth. A phytotoxicity study of Congo red dye solution was performed before and after enzymatic treatment using *Macrotyloma uniflorum* seeds.

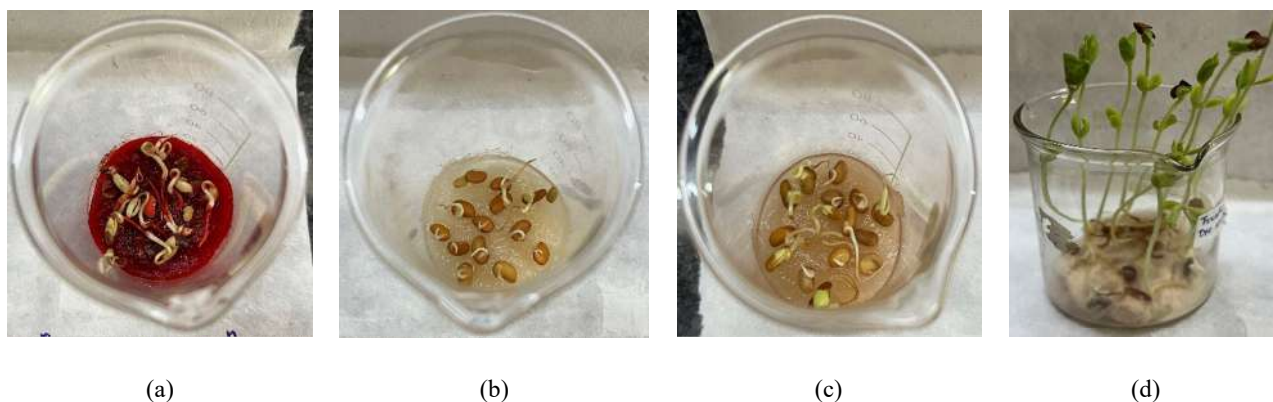


Fig. 9: Phytotoxicity study of (a) Dye solution, (b) Supernatant obtained after enzymatic degradation (c) water (d) plant growth in the Supernatant obtained after enzymatic degradation after 10 days.

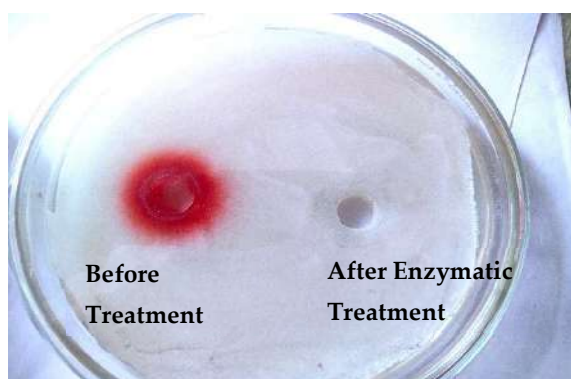


Fig. 10: Microbial Study.

The obtained results indicate that Congo red badly affects the seedlings' growth by penetrating cells (Fig. 9a). While *Macrotyloma uniflorum* seeds grown in supernatant obtained after dye degradation showed comparable growth as in distilled water (Fig. 9(b & c)). The average root length obtained ranges between 2 cm to 3 cm $\pm$ 2 mm. In a similar way, microbial studies gave favorable outcomes for the supernatant obtained after dye degradation. The zone of inhibition for the dye solution before treatment was measured at 3 $\pm$ 0.5 mm. However, no microbial growth inhibition was observed after treatment. A study showed that Congo red dye solution inhibits the growth of microorganisms, whereas the supernatant obtained after enzymatic treatment shows proper growth of microorganisms (Fig. 10), indicating that it is nontoxic.

CONCLUSIONS

Peroxidase extracted from *Macrotyloma uniflorum* was successfully immobilized on PVA-chitosan beads via cross-linking, retaining high activity and enhanced stability compared to the free enzyme. The immobilized enzyme enabled efficient and nearly complete degradation of Congo red

in the presence of H_2O_2 , maintaining activity over eight reuse cycles. This method offers a cost-effective, straightforward, and eco-friendly approach for the bioremediation of Congo red, with practical potential for application in dye effluent treatment of wastewater from the dye industry. Potential scalability of this bioremediation approach for industrial applications is promising due to its high enzyme retention (96%), rapid degradation efficiency (100% decolorization in 10 minutes), and reusability over multiple cycles. The industrial-scale application of this approach remains a subject for future investigation.

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Performance of Venturi Aerator Combined with Zeolite Filter for Removing Iron and Manganese

Euis Nurul Hidayah^{ID}, Okik Hendriyanto Cahyonugroho^{ID}, R. Mohammad Alghaf Dienullah[†]^{ID} and Brillyan Kusuma Pradani

Department of Environmental Engineering, Universitas Pembangunan Nasional Veteran Jawa Timur, Jawa Timur, Rungkut, Surabaya, 60924, Indonesia

[†]Corresponding author: R. Mohammad Alghaf Dienullah; alghaf.ft@upnjatim.ac.id

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ABSTRACT

Groundwater is the primary source of drinking water, but this source is frequently contaminated by heavy metals such as iron (Fe) and manganese (Mn) due to contact with metallic minerals in the soil. This contamination not only causes discoloration and the formation of scale that damages pipes but also poses serious health risks, including neurological disorders and even cancer, if consumed over a long period. Some water treatments, such as aeration and filtration, are capable of removing Fe and Mn concentrations in groundwater. This study aims to solve this problem by testing the effectiveness of a combination of venturi aeration and zeolite filtration in reducing each Fe and Mn concentration in groundwater. Variations were made to the venturi aerator's air hole size with diameters of 12 mm, 10 mm, and 8 mm. During the aeration and filtration process, sampling was conducted at 0 min, 15 min, 30 min, and 60 min. The highest removal Fe and Mn concentration occurred at the 8 mm diameter variation, with a Fe removal efficiency of 97% and an Mn removal efficiency 54% during the aeration process. After that, Mn reduction is up to 99% during the filtration process.

INTRODUCTION

Most developing countries use groundwater as a large source of drinking water because it is considered a lesser pollutant (Islam & Mostafa 2024). In many parts of Asia, such as Indonesia, groundwater is mostly freshwater, but anthropogenic and natural activities degrade and transform groundwater into contaminated water (Irfeey 2023). Almost the groundwater in Indonesia contains a relatively high concentration of Iron (Fe) and manganese (Mn) (Sudiarto et al, 2021). Groundwater has the potential to be contaminated by heavy metals contained within the soil. The presence of heavy metals such as Fe and Mn in the soil can contaminate groundwater in a dissolution form (Arrizal 2021). Fe is the element that is commonly found in almost every place on all geological layers and all bodies of water. In water, it can be dissolved as Fe^{2+} (ferrous) or Fe^{3+} (ferric) and suspended as colloidal grains ($<1 \mu\text{m}$ in diameter). Besides that, Mn in nature is generally found in the form of compounds with various valences. High Mn content can cause turbidity, discoloration, and taste in water (Febrina & Ayuna 2019).

The concentrations of Fe and Mn in soil vary depending on soil conditions and the surrounding environment. (Said 2018). Refers to the World Health Organization (2017), a critical concentration value of Mn in water must not be in excess of 0.05 mg/L, with the range value for its intake being 11 mg.day^{-1} for humans. Whereas for Fe, a critical concentration value of Fe in water must not exceed 0.1 mg.L^{-1} with the range concentration value for humans up to 50 mg.day^{-1} .

Several cases with the excess of Fe and Mn in water cause a yellow coating to build up inside pipes, which can block them if not cleaned (Annisah & Subhan 2020).

Drinking groundwater with heavy metals can be dangerous to health. It can cause problems with nerves, harm unborn babies, and even lead to cancer (Fuad et al. 2018). The excess Fe concentrations can irritate the eyes and skin, and cause intestinal disorders, while excess Mn concentrations cause the element to be neurotoxic (Febrina & Ayuna 2019). Water with high Fe and Mn concentrations requires treatment before it can be able to consumption. Various technologies can be used to reduce Fe and Mn concentrations in water. Aeration and filtration are commonly used technologies for the degradation of Fe and Mn. According to Kalyani et al. (2021), a module water treatment plant containing an aeration unit and a filtration unit is effective for removing manganese and iron in raw water.

Several aeration methods have been developed recently, such as venturi aeration (Said 2018). The Venturi Aerator has several parts consisting of a diverging section, a nozzle, and a suction chamber (Mahmud et al. 2024). According to Yadav et al. (2021), the venturi aerator process works by oxidizing Fe and Mn ions into insoluble hydroxide compounds. Venturi aeration can increase dissolved oxygen levels up to 6.3 mg.L^{-1} within 19 min. Dissolved oxygen concentration in water influences the reduction of Fe and Mn concentrations (Taufan 2010). Venturi aerators operate using the Bernoulli principle, where air from a pipeline is naturally surface flowed in the throat of the pipe, so when compared to other aeration methods such as conventional air transport, stirred tanks, and bubble columns, venturi aerators require less energy (Mahmud et al. 2024). Subsequently, water treatment is conducted using filtration with an adsorbent media such as zeolite. Zeolite is effective in degrading Fe and Mn parameters with efficiencies of up to 97.45% and 95.81%, respectively (Annisah & Subhan 2020). Based on previous research, this study will evaluate the efficiency of Fe and Mn concentrations in groundwater using a combination of venturi aeration and zeolite filtration.

MATERIALS AND METHODS

This research is an experimental study aimed at degrading Fe and Mn parameters commonly found in groundwater. The water samples were collected from Sengon Village, Jombang District, East Java, Indonesia. The water was treated using aeration and filtration processes.

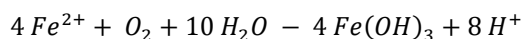
Aeration was conducted using a venturi aerator with 8 – 12 mm air diameter. The venturi aerator diameters used in this study were 12 mm, 10 mm, and 8 mm. The venturi aerator's throat diameter and neck length are kept constant. A constant flow rate of 0.3 L.s^{-1} was used in this study. In the filtration reactor, the zeolite media was used a size 8 × 16 mesh and a filter media height was used 30 cm.

The initial Fe and Mn concentrations of the samples were analyzed. The initial analysis revealed a Fe concentration was 0.743 mg.L^{-1} and a Mn concentration was 1.84 mg.L^{-1} . The water treatment process was conducted continuously and it was collected periodically. Sampling was conducted at each reactor outlet. The sample was collected prior to analysis at the time: 0 min, 15 min, 30 min; and 60 min. The parameters were used such as Dissolved Oxygen (DO), pH, Fe, and Mn. DO concentration was tested and analyzed using the SNI 06-6989 14-2004 standard method. pH was tested by using the standard method of SNI 6989-11 2019. The collected samples were analyzed for residual Fe concentration refers to Standards Methods 3500-Fe Band for Mn concentrations refers to Standard Methods 3500-Mn B. Performance of the process was measured by using an initial concentration of raw water to treated raw water. The performance of the venturi aerator and zeolite filtration units was determined based on their removal efficiency against the Fe and Mn concentration parameters.

RESULTS AND DISCUSSION

The groundwater samples appeared clear upon visual inspection but had a metallic odor. Based on experiment results, the ability of venturi aerators and zeolite filters to remove Fe concentration in water is shown in Fig. 1.

As shown in Fig. 1, the percent removal of Fe concentration in each variant of aerator hole diameter and each sampling time shows 99% or, based on the analysis results, the Fe concentration was 0.0567 mg.L^{-1} . According to Wang and Wang (2022), aeration is one of the best methods to reduce the concentration of Fe because using air as an oxidant for aeration is a simple process to convert the soluble Fe(II) to the form particle Fe(III). Despite in groundwater Fe produces compounds with valence 2 such as FeCO_3 and $\text{Fe}(\text{HCO}_3)_2$ which have high solubility (Febrina & Ayuna 2019), Fe has the property of being easily oxidized by oxygen (Miarti 2023). According to Said (2018), the Fe compound can oxidize continuously, an ionic reaction will occur in which Fe will separate into Fe^{2+} molecules. These compounds will react with oxygen and water to form valence 3 compounds such as $\text{Fe}(\text{OH})_3$ which tend to be insoluble in water. The equation of oxygen reaction in degrading dissolved Fe parameters is:



Based on the equation, it can be said that to oxidize 1 mg.L^{-1} Fe concentration will require $0.14 \text{ mg.L}^{-1} \text{ O}_2$.

According to Mahmud et al. (2024), the high Fe removal efficiency is also supported by the venturi aerator, this is reinforced by the ability of the venturi aerator to

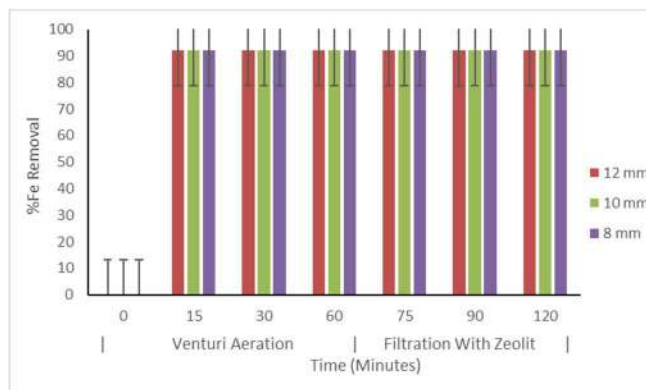


Fig. 1: Fe Removal efficiency based on diameter aerator hole variation.

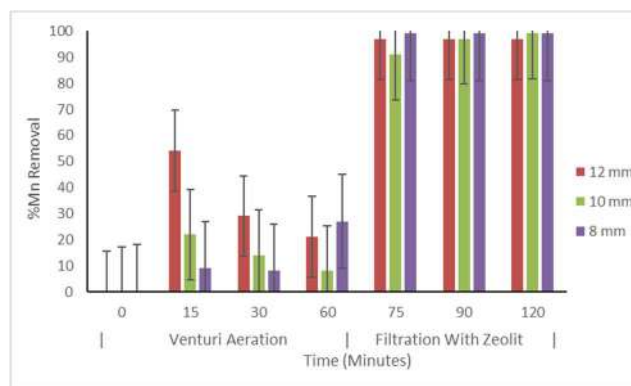


Fig. 2: Mn removal efficiency based on diameter aerator hole variation.

produce bubbles with an average diameter below 100 μm , thus providing a high interface area for mass transfer. Consequently, during operation, the venturi aerator generates higher kinetic energy from the liquid and gas streams creating intense mixing and turbulence leading to high diffusion of air which includes oxygen.

Unlike the parameter Fe, parameter Mn in water is more difficult to oxidize by oxygen. This is because Mn has owing to a higher redox potential (Cheng 2020). The Mn oxidation rate is lower than iron at a pH below 9.5 (Applebaum 1947). The result of the experiment showed that the observed water pH was at a value of 7.1 to 7.8, which resulted in a decrease in Mn concentration during the aeration process that was not efficient. The ability of venturi aerators and zeolite filters to remove Mn concentration in water is shown in Fig. 2.

As shown in Fig. 2, the percent removal of Mn concentration in each variant of aerator hole diameter and each sampling time vary. After the aeration process, the highest Mn removal efficiency is 54% (from Mn concentration 1.84 mg.L^{-1} to 0.85 mg.L^{-1}) when using a venturi aerator with a hole diameter of 12 mm and at

15 min sampling time, while the lowest Mn removal efficiency is 8% (from Mn concentration 1.84 mg.L^{-1} to 1.69 mg.L^{-1}) when using a venturi aerator with a hole diameter of 8 mm (30 min sampling time) and 10 mm (60 min sampling time).

During the filtration process with zeolite, Fig. 2 showed that the percent Mn removal increased dramatically. The percent removal of Mn concentration in each variant of aerator hole diameter and each sampling time shows varied. After the filtration process, the highest Mn removal efficiency is 99% (from Mn concentration 1.84 mg.L^{-1} to 0.014 mg.L^{-1}) when using a venturi aerator with a hole diameter of 12 mm and 8 mm, it is at 15 min; 30 min; and 60 min sampling time, while the lowest Mn removal efficiency is 91% (from Mn concentration 1.84 mg.L^{-1} to 0.16 mg.L^{-1}) when using a venturi aerator with a hole diameter of 8 mm (15 min sampling time).

The results of the efficient aeration for removing Fe concentration need attention because of the filtration process with zeolite. The zeolite filtration mechanism relies on ion exchange and adsorption to heavy metals such as Mn and

Fe (Annisah & Subhan 2020). The filtration results showed that Mn removal efficiency is up to 99% because of zeolite's ability that adsorb Mn at neutral pH levels (Febrina & Ayuna 2019). The aeration stage can make higher DO concentration because of the oxidation of Mn and then Mn forming Mn(IV) oxides that are easily removed from filtration (Cheng 2020). Several factors can affect the filtration performance such as zeolite grain size, contact time, and pH (Fuad et al. 2018). The aeration stage is significant for increasing DO concentration and provides conditions for Mn oxidation, it can make the efficiency of aeration and filtration the same, despite their operational principles.

Fig. 2 shows that the efficiency of reducing Mn levels using aeration tends to be less effective, where the optimal reduction in Mn content in aeration with an aeration hole diameter of 12 mm in the first 15 min is 54%, but there is a decrease in the following min. At the 8 mm diameter variation, the most efficient aeration was obtained at 60 min with an efficiency of 27% and the remaining Mn content of 1.35 mg.L^{-1} . The efficiency achieved by this 8 mm diameter variation tends to be stable where the decrease in Mn levels increases over time. The air vents in the aerator venturi have an important role in the aeration process (Yadav et al. 2019). The larger size of the hole allows more air to enter the reactor. However, on the other hand, larger air holes can lead to the formation of large bubbles, resulting in less surface area of air in contact with water than smaller bubbles (Wijayanti 2008).

The decrease in Mn levels of the aeration process affects the filtration performance. The combination of a venturi aerator with 8 mm air holes with zeolite has a removal efficiency of 99%. This occurs because the process of decreasing Mn levels that occurs during the aeration process increases over time. At 60 min of aeration, the 8 mm diameter variation has a higher effectiveness than other variations. In the 12 mm and 10 mm variations, the process of decreasing Mn levels tends to decrease over time, which can lead to higher zeolite adsorption loads.

The venturi aerator and zeolite filtration can be combined to offer a sustainable water treatment. Compared to conventional air diffusers, the venturi aerator required low energy input same as with the filtration medium. It is widely available and cost-effective (Mahmud et al. 2024). The study found that Venturi aeration requires lower energy input compared to conventional air diffusers, while zeolite, as a filtration medium, is cost-effective and widely available (Mahmud et al. 2024). This study's research can contribute to water management policies for regions with groundwater contamination by providing knowledge about energy-efficient, practical, and other measurable treatment solutions (Kalvani et al. 2021). Adoption technologies can increase

access to drinking water otherwise reducing operational costs for facilities of water treatment.

This study is made up of a unique combination of zeolite filtration and venturi aeration, which can utilize the performance of both oxygenation and adsorption mechanisms to reduce heavy metal concentration. The ability of the venturi aerator to generate small bubbles will increase the surface area for the interaction of liquid-gas, and it can make more efficient oxygen transfer (Yadav et al. 2019). Compared to conventional methods, this research showed using unit venturi aeration before unit filtration will improve zeolite's adsorption efficiency by up to 99% Mn removal. The aerator hole diameters are a novelty. It showed that air inlet size affects dissolved oxygen levels and filtration outcomes. Future research could investigate cost optimization strategies for large-scale that include hybrid treatment systems and automated aeration controls or automated aeration controls (Sudiarto et al. 2021). Testing the method under varying groundwater conditions potential to do, such as different metal concentrations or pH ranges. It is for evaluating the scalability and reliability of groundwater conditions.

CONCLUSIONS

Based on the results of research on the combination of venturi system aeration and adsorption in degrading Fe and Mn parameters, it is concluded that the larger the air hole, the efficiency of dissolved oxygen concentration increases. The most effective Mn concentration reduction also occurred in the largest air hole diameter variation (12 mm) with the largest percentage reduction of 54% in 15 min of aeration time. The concentration of Fe can be removed up to 99% since the beginning of processing because Fe is easy to oxidize.

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