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Tackling Marine Plastic Pollution Through Source-To-Sea Approach and Circular Economy

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ABSTRACT

Marine litter and associated marine pollution are becoming a complex global environmental hazard these days. Among the different faces of marine pollution, by far the largest and probably the most dangerous part is marine plastic litter. Plastic litter can be found in almost every marine environment in the world, including deep ocean beds and frozen polar ice. Unless new sustainable methods of plastic production and waste management are encouraged, marine plastic pollution will continue to pose a severe threat to the natural ecosystems of the world. In this paper, the status of marine plastic litter is reviewed using a DPSIR framework, and it is found that significant changes in the way we live and consume are needed to prevent it. A framework that combines the source-to-sea approach and circular economy is introduced as a possible solution to eliminate plastic waste from the environment as well as from the economy.

INTRODUCTION

It was during the beginning of commercial plastic production in the 1970s, scientists first recognized plastic pollution in marine environments. The first reports which pointed out the threats of plastic litter on marine life such as the presence of polystyrene spherules in the marine waters of southern New England (Carpenter et al. 1972) and the entanglement of northern fur seals on trawl nets (Fowler 1987) did not gain enough attention from the research community at the time (Andrady 2011, Ryan 2015). Within just a few decades since then, plastic debris has made its entry to almost every part of the environment. It is estimated that more than 5.25 trillion plastic particles with a weight of around 268,940 tons are currently floating in the world's oceans (Eriksen et al. 2014). The presence of plastics is not limited to places where there is direct human contact but, it has been found even in the world's largest and only remaining pristine regions, like the Arctic and the Antarctic. Besides, plastic litter in these remote areas reflects the pollution levels of the nearby waters (Bergmann et al. 2013, Eriksson et al. 2013). Similarly, Henderson Island in the South Pacific, regarded as the most remote island of the world, once had an average of 671.6 debris items per square meter on its beaches, which was the highest density of plastic litter reported in the world (Lavers & Bond 2017).

Even though there is no direct relation between litter density inland and seafloor, research shows that materials that are commonly found inland are also common on the seafloor. Therefore, knowing the common waste materials generated inland allows preventing its mismanagement from the production stage and thereby to eliminate marine plastic litter (Roman et al. 2020). However, the majority of current methods to reduce marine plastic problems focus either on one stage of the problem or on one single sector, which is responsible for marine plastic pollution. These strategies often end up as unsustainable and less effective due to the lack of understanding about how natural ecosystems are linked and also due to limited cooperation within the governing system. Therefore, measures that can address the root causes of the issue by connecting and engaging all sectors and stakeholders (formal and informal, private, and public) related to the problem are essential to make a genuine change, and it was the driver of this paper (Mathews & Stretz 2019). Therefore, the primary purpose of this paper is to undertake a systematic assessment of the status of marine plastic litter in the world's oceans, which is done using a DPSIR framework and to develop a framework that can prevent plastic from entering the natural environment.

DPSIR FRAMEWORK FOR MARINE PLASTICS

Drivers and Pressures

'Drivers' of marine plastic pollution are initiated through different socio-economic changes happening in the society, such as increasing population, urbanization, economic

growth, and behavioural changes. To fulfil the basic needs like food, living space, energy, and recreation of these increasing populations, governments are forced to develop new economic initiatives to improve their country's productivity. These activities can lead to the extraction of more and more natural resources, including ocean resources like oil and fisheries. Also, these drivers can cause unsustainable consumption and production patterns across all sectors of society and can generate a vast amount of mismanaged waste (Ecologic.eu 2018, Grida.no 2016, UNEP 2016). This massive rise in natural resource consumption, which is both unsustainable and inefficient, creates many 'pressures' in the environment, such as environmental pollution, declining biodiversity, and exploitation of ocean resources (Atkins et al. 2011). For example, the discharge of pesticides and nutrients from agricultural runoff or improperly treated sewage can harm small marine species like phytoplankton and seagrass. This can lead to the growth of dead zones (low oxygen or hypoxic areas) in oceans where the majority of marine life cannot survive. According to UNEP, currently, around 500 dead zones spreading across 2,45,000 square kilometres are present in the world's oceans. Moreover, plastic pollution made several changes in the aquatic food webs like the rapid multiplication of bacteria, a decline in fish numbers, and the spreading of diseases (Unesco.org 2017).

State

Studies show that coastal environments across the world are in a state of deterioration due to changing environmental situations over the past few decades. Plastic debris can be found almost everywhere including beaches, surface waters, throughout the water column and even with benthos as shown in Fig. 1 (Wright et al. 2013) and can make changes in the 'state' of the oceans such as declination of water quality and ocean warming (Atkins et al. 2011).

Plastics in Water Bodies

In marine waters, plastic debris is distributed among five different sections: coastline, upper or surface ocean, the main water column, the seabed, and the biota. Transfer of

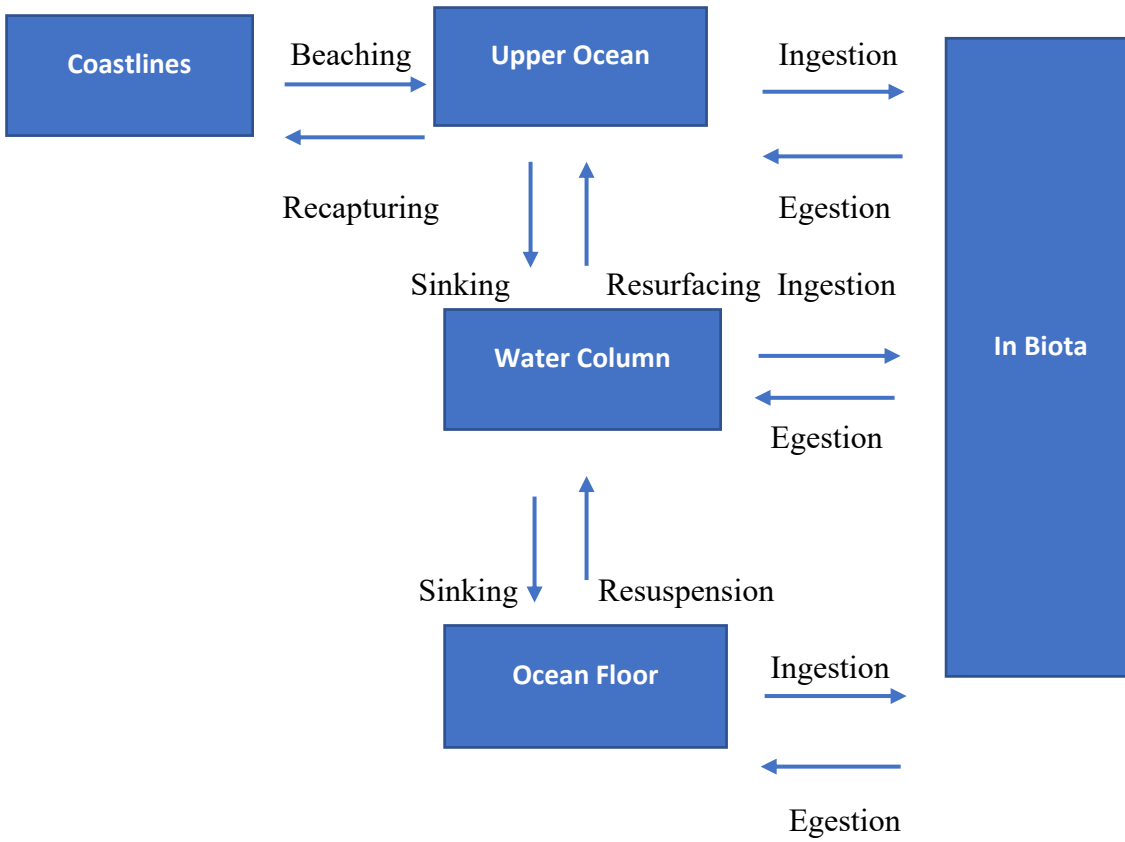


Fig. 1: Distribution of plastics in the marine environment (GESAMP 2016).

plastics between each of these compartments can occur because of natural activities like wind, rainfall, wave action, presence of land and sea-based sources of pollution as well as the exposure of the coastline. Characteristics like density, size, and shape of particles also play crucial roles in the spreading of plastics through marine environments. Even though plastics are usually buoyant, in a marine context, plastics can be positively, neutrally, or negatively buoyant. The positively buoyant ones accumulate near ocean surfaces while negatively buoyant sink to the seabed as they are denser than water. Besides, attachment and growth of fouling and sessile organisms can also cause plastics to sink, extending debris presence throughout the water column (UNEP 2016, Bonanno & Orlando-Bonaca 2018). The major plastic polymers present in oceans are listed in Table 1.

Plastics in Beaches, Shorelines and Sediments

Plastic debris found in the shores is usually a mixture of particles from local sources and those that have been reached the place by ocean currents and wind. Tourism activities and other beach use, influence the type and amount of plastic litter found on a particular beach or shoreline. In addition, places with high concentrations of floating debris called 'hot spots' can be found in marine waters, which is adjacent to high coastal populations with improper waste management methods. Natural factors like rainfall, storm, and tsunamis can also lead to the unexpected accumulation of massive amounts of plastic debris in beaches and shorelines (GESAMP 2016, UNEP 2016, Li et al. 2016).

Debris can also find in sediments of shallow marine environments as well as in ocean depths. In shallow environments, debris is accumulated in sediments and can resuspend by the action of tides, waves and ocean currents, and as a result, they can distribute throughout the water column, whereas in the ocean depths sediments can be a permanent sink for plastic debris (Bonanno & Orlando-Bonaca 2018, UNEP 2016).

Impact

The potential impacts of marine plastics can be mainly classified into ecological, economic, and social (Gall & Thompson 2015, Unesco.org 2017) and are briefly explained below.

Ecological Impacts

Ingestion and entanglement: According to studies, ingestion and entanglement already affected more than 800 marine species globally, including around one-third of the marine turtles and nearly half of all seabirds (CSIRO 2019). Among them, seabirds seem to be particularly susceptible as they confuse tiny particles of plastics with prey and swallow them. The presence of microplastics in the guts of many dead sea birds such as Northern Fulmar and Laysan Albatross had exceeded the environmental quality put forward by OSPAR and reveals the increasing levels of plastic ingestion among birds. Pieces of evidence also show that these polluted marine invertebrates can transfer microparticles to higher predators such as great skua by acting as a pathway (Trevail et al. 2015, UNEP 2016, Wright et al. 2013). Ingestion of plastic debris also facilitates the transport of toxic chemicals into marine species either through the release of chemicals present in plastics from manufacturing processes such as plasticizers or through the accumulation and following release of pollutants such as PBTs and POPs from surrounding seawater (Lusher et al. 2013, UNEP 2016).

Accumulation of microplastic particles in marine animals can cause many health problems such as blockages in the digestive system, starvation, and physical deterioration, which eventually results into a lack of reproductive fitness, weakness in feeding ability, reduced predator avoidance, and even drowning (Wright et al. 2013). In addition to the internal accumulation of debris, external adsorption of plastic particles specifically nanoparticles may obstruct algal photosynthesis by physically blocking air and sunlight while

Table 1: Major plastic polymers present in oceans, their density and field of application (Emmerik & Schwarz 2019).

Polymer	Density (g/cm^3)		Major field of application
	Min	Max	
Polyethylene	0.91	0.97	Packaging
Polypropylene	0.9	0.91	Many applications mainly packaging
Polyester	1.24	2.3	Textiles
Polyethylene terephthalate	1.37	1.45	Packaging
Polystyrene	1.01	1.04	Packaging
Expanded polystyrene	0.016	0.640	Food packaging, construction material
Polyvinyl chloride	1.16	1.58	Building and construction
Polyamide (Nylon)	1.02	1.05	Automotive, textiles

increasing reactive oxygen species production (Bhattacharya et al. 2010, Prata et al. 2019)

At the same time, entanglement in plastic debris mainly affects higher taxonomic groups in different extents, causing chronic and acute injuries or sometimes death (UNEP 2016). Marine animals like dolphins, seals, sea lions, sea turtles, and even whales and sharks can become trapped in marine debris as they swim or while on the beach (NOAA Fisheries 2017). Entanglement in ALDFG or ghost fishing significantly reduce the animal's mobility and can also cause serious physical injuries and infections as the gear and other pieces of equipment cut into their body. Entangled animals may also become less capable of avoiding other obstacles like they usually would, thereby increasing the risk of accidents like vessel strikes. If the gear and nets are too large or heavy, it may result in sudden drowning of smaller marine animals while larger animals can, in most cases, drag or remove the gear or parts of it; however, they do face risks such as infection and exhaustion. Many studies recognized entanglement as the principal cause of human-created mortality in marine species especially among whale species like humpback whales, right whales, and gray whales (Australia State of the Environment Report 2017, NOAA Fisheries 2017)

Habitat loss and introduction of invasive species: Plastic debris can cause scouring, smothering, and breaking of marine habitats, which are the foundation of marine ecosystems and are vital for the existence of all marine species (Impacts | OR&R's Marine Debris Program 2020). Among these, coral reefs are most vulnerable to damage by plastic debris items such as lost fishing gears and ALDFG as these can get hooked on the branches of reefs and can leave significant scars on them by breaking or scratching. Besides, the movement of these nets and ropes can cause abrasion, fragmentation, dislodging, direct suffocation and shading of fouled species colonies which in turn make the corals prone to diseases by facilitating colonization of pathogens (Lamb et al. 2018, The NOAA Marine Debris Program 2018, UNEP 2016, Vegter et al. 2014).

Plastic debris can also accumulate on mangrove forests as they act as a partial sink for plastics (UNEP 2016). Besides, these materials can provide new safe habitats for the growth and survival of foreign species, which can eventually become invasive and aggressive and can harm the local flora and fauna (Gall & Thompson 2015). Moreover, plastic materials can also lower the numbers of invertebrates in marine waters by significantly decreasing the levels of oxygen in sediments (Ec.europa.eu 2019)

Rafting: In the context of marine plastic pollution, rafting can be defined as the transport of organisms attached to debris, which acts as a new artificial substrate in addition to natural materials. This macro and microplastic debris with much higher longevity can host an extensive collection of non-indigenous species like crustaceans, bryozoan, and cephalopods by creating novel habitats. Once colonized by marine biota, these floating debris act as a potential transport vector and facilitate the spreading of the associated organisms at different spatial scales. These debris items usually travel very long distances and reach shorelines where it is foreign, thus disturbing the local fauna and flora (Kiessling et al. 2015, Impacts | OR&R's Marine Debris Program 2020, UNEP 2016). Sometimes they can even reach gyres and combine with eddy currents that can carry these strange hitchhikers across the oceans. Various studies have found that the ability of biota to travel has been doubled with the presence of marine litter, making marine debris a reservoir of potential biological invasions (Garcia-Vazquez et al. 2018).

Socio-economic Impacts

Human health and food safety: Seafood is the major source of protein for around two-thirds of the global population, and contaminating these food sources leads to many chronic and acute health issues. Tiny fragments of plastics can reach human bodies through the consumption of seafood, particularly the whole animal foods like sea cucumbers and sea urchins and filter-feeders such as oysters and mussels as plastic often accumulate in the digestive tracts of these organisms (Murano et al. 2020, Smith et al. 2018). Besides, a wide variety of commercial species like shrimps, shellfish, and other smaller fish types are also found to be polluted with microplastics (UNEP 2016). Also, many studies from different countries found the presence of microplastics in sea salt samples with few studies showed a rate of 94% of the total sample of salt products tested contained microplastics. Furthermore, there is evidence for the presence of tiny fragments of plastics in beer and tap water which proves that plastics can reach the human body through the food chain (IUCN n.d., Iñiguez et al. 2017, Yang et al. 2015, Lee et al. 2019).

Usually, smaller ones like nano plastics and microplastics cause more significant damage to health due to its smaller size, the larger surface to volume ratio, and high reactivity. The most common health impacts of these ingested plastic fragments include inflammatory issues, obesity, cancer, and alteration in chromosomes, which can cause infertility (Smith et al. 2018, Sharma & Chatterjee 2017). Furthermore, nanoparticles can penetrate all human organs, including the placenta and brain, and can cause severe health issues. Endocrine disruption chemicals which have been used extensively on the plastic production processes are another concern (UNEP 2016).

In addition, floating debris possess navigation hazards to ships, boats as well as to scuba divers and surfers. Incidents of injuries and drowning of scuba divers due to entanglement in discarded fishing nets and ropes have found several times in the history of marine pollution (UNEP 2001). Moreover, collisions with partially submerged or floating objects like plastic shipping containers can lead to failures and collapse of ships and boats. In 2005, the USA coastguard reported 269 accidents, which caused 116 injuries and 15 deaths because of collisions with submerged objects in oceans (UNEP 2016).

Loss of income: Marine plastics are an eyesore, detrimental to the beauty and attractiveness of marine environments, thereby negatively affecting maritime activities like recreation, tourism, and shipping. Debris can negatively influence recreational activities like snorkelling and diving, as well as pollutes propellers and jet intakes of recreational boaters and affect recreational fishers by polluting catch or restricted catch. As a result, tourists tend to avoid polluted beaches, and this reduction in visitor numbers can significantly reduce revenues from the tourism industry resulting in a loss of income and reduction in local job opportunities. These effects can be quite severe in places where local economies are dependent too much on the tourism sector as in Hawaii, Bali, and the Maldives. In addition, loads of marine plastics in oceans can significantly reduce the benefits provided by various marine ecosystem services. A survey conducted in 2011 estimated a reduction of 1-5% of marine ecosystem services, which caused a loss of 500-2500 billion USD to the economy due to loss of benefits obtained from these services (Beaumont et al. 2019). Another concerning issue is the clean-up costs of this accumulated debris. Beach clean-up programs and vessel repairing alone cause an annual loss of around one billion dollars in the Asia Pacific (Cbd.int 2016, UNEP 2016) while some power companies in Europe spent more than 75000 USD annually to clear debris from their water intake screens (Vegter et al. 2014). In the fisheries industry, plastic contamination lowers the value of seafood, along with increasing the time and expenses required to clean and repair fishing nets (UNEP 2016).

Responses

Marine plastic pollution can be reduced significantly with proper measures and laws in place and its strict and careful implementation. This can be done in three main ways: a) restricting the use of conventional plastics through political level initiatives and actions such as imposing bans on single-use plastics and forming efficient waste management systems; b) provide economic benefits for reducing traditional plastic usage along with incentives to promote alternative materials; c) facilitate more research on the production and consumption of alternative materials. All these measures along with proper awareness and education can make a real difference in making marine plastic pollution under control (Filho et al. 2019)

Addressing the issues of marine plastic debris is a significant priority in recent years as the awareness about the issue and the political will to tackle this has increased. Accumulation of plastic debris in coastal environments and habitats has been pointed out as a huge waste problem by the UN Environment Assembly (Ec.europa.eu 2019). Many programs and tools are developed and initiated globally and regionally to mitigate these problems. In 2005, the United Nations General Assembly identified the problem of marine litter in its Resolution A/60/L.22- oceans and Laws of the Sea and called for global, national, and regional actions to tackle it. Besides, Goal 14 of the Sustainable Development Goals is aimed at preventing marine pollution and has specific targets, and the important ones are shown in the Fig. 2 (UNEP n.d.).

To achieve these targets following initiatives are taken

- 109 countries committed to a Global Programme of Action for the Protection of the Marine Environment from Land-Based Activities
- More than 143 countries have joined 18 Regional Seas Conventions and Action Plans
- 30 countries already joined the #CleanSeas campaign on marine litter

Another important action taken to prevent marine plastics is the Clean Seas program of UNEP launched in February 2017. This program collaborates private sectors, government, civil societies, and the public to tackle the root causes of

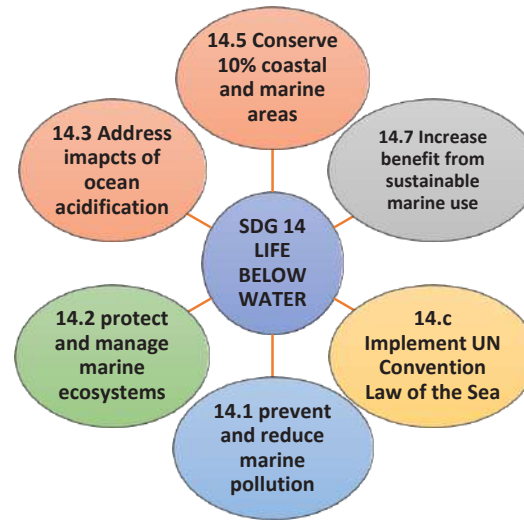


Fig. 2: Main targets of SDG 14 (UNEP n.d.).

marine litter pollution, such as the production and use of single-use or non-recoverable plastic products (Clean Seas 2019).

Some of the other major global and regional initiatives and frameworks adopted to tackle marine pollution are listed below.

International Agreements

- International Convention for the Prevention of Pollution from Ships (MARPOL), 1973
- UN Convention on the Law of the Sea (UNCLOS), 1982
- Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal, 1989
- London Dumping Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, 1996
- UN General Assembly Oceans and the Law of the Sea, 2005
- UNEP/IOC Guidelines on the Survey and Monitoring of Marine Litter, 2009
- Honolulu Strategy, March 2011

Regional Agreements

- Barcelona Convention, 1976
- East Asian Seas Action Plan (COBSEA), 1981
- Cartagena Convention, 1983
- Northwest Pacific Action Plan (NOWPAP), 1994
- Nairobi Convention,1996
- Helsinki Convention
- Strategic Approach to International Chemicals Management (SAICM), 2006
- European Marine Strategy Framework Directive, 2008
- OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic (Lloyd-Smith & Immig 2018, Tiquio et al. 2017, UNEP 2016, Un.org 2016)

In addition to this, most recently in G7 and G20 summits and in the Annual Meeting of World Economic Forum at Davos 2020, the member nations made many commitments to reduce plastic usage and to prevent ocean pollutants.

SOURCE-TO-SEA APPROACH WITH CIRCULAR ECONOMY

Prevention and management of plastic litter require explicit knowledge about its sources, causes, and its impacts on life and the environment. By understanding the social and economic effects of ocean plastic pollution from its sources to the final point, policymakers can develop deeper and systemic changes that reach beyond traditional boundaries by linking land, freshwater, and marine systems together. Frameworks that include river basins are essential to identify the hotspots of leakage, which is essential to prioritize locations and types of interventions needed to prevent it (SIWI 2019). A Source-to-Sea approach combined with Circular Economy principles, bring together the upstream and downstream stages of marine plastic pollution and develop strategies to solve the issue from the base level. River basins play the central part of this framework, as keeping plastics away from waterways can prevent a vast amount of plastic entering the oceans. This framework, which can combine with existing policies and strategies, also addresses behavioural changes from individual to a global scale by creating conditions to facilitate this change (Fig. 3) (Mathews & Stretz 2019).

The Source-to-Sea Approach

Major Definitions

Source-to-Sea System: A source-to-sea system, as shown in the Fig. 4, mainly consists of the following segments:

- the area of land which is drained by a river
- the river basin
- linked aquifers and downstream receivers which includes estuaries and deltas
- coastlines and nearshore waters
- the adjoining sea and the continental shelf
- the open ocean

On a larger scale, a source-to-sea system may also include a sea and its complete drainage area, which may consist of more than one river basin (Mathews & Stretz 2019).

Source-to-Sea Key Flows

The source-to-sea system is connected by six main flows from land systems to open oceans which are shown in Fig. 5. Changes happening in each key flow can alter the state of other segments as well as the first five key flows join to shape the ecosystem services that the source-to-sea system delivers (Mathews & Stretz 2019, Granit et al. 2017).

Six steps in the source-to-sea approach: This six major steps in the approach (Fig. 3) are briefly explained below.

Step 1: Characterize

The first step is to recognize the different sources, types,

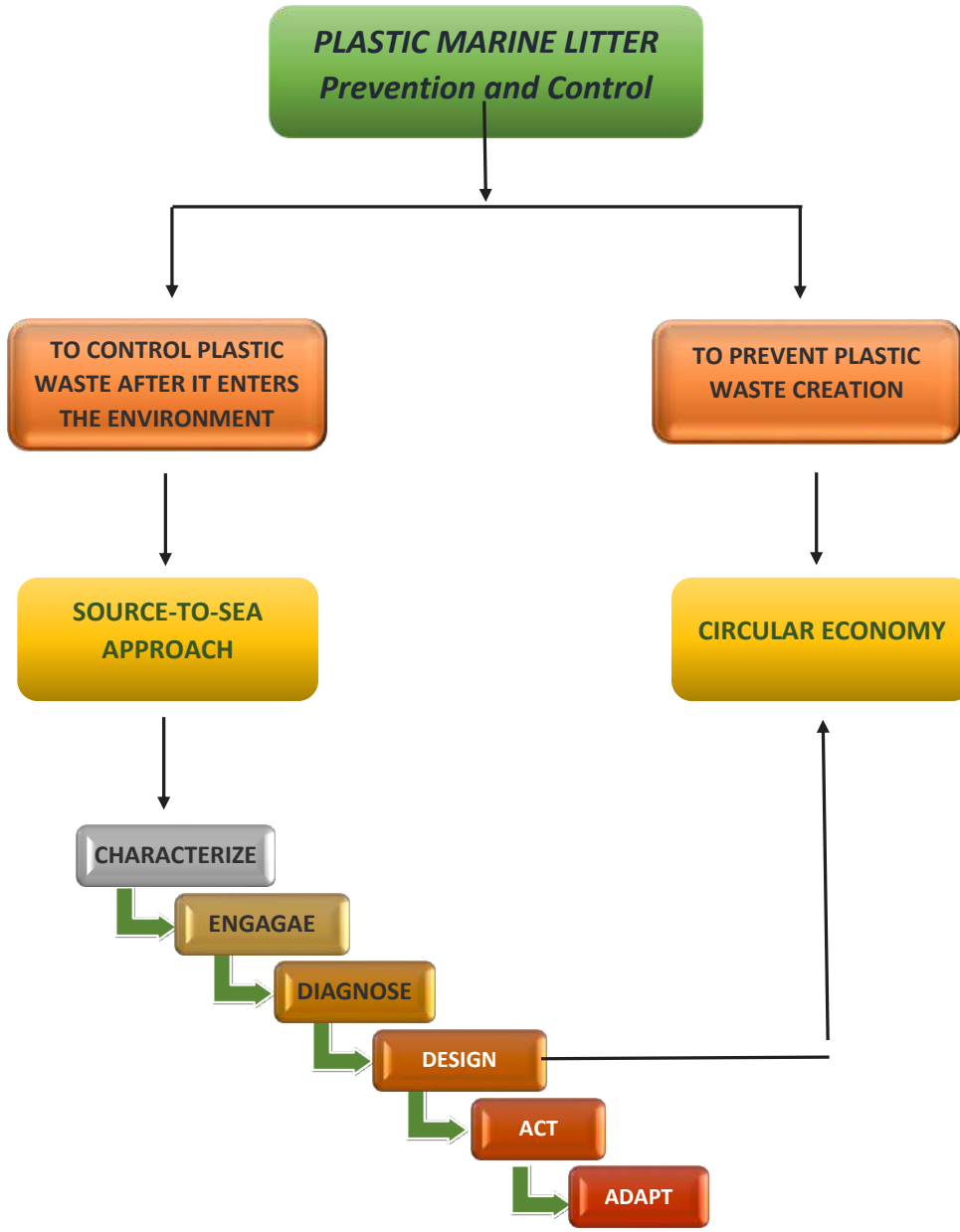


Fig. 3: The proposed framework (Mathews & Stretz 2019).

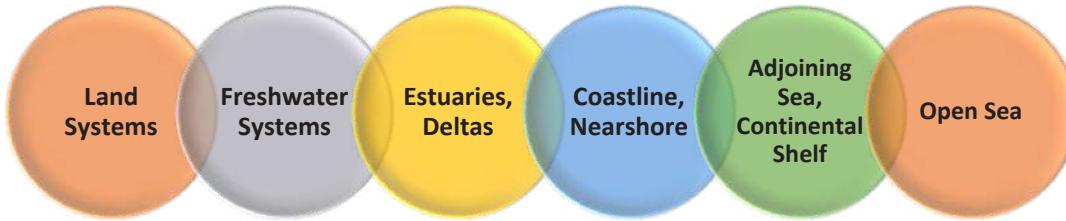


Fig. 4: A source-to-sea system (Mathews & Stretz 2019).

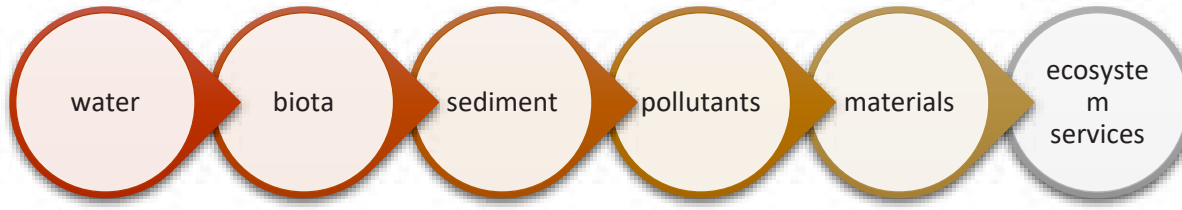


Fig. 5: Six main key flows in the source-to-sea system (Mathews & Stretz 2019).

behaviour, and impacts of plastic waste in freshwater resources as well as in marine environments. As each source requires various measures to adopt and involve different sectors and governance structures, understanding the sources and its routes early on is vital for the successful implementation of this approach. Therefore, understanding the key flows and selecting priority flows which need action is the first phase of this step. Flows can be prioritized by analysing its character in the system, examining how they can be changed, and finding the possible impacts of these changes on the source-to-sea system. Once the key flows to be addressed are prioritized, the next step is to define the system boundaries of the project, which is essential for the following stages. Depending on the geographical locations of sources and their impacts, the geographical scope of a system can range from the local community to the global level (Mathews et al. 2019).

Step 2: Engage

In this step, the main stakeholders related to the project from local to global levels are identified, and a plan is made to engage and build partnerships with them. These partnerships with various stakeholders can help in developing a 'solution space' which brings together a wide range of participants who can provide ideas, solutions, political support, and financial help to achieve the goal, which is to prevent plastic litter. This stakeholder list and engagement plan may contain groups that are not previously considered. For instance, an ocean-focused pollution project may initially consider only ocean clean-ups and ALDFGs, but, in a source-to-sea approach, this same project take account of all upstream factors such as land-based sources as well as all sectors along with the subsequent transport of pollutants through waterways and finally into oceans (Mathews & Stretz 2019, Mathews et al. 2019).

In this framework, there are five major types of stakeholder groups, as described in the Table 2. Bringing together all these five groups from across the source-to-sea system can show paths to new ideas, opportunities, and solutions to tackle marine plastic pollution.

Step 3: Diagnose

In this step, the current system of governance for the prevention of marine plastic litter such as agreements, laws, regulations, policies, plans, procedures and the institutions (community-level groups, local, national, regional or global institutions) that deliver them are examined in order to find drawbacks and limitations. Gaps in the existing system are figured out, and the failures are analysed carefully. To prevent marine plastic pollution, a governance system that acts on the individual, local, national, and global levels as well as address different stages of plastic, from production, to retail, consumption, collection, and disposal are essential. The importance of improving product designs by changing towards a circular economy can be understood in this step (Mathews & Stretz 2019).

Table 2: Types of stakeholders (Mathews & Stretz 2019).

Stakeholder Type	Role
Primary Stakeholders	• Those who are affected by plastic waste and will benefit from its prevention • E.g., fishermen and communities adjacent to waterways
Targeted Stakeholders	• Those who are responsible for plastic leakage. • Includes producers of raw materials, producers, retailers, and consumers of plastic products and materials; waste managers and recyclers of plastic materials.
Enabling Stakeholders	• Individuals, institutions, and organizations that enable opportunities for behavioural changes to occur and help to last over time.
Supporting Stakeholders	• Financiers or development partners • Provide funding, strengthen political will and advise for change
External Stakeholders	• Those who are not included in the system boundary but have common interests and their advocacy can increase attention and awareness globally • E.g., concerned persons and private organizations.

Step 4: Design

Here, a program or a project is designed for the desired outcome, which is preventing plastic litter. For the effective implementation of the project, a theory of change that clearly defines intermediate steps to reach the goals needs to be developed. This theory of change describes the observed causes of the issue and predicted relationships between project or program activities as well as the desired outcomes for the project. During the development of the theory of change, it is important to consider four orders of the outcome, which can help to arrange the design of new strategies.

- *First-order Outcomes*: Enabling conditions that assist behavioural change of the targeted stakeholders found in step 2. This includes policies, laws, regulations, and financial support for preventing plastic pollution, to promote a strong market for recycled products as well as to develop a circular economy for plastics.
- *Second-Order Outcomes*: Required changes identified in step 3 to achieve benefits for the primary stakeholders as well as to accomplish other desired outcomes. This includes a reduction in plastic consumption, improved waste segregation and disposal, and increased demand for repaired and recyclable products.
- *Third Order Outcomes*: Denotes the improvements and changes happening in the state of the source-to-sea system because of preventing waste leakage and thereby restoring priority flows that identified in Step 1. This includes significant stress reduction in the riverine and marine environments as well as inland systems, which result in improved social, economic, and environmental conditions.
- *Fourth Order Outcomes*: Represents the long-term effects such as sustainable development and green growth achieved through the effective application of the intervention strategies (Mathews et al. 2019' Mathews & Stretz 2019).

A theory of change that gives attention to all of these order of outcomes can connect all the enabling conditions needed, such as finance, infrastructure, and governance with the behaviours and practices required to change with the long term effect, which is to prevent plastic leakage into the river basins. It is easier to develop interventions by analysing these links (Mathews & Stretz 2019).

The idea of a circular economy can link into the source-to-sea approach at this stage. When the source-to-sea approach pays particular attention to control plastic waste once it has already created and entered the natural environments, a circular economy, on the other hand, aims at preventing the generation of plastic waste itself. Linking these two approaches presents the greatest opportunity to eliminate marine plastic pollution from its root causes (Mathews & Stretz 2019). The concept and applications of the circular economy are explained in detail in the last section.

Step 5: Act

The financing strategy and the implementation plan for the project are developed in this stage. Hence, this step is about taking action by funding and implementing source-to-sea strategies and putting the project into practice. The significant outcomes expected from this step are developing a funding and implementation plan with the following factors:

- Public or private sources through different mechanisms.
- Linking source-to-sea priorities into public sector action plans and budgets to secure long-term financing
- Actions were taken to develop multi-sectoral engagement in the project to expand opportunities
- Finding the crucial sectors to focus on
- A risk assessment and mitigation plan
- A timeline for the implementation of the plan (Mathews et al. 2019).

Step 6: Adapt

The Source-to-Sea and Circular Economy framework (Fig. 6) is a cyclical process, and therefore, the final stage of this framework is continuous monitoring and assessment of the plan, which circles back to Step 1. By continuously evaluating outcomes of the project, the engaged stakeholders can identify any failures and can make needed changes or can find new priority flows to tackle the problem. This adaptive management cycle is crucial for the success of the project, especially in a source-to-sea approach, which includes different sectors and stakeholders. Without proper monitoring, the effect of actions in one segment of the system on another might be difficult to understand fully.

Circular Economy

Still now, the world's ever-growing economy mainly follows the traditional linear chain of the "take-make-use-dispose" system, which possesses substantial environmental and economic drawbacks (Ellen MacArthur Foundation 2017). In a linear economy, every product is bound to reach its end of life and involves huge resource losses and waste production (Ellen MacArthur Foundation 2013). However, during the past few years, there is an increasing recognition among industries and countries that resource efficiency and its availability are crucial for future economic competitiveness and resilience, and this resulted into a fundamental rethinking

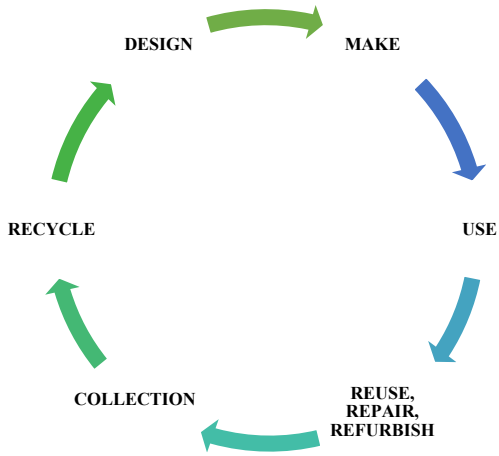


Fig. 6: A simple circular economy model (European Commission 2014).

on the current ways of resource use and methods of production (Preston 2012).

The concept of Circular Economy (CE), in which new products are either made from materials that are sourced from old products or designed in a way that each of its parts can be repaired, recycled or upgraded resulting in zero residual waste can be identified as a solution to this fast-growing resource crunch (Winans et al. 2017). By using the so-called 'wastes' as resources for other products and processes, a CE model produces nothing to throw away, instead give rise to plenty of resources to use continuously in different ways (News European Parliament 2018)

By designing out waste, CE put forward a complete change in the way things are made until now. As opposed to that of a linear model, sustainability and closed-loop thinking should be the key areas for industries and business models which plan to follow a CE, and its implementation needs strong cooperation between consumers, manufactures, businesses, and governments (Barra & Leonard 2018). Table 3 shows six major action areas for companies and governments to shift to a CE, known as the ReSOLVE framework.

By eliminating waste from the industrial chain through recycling, repairing, and reusing, the value of products and resources are maintained in the economy at its highest utility, thereby maximizing material service per resource input (Barra & Leonard 2018). This, in turn, pushes waste up to the waste management hierarchy and leads to improved resource efficiency and security by reducing the extraction and import of new raw materials (ESA 2017). Hence, circular economy principles have the potential to decouple rising economic development from growth in resource consumption. According to studies, transitioning towards a

Table 3: The ReSOLVE framework (Ellen MacArthur Foundation 2015).

The ReSOLVE Framework	
Regenerate	• Use renewable energy sources and materials • Retrieve, maintain and restore damaged ecosystems • Give back retrieved biological resources to nature
Share	• Share goods and properties • Reuse or buy second-hand items • Increase the life span of materials through maintenance, upgrading and designing for durability
Optimise	• Improve product performance and efficiency • Eliminate waste from production processes and from supply chain
Loop	• Remanufacture or recycle products and its parts • Promote anaerobic digestion • Extract biochemicals from organic waste
Virtualise	• Dematerialize directly (e.g., travel, books, DVDs) • Dematerialise indirectly (e.g., online shopping)
Exchange	• Use new processes and technologies like 3D printing • Choose new products and services such as multimodal transport

circular economy can reduce new material consumption by more than 50% by 2050 (News European Parliament 2018, Romero-Hernández & Romero 2018). In addition, CE also bring benefits such as boosting economic growth, stimulate innovation, create jobs, increase competitiveness as well as significantly reduce environmental problems including carbon emissions (Ellen MacArthur Foundation 2015, News European Parliament 2018)

Circular Economy and Marine Plastic Pollution

When it comes to marine plastic pollution, even if various countries and regions implement united abatement efforts to decrease the plastic leakage, the volume of plastic reaching the oceans would be most likely to stabilize rather than decline, indicating the total volume of marine plastics will continue to increase. To change this, new strategies and efforts which aim in long term solutions like the adoption of CE principles on plastic production and consumption are essential (Ellen MacArthur Foundation 2015). This can achieve in six general ways; reduce raw materials, redesign products for reuse and recycling, eliminate single-use plastics, reuse products, recycle products or its parts to avoid plastic waste and recover resources from the end of life products (UNEP 2016).

Methods of Application

Linking CE principles into plastics not only prevents the entry of plastic waste into riverine and marine environments but also maximizes the resource value of plastic materials through recycling, reusing, and redesigning. CE can apply to the world of plastics in different ways such as:

- Produce plastics from alternative renewable feedstocks such as plant fibres
- Develop new techniques and processes to make products with less plastic content
- Design plastic products to reuse, recycle or repair easily
- Develop technologies to effectively reprocess used plastics and encourage innovations to find environmentally friendly alternatives to plastics.
- Initiate extended producer responsibility (EPR) to make the producer completely responsible for their products, even after its use.
- Develop vigorous information platforms to acquire details about the composition of plastic products as well as to trace the movement of plastic resources within the economy.
- Improve awareness among consumers on sustainable consumption and waste management.

Among this, the key to the successful implementation of CE is efficient design instead of trying to deal with the waste after it created. The main things to be considered in the design stage of plastic products are

- Use of less toxic, renewable, bio-degradable, or compostable materials as raw materials for plastic products
- Minimize material used to reduce waste and design using single or a few numbers of polymers that are easy to separate during recycling

Other Possible Solutions for Plastic Waste Management

Plastic waste is a global problem which is increasing every day. In addition to CE principles, several other approaches and solutions can be implemented to reduce plastic waste. An efficient waste management system as in Japan is one of the best possible solutions to the leakage of plastic waste. By increasing waste segregation, collection and recycling activities, a proper waste management system can capture plastic materials before it reaches the environment and becomes a problem (UNEP 2018, OECD 2018). Government policies and regulations are other possible ways of eliminating plastic waste. Many national, state and local governments are implementing bans on single-use plastics such as plastic bags and plastic packaging, particularly from styrofoam. Providing financial assistance in the form of incentives and funds to facilitate the use of natural materials in making bags and packaging can motivate businesses and industries to become more environmentally friendly (UNEP 2018). Polymers created from biomass such as polylactic acid (PLA), polyhydroxyalkanoates (PHA) and thermoplastic styrenic elastomers (TPS) are potential natural alternatives to single use plastic materials (UNEP 2018). However, additional research is needed in this field to examine the behaviour and impacts of these biopolymers in the environment at its end of life as well as the potential recycling options for them. Moreover, policymakers and government should give special attention to check whether products with natural materials are labelled correctly. For example, if a product is defined as biodegradable or bioplastics then they should provide the conditions under which the degradation process happens (UNEP 2018).

Another important approach to eliminate plastic waste is Extended Producer Responsibility or EPR. In this policy, producers are made responsible for the disposal and treatment of their products at the end of life. EPR can prevent plastic waste from creating as businesses will start looking for environmentally friendly product designs and facilitate better recycling options (OECD n.d.). Clean up activities such as ocean clean-ups and other remediation activities like developing devices and technologies like the interceptor to collect plastic litter from oceans help to remove plastics that are already in the environment (OECD 2018, Rivers | The Ocean Cleanup 2020)

CONCLUSION

Plastic contamination has become a severe concern in the marine environments of the world today due to rapid urban industrial transformation. Therefore, the challenge is to promote development and economic growth while keeping environmental sustainability safe and protected. A source-to-sea approach can help this to happen by linking all sectors such as land, water resources, coastal and marine management from land to freshwater to marine environments to develop solutions. This approach, along with a CE model of production and consumption, can become the best possible solution for preventing marine plastics. However, there are many novel challenges in effectively implementing the introduced framework within the current system of production and consumption, which need further research. This includes a lack of specific and standardized guidelines on how to implement the framework, how to properly recognize, categorize, and link all related stakeholders as well as lack of secondary markets for recycled goods.

REFERENCES

Andrady, A. 2011. Microplastics in the marine environment. Marine Pollution Bulletin, 62(8): 1596-1605.

Atkins, J., Burdon, D., Elliott, M. and Gregory, A. 2011. Management of the marine environment: Integrating ecosystem services and societal benefits with the DPSIR framework in a systems approach. Marine Pollution Bulletin, 62(2): 215-226.

Australia State of the Environment Report. 2017. Marine debris. [online] Available at: https://soe.environment.gov.au/theme/marine-environment/topic/2016/marine-debris [Accessed 16 Oct. 2019].

Barra, R. and A.Leonard, S. 2018. Plastics and The Circular Economy. Washington, DC: Scientific and Technical Advisory Panel to the Global Environment Facility.

Beaumont, N., Aanesen, M., Austen, M., Börger, T., Clark, J., Cole, M., Hooper, T., Lindeque, P., Pascoe, C. and Wyles, K. 2019. Global ecological, social and economic impacts of marine plastic. Marine Pollution Bulletin, 142: 189-195.

Bergmann, M., Lutz, B., Tekman, M. and Gutow, L. 2017. Citizen scientists reveal: Marine litter pollutes Arctic beaches and affects wild life. Marine Pollution Bulletin, 125(1-2): 535-540.

Bhattacharya, P., Lin, S., Turner, J. and Ke, P. 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. The Journal of Physical Chemistry C, 114(39): 16556-16561.

Bonanno, G. and Orlando-Bonaca, M. 2018. Ten inconvenient questions about plastics in the sea. Environmental Science & Policy, 85: 146-154.

Carpenter, E., Anderson, S., Harvey, G., Miklas, H. and Peck, B. 1972. Polystyrene spherules in coastal waters. Science, 178(4062): 749-750.

Cbd.int. 2016. Marine Debris: Understanding, Preventing and Mitigating the Significant Adverse Impacts on Marine and Coastal Biodiversity. [online] Available at: https://www.cbd.int/doc/publications/cbd-ts-83-en.pdf [Accessed 28 Nov. 2019].

Clean seas, 2019. about | Cleanseas. [online] Available at: https://www.cleanseas.org/about [Accessed 6 Oct. 2019].

CSIRO 2019. Marine debris Sources, distribution and fate of plastic and other refuse – and its impact on ocean and coastal wildlife. [online] Available at: https://www.csiro.au/~/media/OnA/Files/15-00156_OA_MarineDebris4ppFactsheet_WEB_190405.pdf [Accessed 22 Dec. 2019].

Ecologic.eu. 2018. No More Plastics in the Ocean | Ecologic Institute: Science and Policy for a Sustainable World. [online] Available at: https://www.ecologic.eu/16131 [Accessed 5 Nov. 2019].

Ec.europa.eu. 2019. Biodegradable, oxo-degradable and compostable bags observed over three years in the sea, open air and soil. [online] Available at: https://ec.europa.eu/environment/integration/research/newsalert/pdf/biodegradable_oxodegradable_compostable_bags_observed_in_sea_air_soil_536na4_en.pdf [Accessed 15 Jan. 2020].

Ellen MacArthur Foundation. 2013. Towards the Circular Economy - Economic and business rationale for an accelerated transition. [online] Available at: https://www.ellenmacarthurfoundation.org/assets/downloads/publications/Ellen-MacArthur-Foundation-Towards-the-Circular-Economy-vol.1.pdf [Accessed 10 Oct. 2019].

Ellen MacArthur Foundation, The McKinsey Center, NERA 2015. Delivering the Circular Economy – A Toolkit for Policymakers. Ellen MacArthur Foundation.

Ellen MacArthur Foundation, 2017. The New Plastics Economy: Catalysing Action. [online] Davos: World Economic Forum and Ellen MacArthur Foundation. Available at: <https://www.ellenmacarthurfoundation.org/publications> [Accessed 6 March 2020].

Emmerik, T. and Schwarz, A. 2019. Plastic debris in rivers. WIREs Water, 7(1).

Eriksson, C., Burton, H., Fitch, S., Schulz, M. and van den Hoff, J., 2013. Daily accumulation rates of marine debris on sub-Antarctic island beaches. Marine Pollution Bulletin, 66(1-2): 199-208.

Eriksen, M., Lebreton, L., Carson, H., Thiel, M., Moore, C., Borerro, J., Galgani, F., Ryan, P. and Reisser, J. 2014. Plastic pollution in the world's oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS ONE, 9(12): e111913.

ESA 2017. Planning For A Circular Economy. [online] Available at: <http://www.esauk.org/what-we-say/publications/publications-archive> [Accessed 8 February 2020].

European Commission, 2014. Towards A Circular Economy: A Zero Waste Programme For Europe. Brussels: European Commission.

Filho, W., Havea, P., Balogun, A., Boenecke, J., Maharaj, A., Ha'apio, M. and Hemstock, S. 2019. Plastic debris on Pacific Islands: Ecological and health implications. Science of The Total Environment, 670: 181-187.

Fowler, C. 1987. Marine debris and northern fur seals: A case study. Marine Pollution Bulletin, 18(6): 326-335.

Gall, S. and Thompson, R. 2015. The impact of debris on marine life. Marine Pollution Bulletin, 92(1-2): 170-179

Garcia-Vazquez, E., Cani, A., Diem, A., Ferreira, C., Geldhof, R., Marquez, L., Molloy, E. and Perché, S. 2018. Leave no traces – Beached marine litter shelters both invasive and native species. Marine Pollution Bulletin, 131: 314-322.

GESAMP. 2019. Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean |0 GESAMP. [online] Available at: http://www.gesamp.org/publications/guidelines-for-the-monitoring-and-assessment-of-plastic-litter-in-the-ocean [Accessed 8 Jan. 2020].

Granit, J., Liss Lymer, B., Olsen, S., Tengberg, A., Nõmmann, S. and Clausen, T. 2017. A conceptual framework for governing and managing key flows in a source-to-sea continuum. Water Policy, 19(4): 673-691.

Grida.no. 2016. Marine Litter Vital Graphics | GRID-Arendal. [online] Available at: http://www.grida.no/publications/60 [Accessed 2 Dec. 2019].

Iñiguez, M., Conesa, J. and Fullana, A. 2017. Microplastics in Spanish Table Salt. Scientific Reports, 7(1).

IUCN. (n.d.). Marine plastics. [online] Available at: https://www.iucn.org/resources/issues-briefs/marine-plastics [Accessed 2 Sep. 2019].

Kiessling T., Gutow L. and Thiel M. 2015. Marine litter as habitat and dispersal vector. In: Bergmann M., Gutow L., Klages M. (eds) Marine Anthropogenic Litter. Springer, Cham

Lamb, J., Willis, B., Fiorenza, E., Couch, C., Howard, R., Rader, D., True, J., Kelly, L., Ahmad, A., Jompa, J. and Harvell, C. 2018. Plastic waste associated with disease on coral reefs. Science, 359(6374): 460-462.

Lavers, J. and Bond, A. 2017. Exceptional and rapid accumulation of anthropogenic debris on one of the world's most remote and pristine islands. [online] Proceedings of the National Academy of Science of the United States of America. Available at: https://www.pnas.org/content/114/23/6052 [Accessed 27 Oct. 2019].

Lee, H., Kunz, A., Shim, W. and Walther, B. 2019. Microplastic contamination of table salts from Taiwan, including a global review. Scientific Reports, 9(1).

LI, W., TSE, H. and FOK, L. 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. Science of The Total Environment, 566-567: 333-349.

Lloyd-Smith, M. and Immig, J. 2018. Ocean pollutants guide: toxic threats to human health and marine Life. IPEN: 70-83.

Lusher, A., McHugh, M. and Thompson, R. 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Marine Pollution Bulletin, 67(1-2): 94-99.

Mathews, R.E. and Stretz, J. 2019. Source-To-Sea Framework For Marine Litter Prevention: Preventing Plastic Leakage From River Basins. [online] SIWI. Available at: https://www.siwi.org/wp-content/uploads/2019/08/Marine-LitterPrevention_2019_WEBB.pdf> [Accessed 8 February 2020].

Mathews, R. E., Tengberg, A., Sjödin, J. and Liss-Lymer, B. 2019. Implementing the source-to-sea approach: A guide for practitioners. SIWI, Stockholm

Murano, C., Agnisola, C., Caramiello, D., Castellano, I., Casotti, R., Corsi, I. and Palumbo, A. 2020. How sea urchins face microplastics: Uptake, tissue distribution and immune system response. Environmental Pollution, 264: 114685.

Marinedebris.noaa.gov. 2020. Impacts | OR&R's Marine Debris Program. [online] Available at: <https://marinedebris.noaa.gov/discover-issue/impacts> [Accessed 5 March 2020].

News European Parliament. (2018). Circular economy: definition, importance and benefits. [online] Available at: https://www.europarl.europa.eu/news/en/headlines/economy/20151201STO05603/circular-economy-definition-importance-and-benefits [Accessed 23 Nov. 2019].

NOAA Fisheries. 2017. Entanglement of Marine Life: Risks and Response. [online] Available at: https://www.fisheries.noaa.gov/insight/entanglement-marine-life-risks-and-response [Accessed 25 Nov. 2019].

OECD 2018. Improving Plastics Management: Trends, Policy Responses, And The Role Of International Co-Operation And Trade. Paris: OECD.

Oecd.org. n.d. *Extended Producer Responsibility - OECD*. [online] Available at: <https://www.oecd.org/env/tools-evaluation/extendedproducerresponsibility.htm> [Accessed 1 July 2020].

Prata, J., da Costa, J., Lopes, I., Duarte, A. and Rocha-Santos, T. 2019. Effects of microplastics on microalgae populations: A critical review. Science of The Total Environment, 665: 400-405.

Preston, F. 2012. A Global Redesign? Shaping The Circular Economy. [online] The Royal Institute of International Affairs. Available at: <https://www.chathamhouse.org/publications/papers/view/182376> [Accessed 5 April 2020].

Roman, L., Hardesty, B., Leonard, G., Pragnell-Raasch, H., Mallos, N., Campbell, I. and Wilcox, C. 2020. A global assessment of the relationship between anthropogenic debris on land and the seafloor. Environmental Pollution, 264: 114663.

Romero-Hernández, O. and Romero, S. 2018. Maximizing the value of waste: From waste management to the circular economy. Thunderbird International Business Review, 60(5): 757-764.

Ryan P.G. 2015. A Brief History of Marine Litter Research. In: Bergmann M., Gutow L., Klages M. (eds) Marine Anthropogenic Litter. Springer, Cham.

Sharma, S. and Chatterjee, S. 2017. Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environmental Science and Pollution Research, 24(27): 21530-21547.

SIWI. 2019. Source-To-Sea Framework For Marine Litter Prevention: Policy Brief. Available at: <https://www.siwi.org/publications/source-to-sea-framework-formarine-litter-prevention/> [Accessed 26 March 2020].

Smith, M., Love, D., Rochman, C. and Neff, R. 2018. Microplastics in Seafood and the Implications for Human Health. Current Environmental Health Reports, 5(3): 375-386.

The Ocean Cleanup. 2020. Rivers | The Ocean Cleanup. [online] Available at: <https://theoceancleanup.com/rivers/> [Accessed 2 July 2020].

The NOAA Marine Debris Program. 2018. How Does Marine Debris Affect Coral Reefs? | OR&R's Marine Debris Program. [online] Available at: https://blog.marinedebris.noaa.gov/how-does-marine-debris-affect-coral-reefs [Accessed 4 Feb. 2020].

Tiquio, M., Marmier, N. and Francour, P. 2017. Management frameworks for coastal and marine pollution in the European and South East Asian regions. Ocean & Coastal Management, 135: 65-78.

Trevail, A., Gabrielsen, G., Kühn, S. and Van Franeker, J. 2015. Elevated levels of ingested plastic in a high Arctic seabird, the northern fulmar (Fulmarus glacialis). Polar Biology, 38(7): 975-981.

Unesco.org. 2017. Facts and figures on marine pollution | United Nations Educational, Scientific and Cultural Organization. [online] Available at: http://www.unesco.org/new/en/natural-sciences/ioc-oceans/focus-areas/rio-20-ocean/blueprint-for-the-future-we-want/marine-pollution/facts-and-figures-on-marine-pollution/ [Accessed 1 Nov. 2019].

Un.org. 2016. Inputs to the Secretary-General's Report on Marine Debris, Plastics and Microplastics. [online] Available at: https://www.un.org/depts/los/general_assembly/contributions_2016/UNEP_Contribution_to_ICP_on_marine_debris.pdf

UNEP. (n.d.). GOAL 14: Life below water. [online] Available at: https://www.unenvironment.org/explore-topics/sustainable-development-goals/why-do-sustainable-development-goals-matter/goal-14 [Accessed 5 Oct. 2019].

UN Environment Programme 2001. Marine Litter- Trash That Kills. [online] Available at: https://wedocs.unep.org/bitstream/handle/20.500.11822/9691/-Marine%20Litter%20-%20trash%20that%20kills-2003707.pdf?sequence=3&amp%3BisAllowed= [Accessed 5 Nov. 2019].

UNEP-UN Environment Programme 2018. The State Of Plastics: World Environment Day Outlook 2018. [online] Available at: <https://www.unenvironment.org/resources/report/state-plastics-world-environment-day-outlook-2018> [Accessed 29 June 2020].

UNEP-UN Environment Programme. 2016. Marine Plastic Debris and Microplastics: Global Lessons and Research to Inspire Action and Guide Policy Change. [online] Available at: https://www.unenvironment.org/resources/report/marine-plastic-debris-and-microplastics-global-lessons-and-research-inspire-action.

UNEP 2016. Marine Litter Legislation: A Toolkit for Policymakers. [online] Available at: https://www.unenvironment.org/resources/report/marine-litter-legislation-toolkit-policymakers [Accessed 6 Oct. 2019].

Vegter, A., Barletta, M., Beck, C., Borrero, J., Burton, H., Campbell, M., Costa, M., Eriksen, M., Eriksson, C., Estrades, A., Gilardi, K., Hardesty, B., Ivar do Sul, J., Lavers, J., Lazar, B., Lebreton, L., Nichols, W., Ribic, C., Ryan, P., Schuyler, Q., Smith, S., Takada, H., Townsend, K., Wabnitz, C., Wilcox, C., Young, L. and Hamann, M. 2014. Global research priorities to mitigate plastic pollution impacts on marine wildlife. Endangered Species Research, 25(3): 225-247.

Winans, K., Kendall, A. and Deng, H. 2017. The history and current applications of the circular economy concept. Renewable and Sustainable Energy Reviews, 68: 825-833.

Wright, S., Thompson, R. and Galloway, T. 2013. The physical impacts of microplastics on marine organisms: A review. Environmental Pollution, 178: 483-492.

Yang, D., Shi, H., Li, L., Li, J., Jabeen, K. and Kolandhasamy, P. 2015. Microplastic pollution in table salts from China. Environmental Science & Technology, 49(22): 13622-13627.

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A Review on Green Synthesis of Metal and Metal Oxide Nanoparticles

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ABSTRACT

Metal oxide nanoparticles have captivated scrupulous research interest because of its major relevance in the field of medicine, catalysis, pigment, electronics, biotechnology, sensors, optical devices, adsorption, DNA labelling, drug delivery, kinetics, spintronics and piezoelectricity. Nanoparticles (NPs) became more significant for its reasonable property as a heterogeneous non-toxic catalyst with environmental reimbursement. The biogenic innovation of metal oxide NPs is an enhanced alternative owing to eco-friendliness. In the biological field, the probable efficacy of NPs has been reported by scores of scholars in the treatment of cancer. Owed to munificent returns, NPs explored as a powerful catalyst for several organic transformations. This section unlocks with a short course on to synthesize metal oxide NPs on a natural scale.

INTRODUCTION

Nanotechnology is the greatest dynamic area of exploration in modern material science and has established as the great innovation of things at the nanoscale of 1 to 100 nm. Nano is a Greek word symbolizing "dwarf" in the one-billionth scale (10^{-9}). To synthesis nanomaterial of various shapes and dimensions, two different methods of approaches have been widely used namely bottom-up and top-down approaches (Fig. 1).

Building up of nanomaterials from an atom by atom is a bottom-up approach while trimming down bulk material to smaller nano size is a top-down approach. Some methods of bottom-up approaches are spray pyrolysis, laser pyrolysis chemical vapour deposition, atomic/molecular condensation and sol-gel processes. Mechanical milling, hydrothermal synthesis, photolithographic processing, electron beam lithography, laser ablation, micromachining, electron beam machining, etching and sputtering are the methods of top-down approaches in exploitation. The physical and chemical methods of synthesis utilize high reactive agents, high temperature, pressure, hazardous chemical vapours and defile environment. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride, elemental hydrogen, polyol, Tollen's reagent, N, N-dimethylformamide (DMF) and poly (ethylene glycol)-block copolymers are used in NPs synthesis. The main factor about NPs is its surface to volume ratio, which makes NPs very primitive in the field of technology with specific applications in respective fields like catalysis, adsorption, drug delivery, biotechnology and DNA modelling. NPs are virtualized in its application by its dimension, shape, morphology and size (Vijayaraghavan et al. 2012, Khin et al. 2012, Dimkpa et al. 2012 and Ain et al. 2013) It can be one dimensional (1D), 2D or 3D. NPs used in electronic gadgets and sensing devices are thin-film 1D. 2D carbon nanotubes (CNTs) have more application in the field of catalysis because of its stableness and a high degree of adsorption. Quantum dots and clusters are grouped as 3D NPs. Metal NPs like Ag, Au, Pd, Pt, Zn, Fe are mainly formed from its salt solutions like $AgNO_3$, $AuCl_4$, $PdCl_4$, $PtCl_4$, $ZnSO_4$ by physical and chemical methods. Based on its chemical nature NPs are grouped as metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Correct exploitation of ecologically benevolent solvents and nontoxic chemicals are some of the core subjects in the green synthesis approach contemplations. This review implies the importance of green synthesis of metal NPs in a benign greener way following the 12 principles of green chemistry without defiling the environment (Fig. 2).

MATERIALS AND METHODS

Characterization of Metal and Metal Oxide NPs

Techniques used for structural characterization (size, shape, lattice constants and crystallinity) of NPs are X-ray diffraction technique, electron microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), high resolution transmission electron microscopy

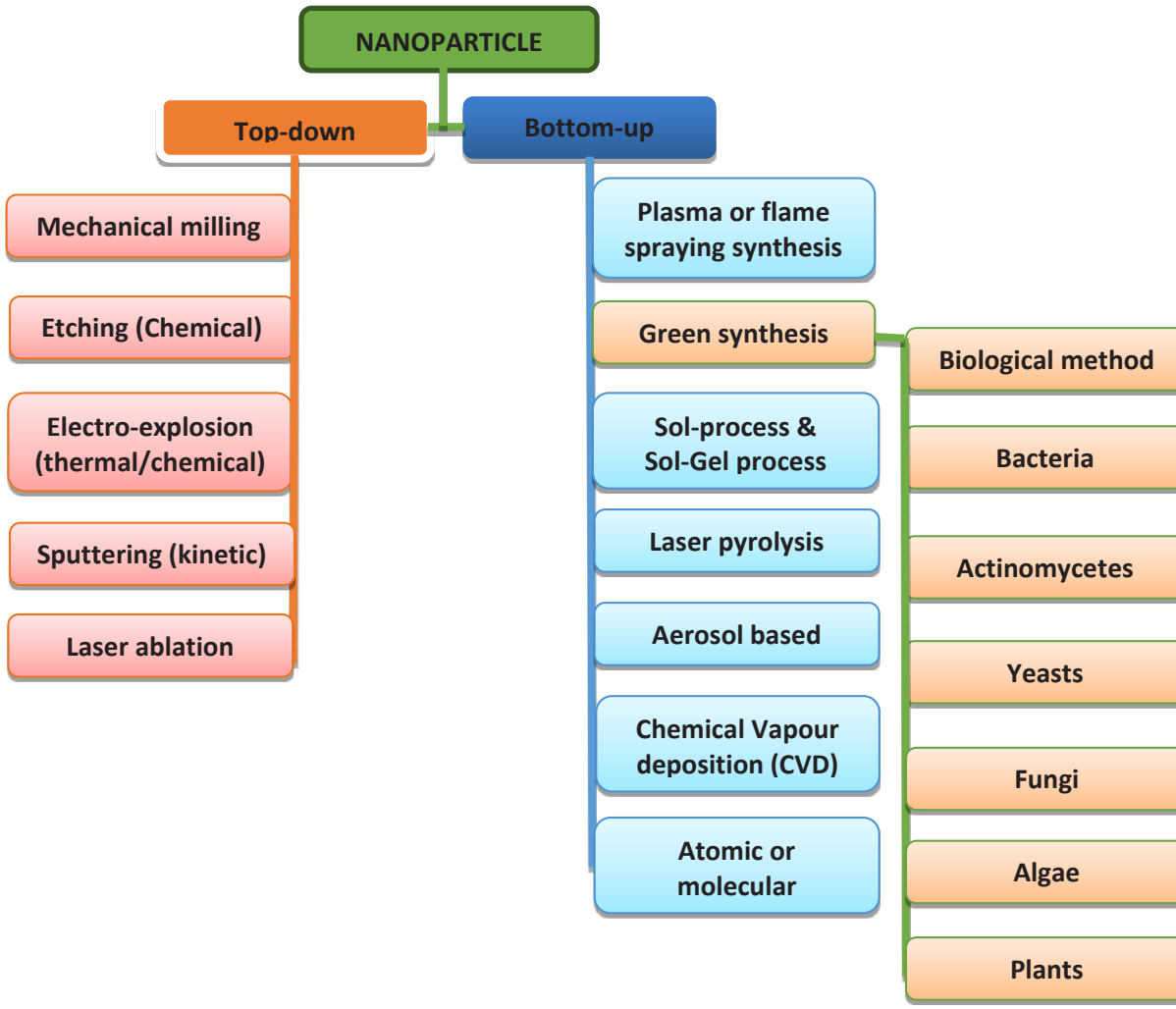


Fig. 1: Various methods of nanoparticles (NPs) synthesis.



Fig. 2: Progression of green chemistry principles over environment deflation.

(HR-TEM), environmental transmission electron microscopy (ETEM) and scanning probe microscopy (SPM). Some internal chemical characterisation techniques used to detect internal chemical constituents of NPs are Fourier transform infrared spectroscopy (FT-IR), energy dispersive x-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), auger electron spectroscopy (AES) and ultraviolet photoelectron spectroscopy (UPS). Two characterisation techniques for structural and internal chemical characterisation is briefed in this paper.

X-Ray Diffraction Method (XRD)

XRD technique is a handy analytical method to detect crystalline forms of the samples using the equation (1)

$$n\lambda = 2d\sin\theta \quad \ldots(1)$$

λ = wavelength of X-ray

d = interplaner spacing

θ = diffraction angle

n = 0, 1, 2, 3, ….

X-ray diffractometer operating (Fig. 3) at 40kV and 30mA with 2θ ranging from 20° - 90° with Cu (Kα) radiation (λ = 1.5416 A°). The Kα doublets were well resolved. From Scherrer's formula (2), the crystallite size of the NPs can be deduced.

$$D = k\lambda/\beta \cos\theta \quad \ldots(2)$$

Where,

k = is a constant

λ = is the X-ray wavelength which equals to 0.15416 nm

β = the full width half maximum intensity (FWHM)

θ = the half diffraction angle

Scanning Electron Microscope (SEM)

SEM is a multipurpose instrument and provides qualitative information of the samples like its topography, morphology, composition and crystallographic arrangements with high resolution (Fig. 4) and produces three-dimensional black and white images with magnification ranging from 20X to approximately 60,000X, spatial resolution of 50 to 100 nm

Fundamental Principles of Scanning Electron Microscopy (SEM)

Incident electron beam sample interactions produce secondary electrons (SE), backscattered electrons (BSE), diffracted backscattered electrons (EBSD), photons, visible light and heat. Secondary electrons are most valuable for showing morphology and topography of samples, backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples to determine crystal structures of orientations and photons are used for elemental analysis (Fig. 5).

Energy Dispersive X-Ray Spectroscopy

Energy dispersive X-ray spectroscopy (EDS or EDX) is an analytical technique used for the elemental analysis of a sample. A high energy beam of electrons or protons or a beam of X-rays is focused into the sample, the number and energy of the X-rays emitted from a specimen can be measured by an energy dispersive spectrometer (EDS).

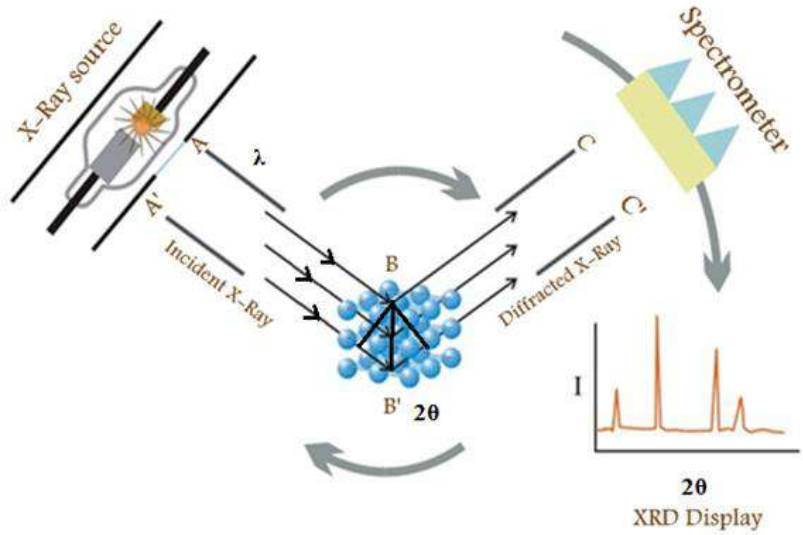


Fig. 3: Bragg's diffraction with XRD display.

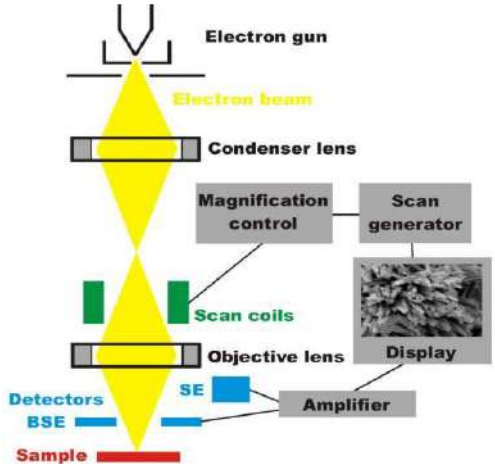


Fig. 4: Instrumentation of scanning electron microscope.

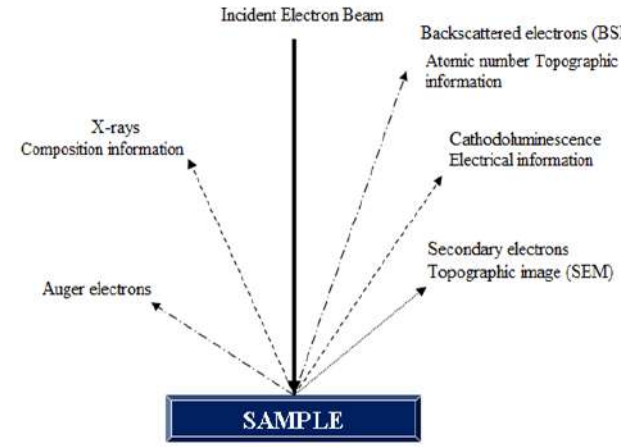


Fig. 5: Fundamental principles of scanning electron microscopy.

Fourier Transform Infrared Spectroscopy (FT-IR)

Sample for FT-IR analysis is mixed with solid KBr pellets uniformly and compressed on a thin transparent film. To determine the functional group of the sample, the compressed thin film is kept in the chamber of the instrument in the scanning range of 4000-400cm^{-1} at a resolution of 4cm^{-1}.

Working principle of FT-IR: Fig. 6 represents the working principle of FT-IR. The instrumentation is as follows.

***The source*:** Glowing black body source emits the infrared energy. This radiation is then allowed to pass through the aperture and then to the sample and detector. The resulted beam undergoes special encoding in the interferometer. This exists as interferogram signals.

***The sample*:** Depending on the type of analysis, the sample may transmit or reflect the interferogram signals in the sample compartment.

***The detector* :** The detectors are specially designed in a way to detect the interferogram signals from the sample compartment.

***The computer*:** The computer converts the measured digitalized signals as spectral lines (Fig. 6). It is then observed by the observer.

RESULTS AND DISCUSSION

Green Synthesis of Metal and Metal Oxide NPs

NPs produced by plants are more steadfast, cost-effective and quiet novel and have fascinated researchers in the field of production of engineered nanomaterials with application in catalysis, adsorption, drug delivery, biotechnology, DNA modelling and have a progression over chemical procedures. In this method, there is no need to use high pressure, energy, temperature and toxic chemicals. Even though bacteria and fungi are used for the synthesis of NPs but the use of plant extracts trim down the cost and do not require any special culture preparation and isolation techniques. Though there are many chemical and physical methods, green synthesis of

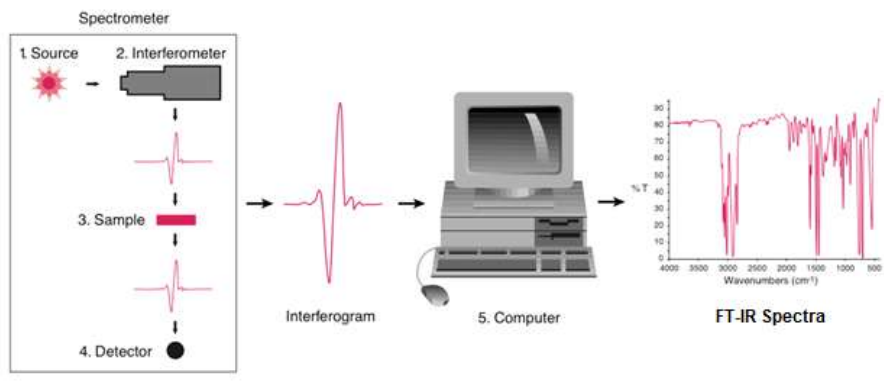


Fig. 6: The interferometer to detect functional group.

nanomaterial is a most promising method depends only on the plant source and organic compound in the crude leaf extract. Green synthesis offers novel challenges for the reduction of environmental pollution. Plants are potent biochemists and have components of phytochemicals acting as natural antioxidants since time immemorial. Presently there has been an augmented interest globally to identify antioxidant compound from plant-derived secondary metabolites which include phenols, tannins, carbohydrates, saponins, flavonoids, amino acids, proteins and polysaccharides (Subbarao et al. 2013, Vijayaraghavan et al. 2010). These molecules prevent agglomeration by acting as reducing and stabilizing agents to synthesis nanoparticle in a benign eco-friendly method that are potent and have no side effect in synthesizing NPs (Table 1).

The metal ions in the salt solution are reduced by the antioxidants or phytochemicals present in the plant extract to make the first phase of formation of NPs more active and leads to the growth and stabilization of NPs by nucleation in the second phase. After nucleation, the so formed NPs are capped and stabilized by the phytochemicals to exhibit various shapes like cubes, spheres, triangles, hexagons, pentagons, rods and wires (Fig. 7). It has also been reported that the amount of extract, pH, time taken and concentration of metabolites gives distinct morphology to NPs.

Cinnamomum zeylanicum leaf extract was used to synthesize crystalline palladium NPs of 15 to 20 nm (Smitha et al. 2009). Cubic shaped silver, nanoparticles of 15 to 500nm, have been premediated from the leaves of *Diospyros kaki* (Song et al. 2010), *Magnolia kobu*, *Carica papaya*. Fruits of *Tanacetum vulgare*, *Emblica officinalis*, *Psidium guajava* produces spherical silver, gold NPs of 16nm, 15 to 25nm, 25 to 30nm. There have been reports (Fig. 8) in the synthesis of silver, gold, zinc, platinum, palladium, indium oxide and zinc oxide NPs using leaf, stem, stem bark, latex, fruit, pulp, seed, root and rhizome extracts of *Aloe barbendis* (Sangeetha et al. 2011), *Azadirachta indica*, *Stevia rebaudiana*, *Calotropis procera*, *Pelargonium graveolens*, *Cuminum cyminum* (Kumar et al. 2010), *Emblica officinalis*, *Tanacetum vulgare*, *Magnolia kobus*, *Coriandrum sativum*, *Jatrapha curcas*, *Cycas, Eucalyptus camaldulensis*, *Cymbopogon flexuosus*, *Oscimum sanctum, Rhizopus nigricans*, *Acalypha indica*, *Zingiber offcinale* and *Musa paradisiaca*.

Table 1: The Taxonomy and phytoconstituents of plants.

S. No	Plant Species	Botanical Name	Family	Vernacular Name	Stabilising and Complexing Phyto-constituents	Plant parts	Traditional use	Earlier Reported NPs & References
1.		*Ocimum sanctum*	Lamiaceae	Tulsi	Flavonoid, Amino acid, Steroid, Protein and Alkaloid	L	Controls diabetes, blood cholesterol, effective in Fever, Cough and Bronchitis.	Silver and Zinc Oxide (Singhal et al. 2011 & Gnana et al. 2015)
2.		*Acalypha Indica*	Euphorbiacea	Kuppaimenii	Terpenoid, Amino acid, Glycoside, Steroid, Fixed oil, Protein and Alkaloid	E	Antibacterial Emetic, Expectorant used in Bronchitis, Asthma, Pneumonia	Silver and Zinc Oxide (Krishnaraj et al. 2010 & Gnana et al. 2014)
3.		*Emblica officinalis*	Euphorbiacea	Amla	Steroid, Flavonoid, Glycoside, Saponnin, Fixed oil, Protein and Alkaloid	A	Restorative, Stomachic, Alterative, Anti-inflammatory used to treat Peptic ulcer	Silver Gold and Zinc Oxide (Ankamwar et al. 2005 & Gnana et al. 2014)
4.		*Azadirachta indica*	Meliaceae	Neem	Amino acid, Terpenoid, Flavonoid, Glycoside, Steroid, Protein and Alkaloid	V	Anthelmintic, Antifungal, Anti-Diabetic, Antibacterial, Antiviral and Sedative.	Silver, Zinc Oxide and Gold (Gnana et al. 2015 & Shiv et al. 2004)
5.		*Corriandrum sativum*	Apiaceae	Corri-an-der	Alkaloid, Terpenoid, Flavonoid, Amino acid, Glycoside, Steroid, Fixed oil and Protein	E S	Analgesic, Carminative, Digestive, Diuretic and Sedative	Silver and Zinc Oxide (Gnan et al. 2014)

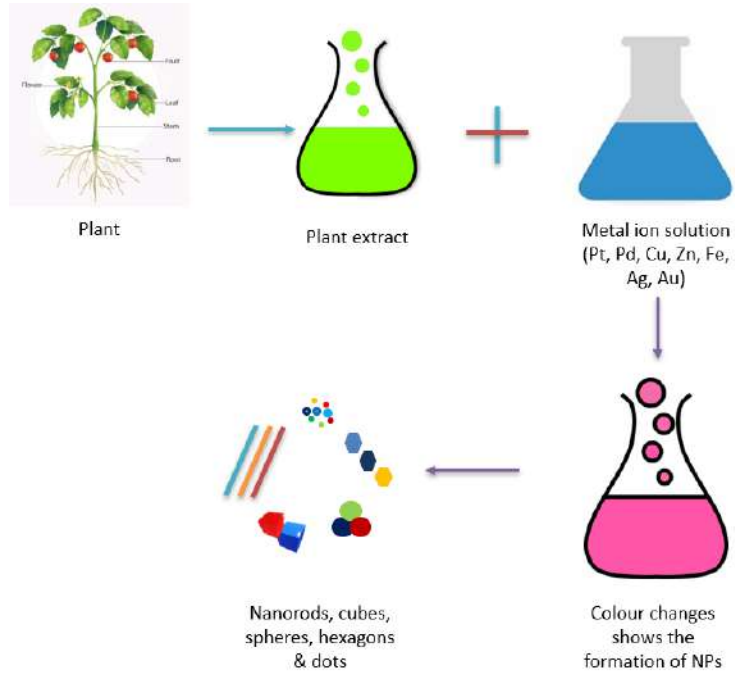


Fig. 7: Schematic representation of green synthesis of NPs.

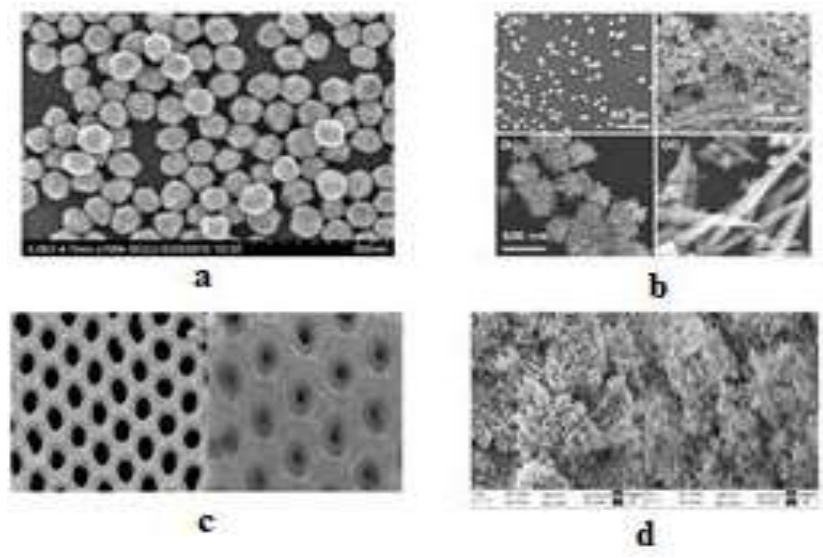


Fig. 8 a, b, c and d: SEM image of zinc oxide, gold and palladium NPs.

Smitha et al. (2009), reported gold nanoprisms with fcc (111) at low concentrations and nanospheres at high concentration using *Cinnamomum zeylanicum* leaf broth as the reducing agent. Crystallinity and morphology of the foresaid nanoprisms of fcc (111) and spheres were characterized by XRD, TEM and SEM. Silver NPs synthesized using leaf extract of *Acalypha indica* by Krishnaraj et al. (2010), shows the formation of NPs within 30 min. The size of the silver NPs was confirmed by high-resolution transmission electron microscopy as 20-30 nm. Song et al. (2009), testified Au, Ag and Au-Ag NPs with fcc (111) of spherical and hexagonal morphology with 20-150 nm in water using the extract of *Volvariella volvacea* by extracellular synthesis method. FT-IR proves (Fig. 9)

that biomolecules responsible for capping and efficient stabilization for Au, Ag and Au-Ag nano prisms are proteins and amino acids.

In addition, Ravindra et al. (2011), reported zinc oxide NPs of 5-40 nm with spherical and granular nature using *Calotropis procera* latex. Petla et al. (2012), reported that TEM images of the palladium NPs of 15 nm confirmed that proteins and amino acids of soybean extract reduced palladium ions. Owing to the excitations of surface plasmon vibrations, steady change in colour from transparent orange (Fig. 10b) to dark brown (Fig. 10c) occurs when soybean leaf extract is added into palladium ion solution. The appearance of brown colour indicates the formation of palladium NPs.

Mallikarjuna et al. (2011) synthesised silver NPs of 20-3nm using leaf broth of *Ocimum sanctum* as reductant and stabilizer with aqueous silver nitrate. Titanium dioxide NPs of 100 to 150nm from titanium isopropoxide solution using N*yctanthes* leaves extract was focused by Sundrarajan et al. (2013). Gnana et al. (2013), fabricated ZnO nanosized flakes from *Ocimum sanctum* with a crystalline size of 81nm. Padil et al. (2013), reported CuO nanoparticles with gum karaya and discovered its probable antibacterial application. Synthesised palladium NPs by Kalaiselvi et al. (2015) at 60°C with *Catharanthus* plant leaves and (PdOAc) have good wastewater remediation property. The shape of the NPs is spherical with a size of 38nm. Additionally, a researcher reported that aqueous solution of *Cochlospermum gossypium* (Gum kodagogu) is used in the development of platinum NPs which was very stable in its synthesis and utility. Aqueous flower extract of *Calotropis gigantea* is used to synthesise TiO_2 NPs with $Ti(OH)_2$ as a predecessor. The mixture of the foresaid was kept in magnetic agitation for a period of 6 h. The resulted mixture is ultrasonicated for 30 min to pull out the agglomerates. Particle size variation of 160 to 220nm is confirmed by SEM micrographs, EDX and XRD (Fig. 11 and Fig. 12).

Hematite (a-Fe2O3) NPs synthesized from green tea (*Camellia sinensis*) leaf extract shows four times higher

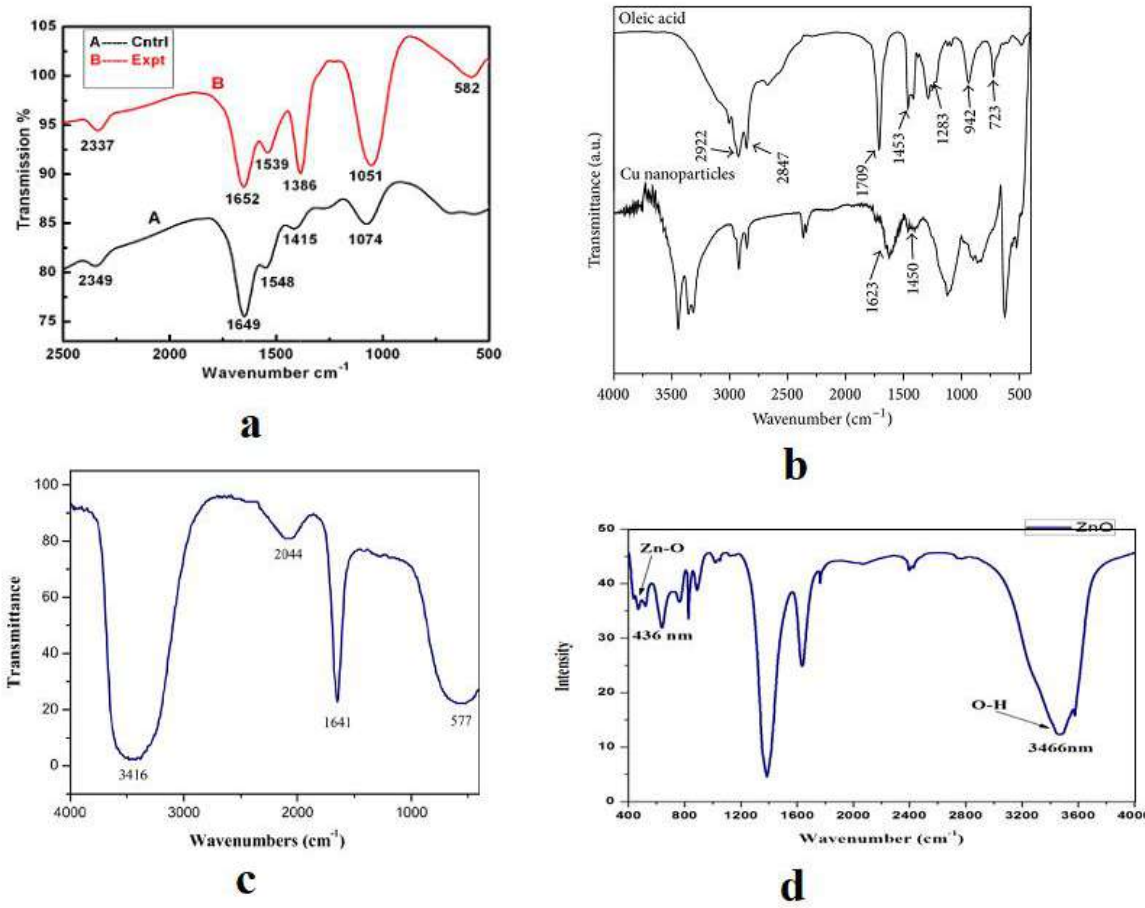


Fig. 9a, b, c and d: FT-IR images of silver, copper, gold and zinc oxide nanoparticles.

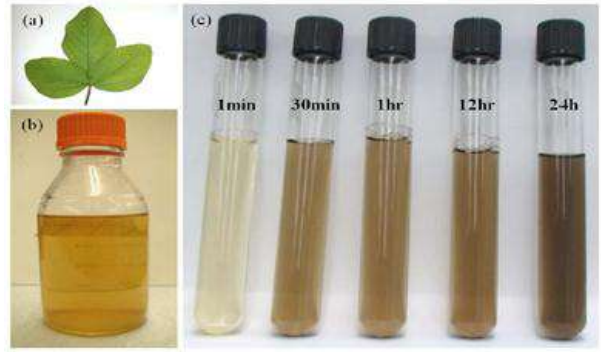


Fig. 10: Photograph of (a) soy leaf; (b) soy leaf extract; and (c) reaction mixture at different time intervals.

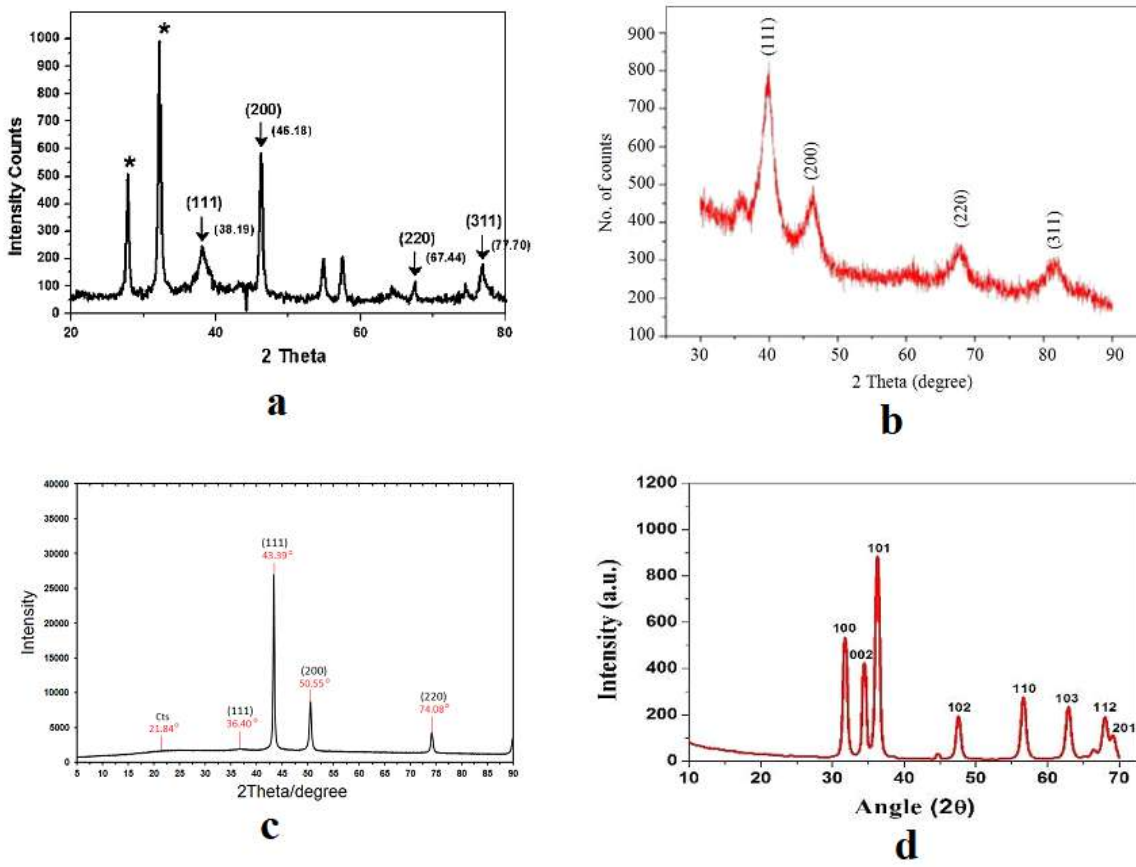


Fig. 11a, b, c and d: XRD images of silver, platinum, copper and zinc nanoparticle.

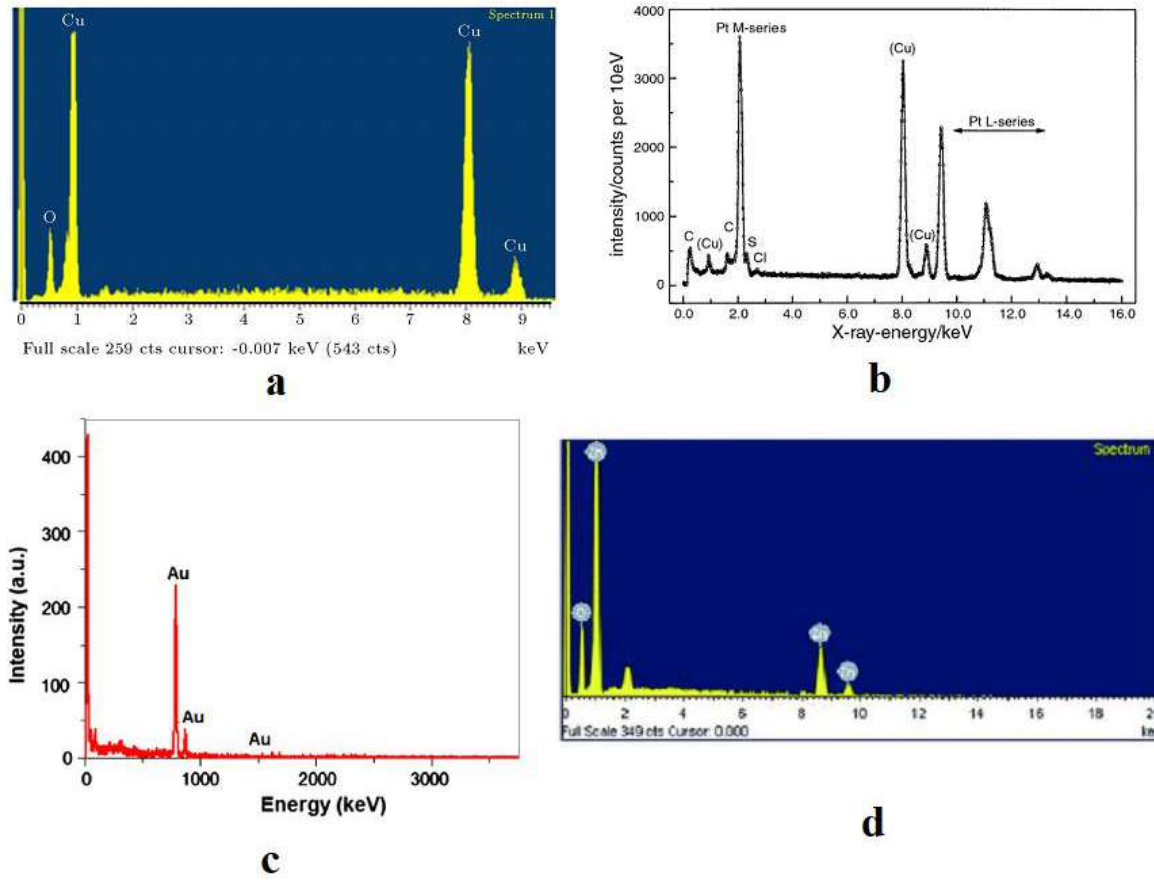


Fig. 12a, b, c and d: EDX images of copper, platinum, gold and zinc oxide nanoparticles.

surface area and two times higher photocatalytic activities (Santhoshkumar et al. (2014)). When applied in a wet-type solar cell the same has enhanced photoelectrochemical activity. Sheny et al. (2012) synthesised PdNPs from dried leaf powder of *Anacardium occidentale*. So formed PdNPs is confirmed with UV-Vis spectrophotometer. Proteins bound to PdNPs are confirmed by FT-IR. The crystallinity, irregularity and rod shape has been confirmed by TEM images. Size of the synthesized nanoparticles was influenced by the quantity of dry leaf powder used. PdNPs exhibited good catalytic activity in the reduction of aromatic nitro-compound. Pb NPs of 47 nm synthesised by a researcher using *Cocos nucifera* with $Pb(COOH)_2$ at 37°C shows the great ability for the absorption of the carcinogenic dye. Mohammed Rafi Shaik et al. (2017) reported palladium NPs using aqueous extract of aerial parts of *Origanum vulgare* L. (OV) which is well known as a rich source of phenolic components and the same is accountable not only for the reduction and progression of NPs, but also to stabilize it. Synthesised ZnO NPs of 9 nm to 18 nm by Saad S M Hassan et al. (2015) show high photocatalytic activity for the degradation of anthracene using zinc acetate as a precursor with *Coriandrum sativum* leaf extract. In a research, Barbosa et al. (2020) authenticated that zinc oxide NPs prepared using madar (*Calotropis procera*) latex and *Ipomoea pes-caprae* showed 40 nm and 20 nm of spherical shape. Table 2 summarizes the abundance of NPs from plant extracts.

CONCLUSIONS

Rigorous agricultural practices, swift development and progressively erudite existences have loaded chemicals into the environment in all forms. Green NPs due to their special features scored many researchers worldwide as it is benign and have extensive applications in the field of medicine agriculture and water remediation. In this work metal and metal oxide nanoparticles like silver, gold, copper, platinum, titanium, zinc and palladium from various parts of leaves, fruits, seeds, barks, root, stem, pulp and rhizome were reported. As benign NPs are nontoxic, it can be used as a novel material to degrade environmental pollutants in multiple ways. These

Table 2: Green NPs from plant leaves, seeds, fruits, barks and roots.

S. No.	Plants	Parts	Nano-particles	Size (nm)	Shapes	References
1.	*Cinnamomumzeylanicum*	Leaves	Au	25	Spherical Prism	Sathishkumar et al. (2009)
2.	*Magnolia kobus*	Leaves	Ag	15-500	Cubic	Song et al. (2009)
3.	*E. coli DH*	E.Coli	Au	8-25	Spherical	Du et al. (2007)
4.	*Glycine max*	Leaves	Pd	15 nm	Spherical	Petla et al. (2012)
5.	*Eucalyptus camaldulensis*	Leaves	Au	5.5–7.5	Crystalline	Ramezani et al. (2008)
6.	*Ocimum sanctum*	Leaves	ZnO	81nm	Flakes	Gnana et al. (2015a)
7.	*Carica papaya*	Fruit	Ag	15 nm	Cubic	Jain et al. (2009)
8.	*Tanacetum vulgare*	Fruit	Ag	I6nm	Spherical	Dubey et al. (2010)
9.	*Jatropha curcas*	Seed	Ag	15-50	Spherical	Bar et al. (2009)
10.	*Rhizopus nigricans*	Black Bread Mold (Fungi)	Ag	35-40	Round	Ravindra et al. (2014)
11.	*Pinus resinosa*	Bark	Pt	6–8	Irregular	Coccia et al. (2012)
12.	*Pinus resinosa*	Bark	Pd	16–20	Spherical	Coccia et al. (2012)
13.	*Cinnamomum zeylanicum*	Bark	Pd	15–20	Crystalline	Sathishkumar et al. (2009)
14.	*Zingiber offcinale*	Rhizome	Ag	6–20	Spherical	Kumar et al. (2012)
15.	*Ocimum sanctum*	Root	Ag	5-10	Spherical	Singhal et al. (2011)
16.	*Pelargonium graveolens*	Leaves	Au	20–40	Decahedral, Icosahedral	Shankar et al. (2003)
17.	*Penicillium brecompactum*	Mould Sp.	Ag	23–105	Crystalline Spherical	Shaligram et al. (2009)
18.	*Diopyros kaki*	Leaves	Ag	15-500	Cubic	Song et al. (2010)
19.	*Syzygium cumini*	Seed	Ag	73–92	Spherical	Kumar et al. (2010)
20.	*Acalypha indica*	Leaves	ZnO	80	Cubes	Gnana et al. (2014a)
21.	*Syzygium cumini*	Seed	Ag	3.5	---	Banarjee et al. (2011)
22.	*Emblica officinalis*	Leaves	ZnO	16	Rods	Gnana et al. (2014b)
23.	*Emblica officinalis*	Fruit	Au	15-25	--	Ankamwar et al. (2005)
24.	*Cinnamomum zeylanicum*	Bark Powder	Ag	31–40	Spherical	Sathish kumar et al. (2009)
25.	*Boswellia ovalifoliolata*	Stem Bark	Ag		Spherical	---
26.	*Verticillium*	Fungi	Ag	21–25	Spherical	Mukherjee et al. (2001)
27.	*Shoreatum buggaia*	Stem Bark	Ag		Spherical	Savithramma et al. (2011)
28.	*Stevia rebaudiana*	Leaves	Au	8–20	Octahedral	Mishra et al. (2010)
29.	*Psidium guajava*	Fruit	Au	25-30	Spherical	---
30.	*Azadirachta indica*	Leaves	ZnO	51	Flowers	Shiv et al. (2004)
31.	*Diospyros kaki*	Leaves	Pt	2–12	Crystalline	Song et al. (2010)
32.	*Azadirachta indica*	Leaves	Au	5.5–7.5	Crystalline	Gnana et al. (2015b)
33.	*Tanacetum vulgare*	Fruit	Au Ag	11 16	Triangular & Spherical	Dubey et al. (2010)
34.	*Ginko biloba*	Leaves	Ag	15-500	Cubic	Song et al. (2010)
35.	*Mangifera indica*	Leaves	Ag	20	Spherical, Triangular, Hexagonal	Phillip et al. (2011)
36.	*Aspergillus niger*	Common Mold Fungi	Ag	20	Spherical	Gade et al. (2008)
37.	*Emblica officinalis*	Fruit	Au Ag	20 25	Triangular	Ankamwar et al. (2005)
38.	*Musa paradisiaca*	Fruit	Pd	50	Crystalline Irregular	Bankar et al. (2010)
39.	*Bacillus cereus*	Bacteria	Ag	20-40	Spherical	---

S. No.	Plants	Parts	Nano-particles	Size (nm)	Shapes	References
40.	*Aloe barbadensis* Miller	Leaves	In_2O_3	5-50	Spherical	Maensiri et al. (2008)
41.	*Cymbopogon flexuosus*	Leaves	Au	200-500	Spherical, Triangular	Shankar et al. (2005)
42.	*Garcinia mangostana*	Frut	Ag	35	Spherical	Veerasamy et al. (2010)
43.	*Syzygium aromaticum*	Fruit	Au	5-100	Irregular	---
44.	*Lactobacillus casei*		Ag	20–50	Spherical	Korbekandi et al. (2012)
45.	*Coriandrum sativum*	Leaves	ZnO	66	Composites	Gnana et al. (2015c)
46.	*Cuminum cyminum*	Seed	Au	1–10	Spherical	Krishnamurthy et al. (2011)
47.	*Aloe barbadensis* Miller	Leaves	Au Ag	10-30	Spherical, Triangular	Chandran et al. (2006)

metal oxide NPs also has considerable attention due to its UV filtering properties. So the exploration of the plant systems as the potential nano factories has heightened interest in the biological synthesis of nanoparticles.

REFERENCES

Ain, Samat N. and Md Nor, R. 2013. Sol-gel synthesis of zinc oxide nanoparticles using *Citrus aurantifolia* extracts. Ceramics International, 39: S545-S548.

Ankamwar, B., Damle, C., Ahmad, A. and Sastry, M. 2005. Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. Journal of Nanoscience and Nanotechnology, 5: 1665-1671.

Banerjee, J. and Narendhirakannan, R.T. 2011. Biosynthesis of silver nanoparticles from *Syzygium cumini* (L.) seed extract and evaluation of their in vitro antioxidant activities. Dig. J. Nanomater. Biostruct., 6: 961-968.

Bankar, A., Joshi, B., Kumar, A.R. and Zinjarde, S. 2010. Banana peeled extract mediated noval route for the synthesis of palladium nanoparticles. Mater. Lett., 64: 1951-1953.

Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., Pyne, S. and Misra, A. 2009. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Colloids Surf. A, 348: 212-216.

Barbosa, Mayck S., Souza, and Bruna da Silva 2020. Antifungal propeins from plant latex. Current Protein and Peptide Science, 21: 497-506.

Chandran, S.P., Chaudhary, M. and Pasricha, R. 2006. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol. Prog., 22: 577-583.

Coccia, F., Tonucci, L., Bosco, D., Bressan, M. and d'Alessandro, N. 2012. One pot synthesis of lignin-stabilized platinum and palladium nanoparticles and their catalytic behaviours in oxidation and reduction reactions. Green Chem., 14: 1073-1078.

Dimkpa, C.O., McLean, J.E., Britt, D.W. and Anderson, A.J. 2012. Bioactivity and biomodification of Ag, ZnO, and CuO nanoparticles with relevance to plant performance in agriculture. Industrial Biotechnology, 8: 344-357.

Du, L., Jiang, H., Liu, X. and Wang, E. 2007. Biosynthesis of gold nanoparticles assisted by *Escherichia coli* DH5α and its application on direct electrochemistry of hemoglobin. Electrochem Commun., 9: 1165-70.

Dubey, S.P., Lahtinen, M. and Sillanpää M. 2010. Tansy fruit mediated greener synthesis of silver and gold nanoparticles. Process Biochem., 45: 1065-1071.

Gade, A.K., Bonde, P. and Ingle, A.P. 2008. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J. Biobased Mater. Bioenergy, 2: 243-7.

Gnanasangeetha D. and Sarala Thambavani, D. 2014a. Biogenic production of zinc oxide nanoparticles using *Acalypha indica*. Journal of Chemical, Biological and Physical Sciences, 4: 238-246.

Gnanasangeetha D. and Sarala Thambavani, D. 2014b. Green synthesis of benevolent ZnO nanorods using *Emblica officinalis*. Asian Journal of Chemistry, 27: 3054-3056.

Gnanasangeetha D. and Sarala Thambavani, D. 2015a Green Zinc oxide nanoparticle ingrained on activated silica for the removal of As(III) from aqueous solution using *Ocimum sanctum* and *Azadirachta indica.* International Journal of Chem. Tech. Research, 8: 44-52.

Gnanasangeetha D. and Sarala Thambavani, D. 2015b. Modelling of As^{3+} adsorption from aqueous solution using *Azadirachta indica* by artificial neural network. Desalination and Water Treatment, 56: 1839-1854.

Gnanasangeetha D. and Sarala Thambavani, D. 2015c. Modelling and biosorption competence of zinc oxide nanoparticle. Nature Environment and Pollution Technology 14(2): 445-449.

Jain, D., Daima, H.K., Kachhwala, S. and Kothari, S.L. 2009. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. Dig. J. Nanomater. Biostruct., 4: 557-563.

Kalaiselvi, A., Roopan, S.M., Madhumitha, G., Ramalingam, C. and Elango, G. 2015. Synthesis and characterization of palladium nanoparticles using *Catharanthus roseus* leaf extract and its application in the photo-catalytic degradation. Spectrochimica acta. Part A, Molecular and Biomolecular Spectroscopy, 135: 116-119.

Khin, M.M., Nair, A.S., Babu, V.J., Murugan, R. and Ramakrishna, S. 2012. A review on nanomaterials for environmental remediation. Energy and Environmental Science, 5: 8075-8109.

Korbekandi, H., Iravani, S. and Abbasi, S. 2012. Optimization of biological synthesis of silver nanoparticles using *Lactobacillus casei* subsp. *casei*. J. Chem. Technol. Biotechnol., 87: 932-937.

Krishnamurthy, S., Sathishkumar, M., Lee, S.Y., Bae, M.A. and Yun, Y.S. 2011. Biosynthesis of Au nanoparticles using cumin seed powder extract. J. Nanosci. Nanotechnol., 11: 1811-1814.

Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T. and Mohan, N., 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces, 76: 50-56.

Kumar, K.P., Paul, W. and Sharma, C.P. 2012. Green synthesis of silver nanoparticles with *Zingiber officinale* extract and study of its blood compatibility. Bio Nano Sci., 2: 144-152.

Kumar, V., Yadav, S.C. and Yadav, S.K. 2010. *Syzygium cumuni* leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization. J. Chem. Technol. Biotechnol., 85: 1301-1309.

Maensiri, S., Laokul, P. and Klinkaewnarong, J. 2008. Indium oxide (In_2O_3) nanoparticles using *Aloe vera* plant extract: Synthesis and optical properties. J. Optoelectron Adv. Mater., 10: 161-165.

Mallikarjuna, N., Saxena, K. B. and Jadhav, D. R., 2011. Cajanus. In: Chittaranjan Kole (Ed.). Wild Crop Relatives: Genomic and Breeding Resources - Legume Crops and Forages. Springer-Verlag Berlin Heidelberg, 21-23.

Mishra, A.N., Bhadauria, S., Gaur, M.S., Pasricha, R. and Kushwah, B.S. 2010. Synthesis of gold nanoparticles by leaves of zero-calorie sweetener herb (*Stevia rebaudiana*) and their nanoscopic characterization by spectroscopy and microscopy. Int. J. Green Nanotechnol. Phys. Chem., 1: 118-124.

Mohammed Rafi Shaik, Zuhur Jameel Qandeel Ali, Mujeeb Khan, Mufsir Kuniyil, Mohamed E., Assal, Hamad Z., Alkhathlan, Abdulrahman Al-Warthan, Mohammed Rafiq H., Siddiqui, Merajuddin Khan and Syed Farooq Adil 2017. Green synthesis and characterization of palladium nanoparticles using *Origanum vulgare* L. extract and their catalytic activity. Molecules, 22: 165: 1-12.

Mukherjee, P., Ahmad, A. and Mandal, D. 2001. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. Nano Lett., 1: 515-519.

Padil, V.V.T. and Cernik Miroslav. 2013. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. International Journal of Nanomedicine, 8: 889-898.

Petla, R.K., Vivekanandhan, S., Misra, M., Mohanty, A.K. and Satyanarayana, N. 2012. Soybean (*Glycine max*) leaf extract based green synthesis of palladium nanoparticles. J. Biomater. Nanobiotechnol., 3: 14-19.

Phillip, D. 2011. *Mangifera indica* leaf-assisted biosynthesis of well dispersed silver nanoparticles. Spectrochim. Acta, Part A, 78: 327-331.

Ramezani, N., Ehsanfar, Z., Shamsa, F., Amin, G., Shahverdi, H.R., Esfahani, H.R.M., Shamsaie, A., Bazaz, R.D. and Shahverdi, A.R. 2008. Screening of medicinal plant methanol extracts for the synthesis of gold nanoparticles by their reducing potential. Z. Naturforsch., 63: 903-908.

Ravindra, P. Singh., Vineet K. Shukla., Raghvendra S. Yadav., Prashant K. Sharma., Prashant K. Singh and Oandey, A. C. 2011. Biological approach of zinc oxide nanoparticles formation and its characterization. Advance Material Letter, 2: 313-317.

Ravindra, B.K. and Rajasab, A.H. 2014. A comparative study on biosynthesis of silver nanoparticles using four different fungal species. Int. J. Pharm. Pharm. Sci., 6: 372-376.

Saad, S. M. Hassan, El Azab, W.I., Ali, H.R. and Mansour, M.S. 2015. Green synthesis and characterization of ZnO nanoparticles for photocatalytic degradation of anthracene. Adv. Nat. Sci.: Nanosci. Nanotechnol. 6(4) : 045012.

Sangeetha, G., Rajeshwari, S. and Venckatesh, R. 2011. Green synthesis of zinc oxide nanoparticles by *Aloe barbadensis* Miller leaf extract: Structure and optical properties. Materials Research Bulletin, 46: 2560-2566.

Santhoshkumar, T., Rahuman, A.A., Jayaseelan, C., Rajakumar, G., Marimuthu, S. and Kirthi, A.V. 2014. Green synthesis of titanium dioxide nanoparticles using *Psidium guajava* extract and its antibacterial and antioxidant properties. Asian Pac. J. Trop. Med., 7: 968-976.

Sathishkumar, M., Sneha, K., Kwak, I.S., Mao, J., Tripathy, S.J. and Yun, Y.S. 2009. Phyto-crystallization of palladium through reduction process using *Cinnamon zeylanicum* bark extract. J. Hazard. Mater., 171: 404-404.

Sathishkumar, M., Sneha, K., Won, S.W., Cho, C.W., Kim, S. and Yun, Y.S. 2009. *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids Surf., B, 73: 332-338.

Savithramma, N., Linga Rao M. and Suvarnalatha Devi P. 2011. Evaluation of antibacterial efficacy of biologically synthesized silver nanoparticles using stem barks of *Boswellia ovalifoliolata* Bal. and Henry and *Shorea tumbuggaia* Roxb. J. Biol. Sci., 11: 39-45.

Shaligram, N.S., Bule, M. and Bhambure, R. 2009. Biosynthesis of silver nano-particles using aqueous extract from the compact in producing fungal strain. Process Biochem., 44: 939-943.

Shankar, S.S., Ahmad, A., Pasricha, R. and Sastry, M. 2003. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. J. Mater. Chem., 13: 1822-1826.

Shankar, S.S., Rai, A., Ahmad, A. and Sastry, M. 2005. Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings. Chem. Mater., 17: 566-572.

Sheny, D.S., Philip, D. and Mathew J. 2012. Rapid green synthesis of palladium nanoparticles using the dried leaf of *Anacardium occidentale*. Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy, 91: 38-35.

Shankar, S.S., Rai, A., Ahmad, A. and Sastry, M. 2004. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. Journal of Colloid and Interface Science, 14: 445-449.

Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A.R. and Singh, R.P. 2011. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. Journal of Nanoparticle Research, 13: 2981-2988.

Smitha, S.L., Philip, D. and Gopchandran, K.G. 2009. Green synthesis of gold nanoparticles using *Cinnamomum zeylanicum* leaf broth. Spectrochim. Acta, Part A, 74(3): 735-739.

Song, J.Y. and Kim, B.S. 2009. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst. Eng., 32: 79-84.

Song, J.Y., Kwon, E.Y. and Kim, B.S. 2010. Biological synthesis of platinum nanoparticles using *Diospyros kaki* leaf extract. Bioprocess Biosyst. Eng., 33: 159-164.

Subbarao, Y., Kotakadi, V.S., Prasad, T.N.V.K.V., Reddy, A.V. and Sai Gopal, D.V.R. 2013. Green synthesis and spectral characterization of silver nanoparticles from Lakshmi tulasi (*Ocimum sanctum*) leaf extract. Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy, 103: 156-159.

Sundrarajan, M., Selvam, S. and Ramanujam, K. 2013. Synthesis of sulfated αcyclodextrin/cotton/ZnO nanocomposite for improve the antibacterial activity and dyeability with *Azadirachta indica*. Journal of Applied Polymer Science, 128: 108-114.

Veerasamy, R., Xin, T.Z. and Gunasagaran, S. 2010. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their anti-microbial activities. J. Saudi Chem. Soc., 15: 113-120.

Vijayaraghavan, K. and Nalini, S.P.K. 2010. Biotemplates in the green synthesis of silver nanoparticles. Biotechnology Journal, 5: 1098-1110.

Vijayaraghavan, K., Nalini, S.P.K., Prakash, N.U. and Madhankumar, D. 2012. Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum*. Materials Letters, 75: 33-35.

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Spatio-Temporal Change Detection and Its Impact on the Waterbodies by Monitoring LU/LC Dynamics - A Case Study from Holy City of Ratanpur, Chhattisgarh, India

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ABSTRACT

The holy city of Ratanpur is situated in the Bilaspur district of the Central Indian state of Chhattisgarh. In the past few decades, the wetlands and water bodies of Ratanpur have been subjected to various anthropogenic pressures and undergone changes in land use land cover (LULC) patterns. The paper focuses on assessing the changes in land use and land cover in and around Ratanpur city from 1989 to 2015 using LANDSAT satellite imageries. The processing of satellite imageries and quantitative assessment of LULC data was done using ArcGIS and ERDAS Imagine software. From the current study, it is evident that the numbers and quality of ponds have decreased resulting in decreased numbers and frequency of avian fauna in the area. Earlier water bodies covered an area of 3.76% which has decreased to 2.06%. The reduction in areas covered under water bodies has increased in the dry watercourse area (3%) and river bed area (0.80%). As seen from 2015 data the built-up land areas have expanded by 2.22% as compared to 1989. A considerable decrease in open forest area (8.21%) and agricultural land (3.97%) has been witnessed, whereas the area occupied by scrubland (6.42%), wasteland (1.18%), and built-up land (1.99%) has increased. The Spatio-temporal LULC changes of the study area can be used to monitor, plan, and implement proper town and country planning to maintain the sustainable environment of Ratanpur city. The adverse impact of urban growth in the surface water bodies/ponds must be regulated by taking suitable conservation measures at the individual and community level for maintaining the biodiversity and aesthetic beauty of the area.

INTRODUCTION

Freshwater is a finite resource as it accounts for less than 1% of the earth's available water. As the availability of freshwater is uneven on the earth's surface, arid and semi-arid regions receive only 2% of all surface runoff yet account for 40% of the global land area. Thus, there is huge anthropogenic pressure on surface water bodies to satisfy their ever-increasing demand. Waterbodies play a very important role to fulfil human water needs. Apart from this, water bodies help in regulating the local climatic conditions, provides shelter to aquatic and avian biodiversity, recharges the groundwater aquifers, and also enhances the aesthetic beauty of the area. Globally, the surface water bodies are shrinking due to multiple factors such as an increase in population, industrialization, increased agricultural activities, etc.

The land use land cover (LULC) change is a continuous process (Mondal et al., 2016). Rapid replacement of Landcover is observed by various land-use patterns (Lambin & Geist 2001). The pace of change in LC to LU depends upon the growth rate of the population of the area and their needs along with the natural and economic drivers (Hund et al., 2013, Prasad et al., 2012). The choice of economic growth over ecological sustainability leads to rapid and drastic changes in LULC patterns thereby resulting in depletion of natural resources like forest, water bodies, soil, etc.(Beckerman 1992). In and around the forest area, indigenous flora and fauna are threatened by the budding of residential and built-up areas. With time the major land cover comprising of forest and water gets transformed into urban areas, settlements, built-up areas, and agricultural lands. Over a period, agricultural lands also get metamorphose into built-up areas, thereby resulting in depletion in hydrological resources and greenery (Salghuna et al.2018). The Supervised classification method has been used by various researchers (Butt et al. 2015, Rawat & Kumar 2015, Boori et al. 2015, Islam et al. 2018, Kar et al. 2018, Nagarajan & Poongothai 2011, Rouse et al. 1973, Alcamo et al., 2010) to study the LULC change of the global landscapes.

Alteration in wetland due to LULC change has been observed by various researchers (Ozsahin et al., 2018, Praveen et al., 2018 and Alam et al., 2011). The anthropogenic activities and development processes affect the habitat structure thereby affecting the natural properties such as water, wetlands, and biodiversity (Gibbard et al., 2005, Dhere & Pondhe 2018). analyses the impact of unregulated and unplanned development on the Bhima river of the Pune district. Spatial changes in the LULC pattern of the Manas River Basin have been studied and analyzed between the periods of 1976-2006 (Zhang et al., 2012). Two decadal changes in the LULC pattern of Delhi NCR (National Capital Region) has been studied by (Suzanchi & Kaur 2011) by using RS & GIS technology.

The holy city of Ratanpur situated in the Central Indian state of Chhattisgarh has a long historical record. The city was established by Ratnaraja (Grandson of Kalingaraja). In 1407, the Ratanpur kingdom was divided into two parts- one is Ratanpur and the other was Raipur (Jha 1997). The ancient capital of Chhattisgarh is famous for its historical importance and culture. The administrative unit of Ratanpur lies under Nagar panchayat of Bilaspur district of Chhattisgarh. Ratanpur is famous for its religious monuments such as Mahamaya Temple, Baba Bhairavnath Temple, Bhuddeshwar Shiva Temple, Ratneshwar Mahadev Temple, Girijaban Hanuman Temple, Lakhani Devi Temple, Ram Tekri, Ashok Vatika, and Jagannath Temple, etc. Apart from that, the area is bestowed by good scenic beauty such as undulating landscapes, rich forests, agricultural fields, and a large number of ponds which attracts the attention of a large number of tourists. Various studies have also been done on the wetlands and aquatic fauna and floral diversity of the Ratanpur area (Dwivedi 2014, Singh & Shahi 2017, Porte et al. 2018, Dwivedi & Singh 2017) but no studies have been conducted on LULC changes.

STUDY AREA

The Ratanpur city is located in Bilaspur district of Chhattisgarh, India. It is also known as a city of ponds due to the presence of numerous surface water bodies and is situated in Kota block at a distance of about 25 kilometres from Bilaspur city (Fig. 1). The geographical extent of the place lies between 82°16′66.18″E-82°13′14.28″E and 22°30′51.20″N-22°26′71.58″N latitude, having an altitude of 306 m above mean sea level. The area is characterized by three distinct seasons such as summer, monsoon, and winter. The average annual rainfall of the Bilaspur district between the periods of 1990-2015 was 1211.89 mm (Fig. 2).

Population Status of Ratanpur

Ratanpur area comes under Nagar Palika of Bilaspur district of Chhattisgarh state, covering the 15 wards. According to the Census 2011, it consists of a total of 10,806 households, with a population of 49,272 (25268 males and 24004 females). During the year 2001, 39678 individuals resided in the area. In the year 2001 the population density of the area from 468.01 people/km^2 while in the year 2011 it reached 581.17 people/km^2. A demographic detail of the study area was collected from Census data for the years 2001 and 2011 by the Government of India.

MATERIALS AND METHODS

Data Used

The satellite imageries were provided by NASA-GLCF (Global Land Cover Facility) and USGS (United States Geological Survey), the satellite imageries were downloaded from the USGS website (www.usgs.com). The Landsat satellite imageries for 1989, 1995, 2000, 2005, 2010, and 2015 have been used for image processing and interpretation. Cloud free satellite imageries during the winter months were selected to minimize the seasonal variation in data due to spectral reflectance. The Survey of India (SOI) Toposheet number

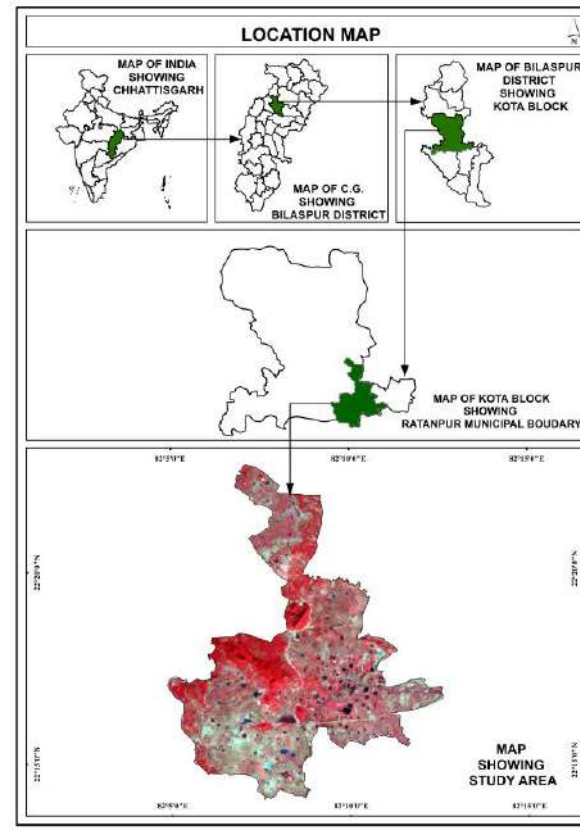


Fig. 1: Location map of Ratanpur.

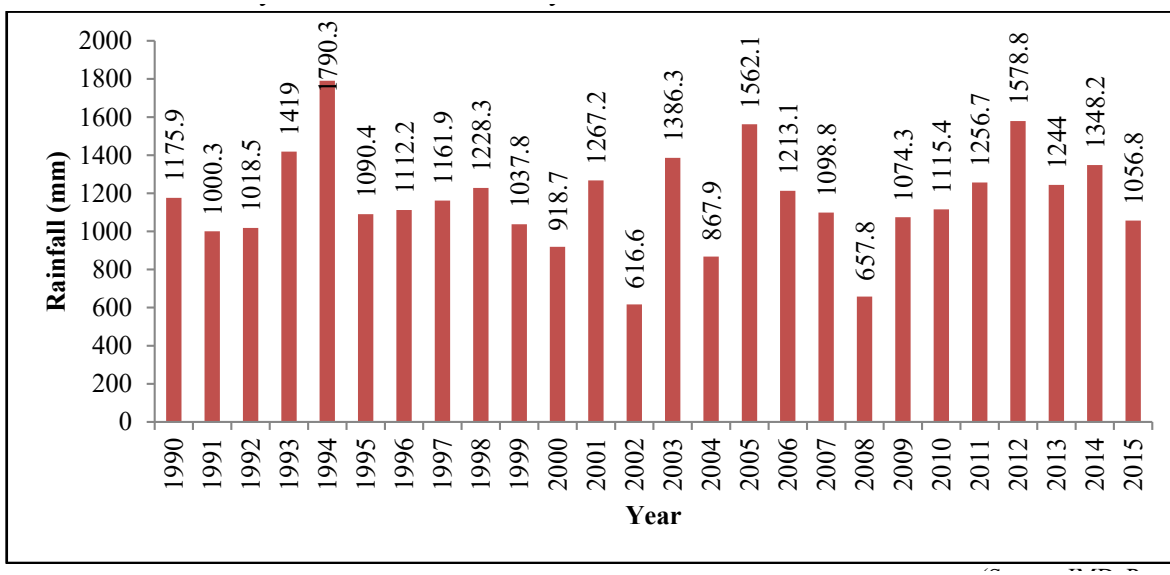
64J03, 64J04 at 1:50,000 scales were used for the study. The master plan of Ratanpur municipal area is downloaded from Town and Country Planning, Government of Chhattisgarh website (www.town&countryplanning.com). Characteristics of Landsat satellite data are illustrated in Table 1.

Image Processing

The LULC change detection and analysis is done using Landsat satellite data. The LULC map for the years 1989, 1995, 2000, 2005, 2010 and 2015 was prepared using ArcGIS, and supervised classification has been done using ERDAS Imagine software. Geo-referencing of master plan map was performed using 4-6 Ground Control Points (GCP) collected from Landsat satellite data of 2015 and digitalization of study area boundary using Georeferenced Ratanpur Nagar Palika map. Pre-processing of satellite imageries includes processes such as layer stacking False Colour Composition (FCC) format of band combination 3, 4, and 5 bands followed by masking of the study area by using the subset tool. The subset imageries are then projected to UTM (Universal Transverse Mercator) zone 43 N. As six different satellite imageries were used during the study, supervised classification of the satellite data was performed using Maximum Likelihood Method taking into consideration the LULC classes of the area. Supervised classification was done in ERDAS Imagine software and map composition has been prepared using ArcGIS software. The thematic maps derived were assessed for change in LULC classes from 1989 to 2015. The later LULC maps (2010 and 2015) were considered for changes mostly focusing on built-up land. Validation of past LULC changes with 2015 data was useful in knowing the extent of increase in built-up class, dry watercourse and reduction in water bodies, etc.

Change Detection

For each LULC categories, the magnitude of change was



(Source: IMD, Pune)

Fig. 2: Average annual rainfall (mm) of Bilaspur district.

Table. 1: Description of the satellite imageries.

Satellite	Sensor	Path/ Row	Acquisition Date	Spatial Resolution (m)
Landsat 4	TM	142/45	09/12/1989	30
Landsat 4	TM		10/12/1995	30
Landsat 7	ETM+		15/12/2000	30
Landsat 4	TM		19/11/2005	30
Landsat4	TM		19/12/2010	30
Landsat 8	OLI/TIRS		01/12/2015	30

Note: TM - (Thematic Mapper), ETM+ - (Enhanced Thematic Mapper Plus), OLI/TIRS - (Operational Land Imager/Thermal Infra-Red Sensor)

calculated by subtracting the area covered by the second year and the initial year as shown in the equation (1)

$$\text{Magnitude of change} = \text{magnitude of new year} - \text{magnitude of previous year} \quad ...(1)$$

Change in percentage for each LULC categories was calculated by dividing the magnitude of change by the initial year and multiplied by 100 as shown in the equation (2)

$$\text{Change (\%)} = \frac{\text{magnitude of change} \times 100}{\text{Initial year}} \quad ...(2)$$

The annual rate of change for each LULC categories was calculated by subtracting the final year to initial year, which was further divided by the number of study year, i.e. 1989-1995 (6 years), 1995-2000 (5 years), 2000-2005 (5 years), 2005 -2010 (5 years), 2010-2015 (5 years) respectively using the equation (3)

$$\text{Annual rate of change} = \frac{\text{Final year} - \text{initial year}}{\text{Number of years}} \quad ...(3)$$

Accuracy Assessment

A classification is incomplete until its accuracy has been assessed. The term accuracy means the level of agreement between labels assigned by the classified and class allocation based on ground data collected by the user, known as test data (Pal & Mather 2006). The accuracy assessment of supervised classification imageries was performed in ERDAS IMAGINE software using Google earth by synchronizing the Ground Location Points. About 100 ground locations were randomly selected in the classified imageries and with the help of the Google earth synchronization tool accuracy assessment of the supervised imageries was performed. It includes an assessment of overall accuracy, kappa statistics, producer's accuracy, and user's accuracy of the LULC classes for the supervised imageries of the year 1989, 1995, 2000, 2005, 2010, and 2015, respectively.

RESULTS AND DISCUSSION

The LULC classes are categorized into nine groups including (1) Agriculture land (2) Dense forest (3) Open forest (4) Scrubland (5) Wasteland (6) Built-up land (7) Waterbody (8) Dry watercourse and (9) Riverbeds. Water bodies include all the surface water bodies of the area filled with water. The dry watercourse is the area which was earlier covered by water bodies and is dried at present and the area of the riverbed is the areas adjoining the water body which are covered by sand. The aerial distribution of various LULC classes for the years 1989, 1995, 2000, 2005, 2010, and 2015 was prepared and their change scenario in between different time frames are shown in Table 2 and Table 3. After the classification of satellite imageries based on the above LULC categories, relevant map layouts were prepared on 1:70,000 scales (Fig. 3)

Dynamics of LULC Change of Ratanpur during the Year 1989 and 1995

Landsat TM imageries have been used to assess the past LULC pattern of the study area. The 9 LULC categories have been identified for the years 1989 and 1995 as shown in Table 2 and fig. 3. A total of 84.78 km^2 was estimated for the whole Ratanpur area. Among the above land use categories, agricultural land remained the dominant land use type (covering 52.10 km^2 and 52.16 km^2) followed by open forest during the years 1989 and 1995. The areas covered under open forest decreased from 16.82 km^2 (19.84 %) in the year 1989 to 10.40 km^2 (12.27 %) in 1995. During six years, the areas covered under scrubland, wasteland, and built-up land increased by 4.53%, 1.55 %, and 0.65%, whereas a decline in trend has been observed for the dense forest and open forest area by 0.32% and 7.57% respectively. The surface water bodies covered an area of 3.19 km^2 (3.76 %) during the year 1989 which reduced to 2.16km^2 (2.55 %) by the year 1995. This reduction in the water body area positively coincides with an increase of dry watercourse area (1.97 %) and riverbed occupied area (0.34 %) between the periods of 1989 -1995.

Dynamics of LULC Change of Ratanpur During the Year 1995 and 2000

Between the years 1995 and 2000, the agricultural area increased by 2.17 km^2 (2.56 %). Further reduction in areas covered under open forest area (1.74 %), wasteland (1.15 %), riverbed (0.37 %). The LULC categories which showed an increasing trend during the period was dense forest (1.45 %) and scrubland (0.16 %). The water body area increased by 0.53 km^2 (0.62 %), which resulted in a reduction in dry watercourse area by 1.52 km^2 (1.79 %), this rise in water body area is due to good rainfall over the study area and its catchments during this period.

Dynamics of LULC Change of Ratanpur During the Year 2000 and 2005

A total of nine LULC classes were identified similar to the previous years. It was noticed that the agricultural land was still the dominant land use type which shared 54.33 km^2 and 48.91 km^2during 2000 and 2005. The areas covered under open forest and scrubland have increased by 5.72% and 0.55%, whereas dense forest area reported decreasing by 2.01 %. The dry watercourse area increased by 1.78 %, followed by the riverbed area by 0.24%.

Dynamics of LULC Change of Ratanpur During the Year 2005 and 2010

During the years 2005 and 2010, agricultural land increased from 48.91 km^2 to 50.29 km^2. The other LULC categories

such as built-up land (0.37%), dense forest (0.72%), dry watercourse (0.90%), river bed (0.46 %), scrubland (0.44 %), scrubland (0.33%) have shown increasing trend between the interval of 5 years. The decreasing trend has been reported in areas covered under open forest (3.63%) and water bodies (1.22%).

Dynamics of LULC Change of Ratanpur During the Year 2010 and 2015

During this period, the open forest area continues to decrease from 10.7 km^2 (12.62%) to 9.86 km^2 (11.63%), reduction in water body area have also been reported by 1.44 km^2 (1.69%) followed by dense forest by 0.13 km^2 (0.15%). The conversion of dense forest area, open forest area, and agricultural land into scrubland (6.42%) has also been reported during this time frame. The LULC categories which recorded an increasing trend are dry watercourse area (3%), built-up land (1.99%), riverbed area (1.43%), and wasteland (1.19%).

The LULC pattern of the study area was depicted in Fig. 3. Here the yellow patch indicates agricultural land which was more prominent in the Ratanpur area as agricultural activities are the main source of income for the residents. The dark green patch indicates the dense forest area in the western and south western region of the study area, whereas the light green colour indicates the open forest area. Red colour shows the built-up land, pink indicates a dry watercourse, brown colour indicates the wasteland area. A blue shade represents water bodies (dark blue) and riverbed (light blue).

Table 2: Category wise LULC distribution of Ratanpur (1989-2015).

LULC category	1989		1995		2000		2005		2010		2015	
	Area (sq. km)	Area (%)	Area (sq. km)	Area (%)	Area (sq. km)	Area (%)	Area (sq. km)	Area (%)	Area (sq. km)	Area (%)	Area (sq. km)	Area (%)
Agriculture Land	52.10	61.45	52.16	61.52	54.33	64.08	48.91	57.69	50.29	59.32	48.74	57.49
Built Up Land	1.88	2.22	2.43	2.87	2.65	3.13	2.66	3.14	2.98	3.51	3.57	4.21
Dense Forest	4.07	4.80	3.8	4.48	5.03	5.93	3.32	3.92	3.93	4.64	3.94	4.65
Dry Water Course	0.90	1.06	2.57	3.03	1.05	1.24	2.56	3.02	3.32	3.92	3.44	4.06
Open Forest	16.82	19.84	10.4	12.27	8.93	10.53	13.78	16.25	10.7	12.62	9.86	11.63
River Beds	0.68	0.80	0.97	1.14	0.65	0.77	0.86	1.01	1.25	1.47	1.89	2.23
Scrub Land	3.19	3.76	7.03	8.29	7.16	8.45	7.63	9.00	8	9.44	8.63	10.18
Waste Land	1.95	2.30	3.26	3.85	2.29	2.70	2.12	2.50	2.4	2.83	2.96	3.49
Water Body	3.19	3.76	2.16	2.55	2.69	3.17	2.94	3.47	1.91	2.25	1.75	2.07
Total	84.78	100.00	84.78	100.00	84.78	100.00	84.78	100.00	84.78	100.00	84.78	100.00

Source: Landsat satellite imageries from 1989-2015 and data extraction as well as compilation done by authors using ArcGIS and ERDAS IMAGINE software.

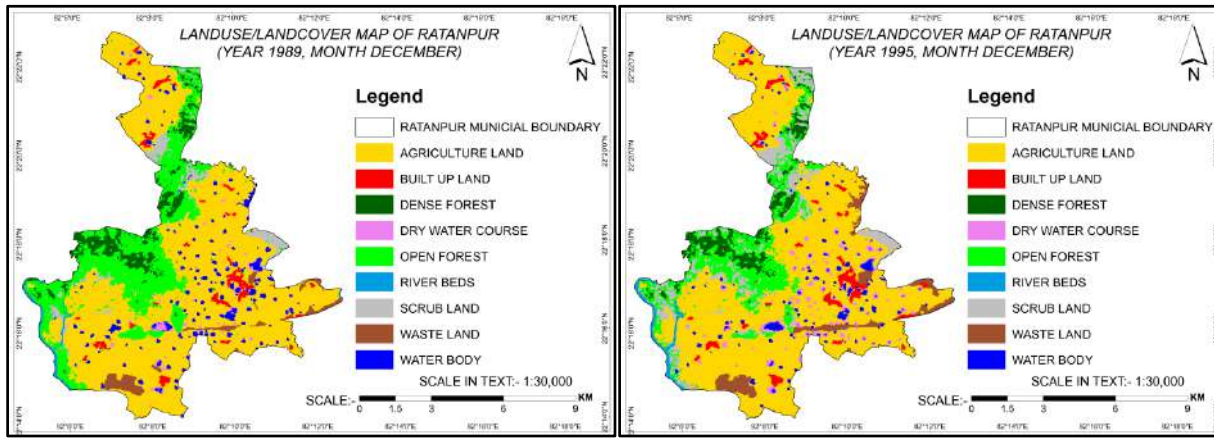


Fig. Cont....

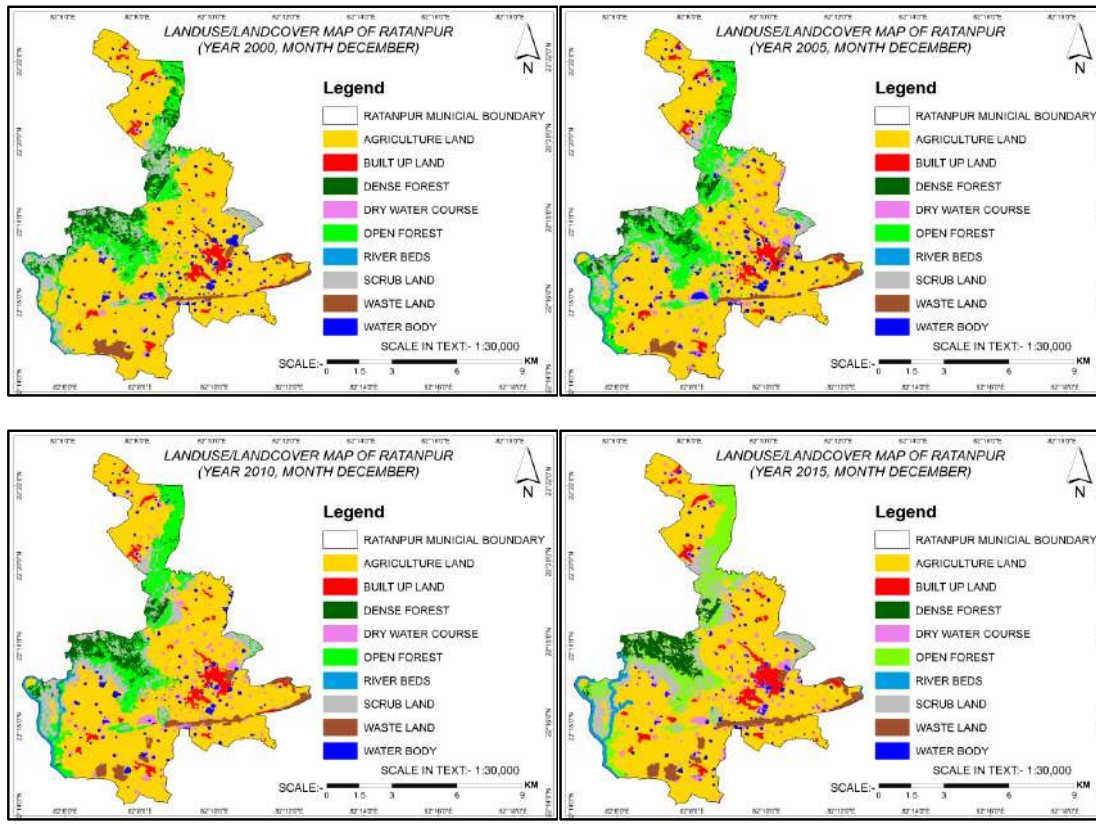


Fig. 3: Spatiotemporal LULC map of Ratanpur for the years 1989, 1995, 2000, 2005, 2010, and 2015.

Accuracy Assessment of the Supervised Classification Imageries

The accuracy assessment of the supervised imageries was performed taking into consideration the LULC classes. The producers' and users' accuracy of the LULC classes were illustrated in Table 4. The overall accuracy of 92 %, 97 %, 95%, 96%, 93% and 87% were recorded for the year 1989, 1995, 2000, 2005, 2010 and 2015. The Kappa coefficient which lies on a scale between 0 (no reduction in error) and 1 (complete reduction of error) was also analyzed for the above-mentioned imageries. The Kappa statistics value of 0.8619, 0.9447, 0.8963, 0.9406, 0.8942 and 0.7981 was recorded for the year 1989, 1995, 2000, 2005, 2010 and 2015.

Relative Changes in LULC Dynamics of the Study Area from 1989-2015

Overall change dynamics in the LULC pattern of the Ratanpur area show a major reduction in open forest area (8.21%) followed by agricultural land (3.97%). The decline in agricultural activities can be positively correlated with a decrease in water bodies (1.70%), as most of the agricultural land of the area is rain-fed. Reduction in surface water bodies resulted in a rise in the dry watercourse area (3%) which was earlier covered with water. The extent of change in areas covered by water bodies changes from year to year due to variation in temperature and rainfall of the region. Due to the dumping of wastes and effluents, the water bodies are converting into a eutrophic state resulting in the growth of aquatic weeds floating over the water as shown in Fig. 4

(Porte & Gupta 2017) have studied the comparative distribution pattern of wetland birds of polluted and unpolluted ponds of Ratanpur. Their study revealed that scared ponds such as Mahariya, Jagannath, Gireejaban, Dulahara, Bikma, and Bairagban Ponds remain unpolluted due to regional beliefs, but the ponds without having religious importance (Kaira, Bhedimuda, Krishnarjuni and Rateshwar Ponds) are more polluted due to anthropogenic influences. Apart from irrigating the crops these water bodies provide shelter to a large number of wetland birds along with migratory avian species, thereby very important to maintain the ecological integrity of the area.

Various developmental activities, a rise in tourist visitors, and human encroachment have increased in settlements. Accelerated anthropogenic activities such as increased irrigation, dumping of garbage and sewage in the water bodies

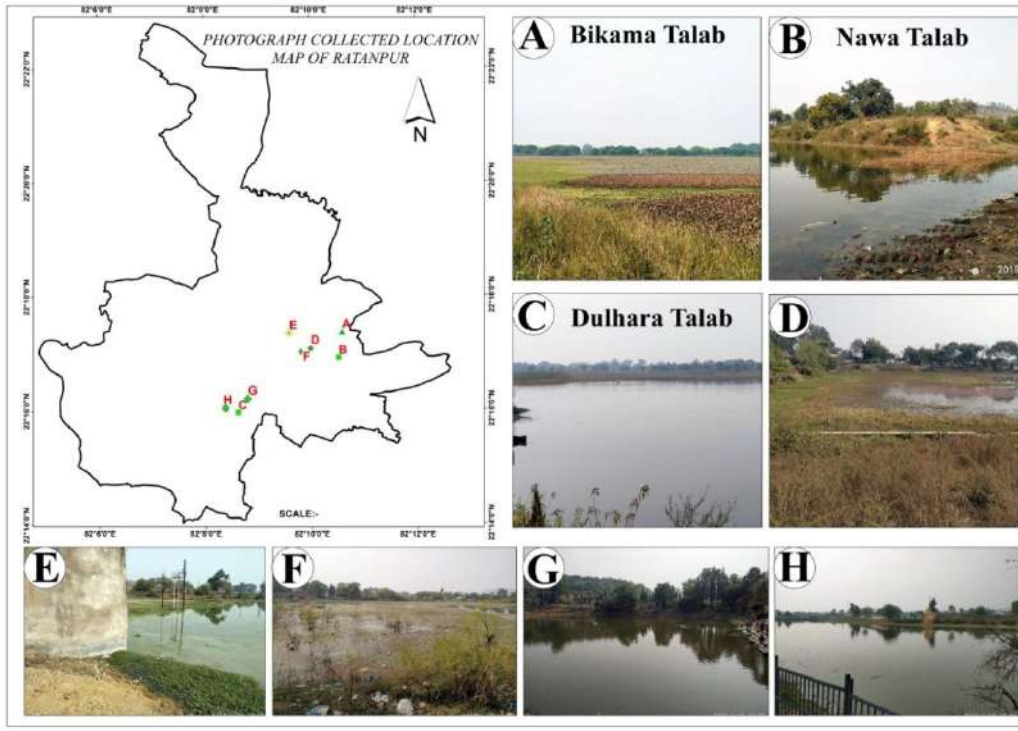
Table 3: LULC change assessment of Ratanpur based on time frame data (1989-2015).

LULC category	LULC change (1989-2000)			LULC change (2000-2010)			LULC change (2010-2015)			LULC change (1989-2015)		
	Magnitude Area (sq. km)	Area (%)	Annual rate of change	Area (sq. km)	Area (%)	Annual rate of change	Area (sq. km)	Area (%)	Annual rate of change	Area (sq. km)	Area (%)	Annual rate of change
Agriculture Land	(+) 2.23	(+) 2.63	(+)0.20	(-) 4.04	(-) 4.77	(-) 0.40	(-) 1.55	(-) 1.83	(-) 0.31	(-) 3.36	(-) 3.96	(-) 0.13
Built Up Land	(+) 0.77	(+) 0.91	(+) 0.07	(+) 0.33	(+) 0.39	(+) 0.03	(+) 0.59	(+) 0.70	(+) 0.12	(+) 1.69	(+) 1.99	(+) 0.07
Dense Forest	(+) 0.96	(+) 1.13	(+) 0.09	(-) 1.10	(-) 1.30	(-) 0.11	(+) 0.01	(+) 0.01	(+) 0.00	(-) 0.13	(-) 0.15	(-) 0.01
Dry Water Course	(+) 0.15	(+) 0.18	(+) 0.01	(+) 2.27	(+) 2.68	(+) 0.23	(+) 0.12	(+) 0.14	(+) 0.02	(+) 2.54	(+) 3.00	(+) 0.10
Open Forest	(-) 7.89	(-) 9.31	(-) 0.72	(+) 1.77	(+) 2.09	(+) 0.18	(-) 0.84	(-) 0.99	(-) 0.17	(-) 6.96	(-) 8.21	(-) 0.27
River Beds	(-) 0.03	(-) 0.04	(+) 0.00	(+) 0.60	(+) 0.71	(+) 0.06	(+) 0.64	(+) 0.75	(+) 0.13	(+) 1.21	(+) 1.43	(+) 0.05
Scrub Land	(+) 3.97	(+) 4.68	(+) 0.36	(+) 0.84	(+) 0.99	(+) 0.08	(+) 0.63	(+) 0.74	(+) 0.13	(+) 5.44	(+) 6.42	(+) 0.21
Waste Land	(+) 0.34	(+) 0.40	(+) 0.03	(+) 0.11	(+) 0.13	(+) 0.01	(+) 0.56	(+) 0.66	(+) 0.11	(+) 1.01	(+) 1.19	(+) 0.04
Water Body	(-) 0.50	(-) 0.59	(-) 0.05	(-) 0.78	(-) 0.92	(-) 0.08	(-) 0.16	(-) 0.19	(-) 0.03	(-) 1.44	(-) 1.70	(-) 0.06

Note: (I) LULC change assessment based on interim years 1989-2000, 2000- 2010, 2010-2015 and overall 1989-2015 respectively and (II) (+) sign denotes increase and (-) sign denotes a decrease of magnitude of change of LULC categories in the different time frame.

Table 4: Accuracy assessment for the supervised classification imageries showing producers accuracy, users accuracy, overall accuracy, and Kappa statistics for the time frame data (1989-2015).

LULC categories	1989		1995		2000		2005		2010		2015	
	Producers accuracy	Users accuracy	Producers accuracy	Users accuracy	Producers accuracy	Users accuracy	Producers accuracy	Users accuracy	Producers accuracy	Users accuracy	Producers accuracy	Users accuracy
Agriculture Land	98.31	90.63	100.00	98.48	100.00	93.15	100.00	94.44	100.00	92.73	96.36	89.83
Built-Up Land	60.00	100.00	100.00	100.00	60.00	100.00	50.00	100.00	85.71	100.00	50.00	75.00
Dense Forest	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Dry Water Course	-	-	100.00	66.67	100.00	100.00	83.33	100.00	100.00	100.00	66.67	66.67
Open Forest	100.00	94.74	92.86	100.00	88.89	100.00	100.00	100.00	92.86	81.25	91.67	91.67
River Beds	-	-	100.00	100.00	-	-	100.00	100.00	-	-	100.00	66.67
Scrub Land	62.50	83.33	100.00	88.89	90.91	100.00	100.00	100.00	78.95	100.00	66.67	75.00
Waste Land	50.00	100.00	-	-	100.00	100.00	33.33	100.00	50.00	100.00	66.67	100.00
Water body	100.00	100.00	50.00	100.00	-	-	100.00	50.00	-	-	50.00	100.00
Overall accuracy	92.00		97.00		95.00		96.00		93.00		87.00	
Kappa statistics	0.8619		0.9447		0.8963		0.9406		0.8942		0.7981	



(Photo courtesy: Mr. Jai Prakash Koshale)

Fig. 4: Drivers of change in wetlands A) Growth of weed (A, D, E) ii) Dumping of waste, garbage, etc. on the ponds (B, F).

resulting in habitat fragmentation and loss thereby leading to a decline in numbers and quality of the existing ponds in the area. The growing demand for water resources is likely to have negative consequences for ecosystems and the wildlife they support (Stommel et al. 2016). Large-scale utilization of water is responsible for a significant reduction in water level and its quality (Stommel et al. 2015) during the dry seasons.

During 26 years, the built-up land has increased by 1.99% with an annual rate of change of 0.07 km^2. (Suzanchi & Kaur 2011) reported the transformation of 13.25% of water bodies into built-up land in the NCR region, India which noticed the decline in water bodies by (49.5%) during 1998-2006. Wasteland, scrubland and river bed area has also reported an increase in the due course of time. Increased urban area and settlement during the period are depicted in Fig. 3. The influence of anthropogenic disturbance was recorded higher in the central portion of the area, affecting the ponds of that area. Information obtained by LULC change detection will help in providing better options for effective management of the land and water resources and also cope with the rise in population due to urbanization. Thus, the dynamics of LULC changes and its consequences is essential to study and implement better planning for developmental projects along with sustained survival of biodiversity and hydrology of the area.

CONCLUSION

Geospatial technology such as RS and GIS has been used to study the LULC change dynamics of Ratanpur city between the period of 1989 and 2015. The results show a major reduction in the open forest area (8.21%) which is directly or indirectly related to the conversion of scrubland, wasteland, and built-up land. The built-up area of the study area has been reported to increase by 1.69 km^2 (1.99%). Huge urban expansion and developmental activities were developed around the area which is damaging the wetlands and ecology of the area. The water bodies covered an area of 3.76% during the year 1989 decreased to 2.07% by the year 2015. This decrease in water body area increased the dry watercourse area (3%) and river bed area (1.43%). As, the agricultural activity is rain-fed and partially on the irrigation by the available surface water bodies, resulting in a decrease in the agricultural area by 3.97 % during this

period. The study may also be used to assess the change in water bodies and their conversion into other land use categories. Thus, the present study may provide very important data for urban expansion and utilization/overexploitation of water resources. The information may also use for the restoration and conservation of water bodies in and around the city.

The study demonstrates the potential of using Landsat satellite imageries in LULC change detection and assessment and provides huge potential for implementing proper urban planning and management. Because of capital city development, emphasis must be given to sustained economic development by taking into consideration environmental and natural resources such as water bodies, land, and forest as important factors. Also, there is an urgent need for sustainable utilization of water resources, formulation of climate adaption strategies, making the people and government aware about shrinking water resources as well as taking urgent action for conservation and harvesting of water.

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REFERENCES

Alam, A., Rashid, S. M., Bhat, M. S. and Sheikh, A. H. 2011. Impact of land use/land cover dynamics on Himalayan wetland ecosystem. Journal of Experimental Sciences, 2(3): 60-64.

Alcamo, J., Flörke, M., Märker, M., Alcamo, J., Flörke, M. and Märker, M. 2010. Future long-term changes in global water resources driven by socio-economic and climatic changes. Hydrological Sciences Journal, 52(2): 247-275.. https://doi.org/10.1623/hysj.52.2.247

Butt, A., Shabbir, R., Ahmad, S. S., Aziz, N., Nawaz, M. and Shah, M. T. A. 2015. Land cover classification and change detection analysis of Rawal watershed using remote sensing data. J. Biol. Environ. Sci., 6(1): 236-248.

Beckerman, W. 1992. Economic growth and the environment: Whose growth? Whose environment? World Development, 20(4), 481-496. https://doi.org/10.1016/0305-750X(92)90038-W

Boori, M. S., Voženílek, V. and Choudhary, K. 2015. Land use/cover disturbance due to tourism in Jeseníky Mountain, Czech Republic: A remote sensing and GIS-based approach. The Egyptian Journal of Remote Sensing and Space Science, 18(1): 17-26. https://doi.org/10.1016/J.EJRS.2014.12.002

Dhere, A. M. and Pondhe, G. M. 2018. Application of geo-spatial technology for analyzing impact on Bhīma River Pune district. Eco Chronicle, 24-30.

Dwivedi, S. 2014. Culture and Tradition of Chhattisgarh. 3(10): 13-15.

Dwivedi, U. and Singh, R. K. 2017. Biodiversity of migratory birds in Khutaghat, Ratanpur District, Bilaspur (C.G.). World Journal of Pharmacy and Pharmaceutical Sciences, 6(7): 1148-1155

Gibbard, S., Caldeira, K., Bala, G., Phillips, T. J. and Wickett, M. 2005. Climate effects of global land cover change. Geophysical Research Letters, 32(23): 1-4. https://doi.org/10.1029/2005GL024550

Hund, K., Megevand. C., Gomes. E. P., Miranda, M. and Reed. E. 2013. Deforestation Trend in the Congo Basin: Mining World Bank, Washington, DC.

Islam, K., Jashimuddin, M., Nath, B. and Nath, T. K. 2018. Land use classification and change detection by using multi-temporal remotely sensed imagery: The case of Chunati wildlife sanctuary, Bangladesh. The Egyptian Journal of Remote Sensing and Space Science, 21(1): 37-47. https://doi.org/10.1016/J.EJRS.2016.12.005

Jha, M. 1997. Anthropology of Ancient Hindu Kingdoms: A Study in Civilizational Perspective. MD Publications Pvt. Ltd., pp 65.

Kar, R., Obi Reddy, G. P., Kumar, N. and Singh, S.K. 2018. Monitoring spatio-temporal dynamics of urban and peri-urban landscape using remote sensing and GIS - A case study from Central India. The Egyptian Journal of Remote Sensing and Space Science, 21(3): 401-411. https://doi.org/10.1016/J.EJRS.2017.12.006

Lambin, E.F. and Geist, H. J. 2001. Global land-use and land-cover change: What have we learned so far. Global Change Newsletter, 46: 27-30.

Mondal, M. S., Sharma, N., Garg, P. K. and Kappas, M. 2016. Statistical independence test and validation of CA Markov land use land cover (LULC) prediction results. Egyptian Journal of Remote Sensing and Space Science, 19(2): 259-272. https://doi.org/10.1016/j.ejrs.2016.08.001

Nagarajan, N. and Poongothai, S. 2011. Trend in Land Use/Land Cover Change Detection by RS and GIS Application. 3(September): 263-269.

Ozsahin, E., Duru, U. and Eroglu, I. 2018. Land use and land cover changes (LULCC), a key to understand soil erosion intensities in the Maritsa Basin. Water, 10(335): 1-15

Porte, D.S. and Gupta S. 2017. Assessment of distribution patterns of wetland birds between unpolluted and polluted ponds at Ratanpur; district Bilaspur, Chhattisgarh, India, Indian J. Sci. Res., 12(2): 204-215

Pal, M. and Mather, P.M. 2006. Some issues in the classification of DAIS hyperspectral data. Int. J. Remote Sens., 27(14): 2895-2916.

Porte D.S., Gupta, S. and Kankariya 2018. Temporal pattern in foraging behavior of the Indian pond heron, *Ardeola grayii* at Ratanpur, Chhattisgarh, India. Biological Rhythm Research, 49(5): 761-771.

Prasad, P., Rama Chandra, Lakshmi, P., Mamtha, Rajan, K. S., Bhole, Vijaya and Dutt, C.B.S. 2012. Tsunami and tropical island ecosystem - A mega-analysis of the studies in Andaman and Nicobar Islands. Biodiversity Conservation, 21(2): 309-322.

Praveen, S., Basheer, J. and Praveen, B. 2018. A literature review on land use land cover changes. Int. J. Adv. Res., 6(7): 1-6.

Rouse, J. W., Haas, R. H., Schell, J. A. and Deering, D.W. 1973. Monitoring vegetation systems in the Great Plains with ERTS. Third ERTS Symposium, NASA SP-351, Washington DC, 10-14 December 1973, 309-317.

Rawat, J. S. and Kumar, M. 2015. Monitoring land use/cover change using remote sensing and GIS techniques: A case study of Hawalbagh block, district Almora, Uttarakhand, India. The Egyptian Journal of Remote Sensing and Space Science, 18(1): 77-84. https://doi.org/10.1016/J.EJRS.2015.02.002

Salghuna, N. N., Rama Chandra Prasad, P. and Asha Kumari, J. 2018. Assessing the impact of land use and land cover changes on the remnant patches of Kondapalli reserve forest of the Eastern Ghats, Andhra Pradesh, India. The Egyptian Journal of Remote Sensing and Space Science, 21(3): 419-429. https://doi.org/10.1016/J.EJRS.2018.01.005

Singh, R.S. and Shahi S. 2017. Diversity of medicinal plants of Ratanpur region of Bilaspur district (Chhattisgarh), Journal of Medicinal Plants Studies, 5(2): 276-281.

Stommel, C., Hofer, H. and East, M.L. 2015. The effect of reduced water availability in the Great Ruaha River on the vulnerable Common Hippopotamus in the Ruaha National Park, Tanzania. PLOS ONE,11(6): 1-18.

Stommel, C., Hofer, H., Grobbel, M. and East, M.L. 2016. Large mammals in Ruaha National Park, Tanzania, dig for water when water stops flowing and water bacterial load increases. Mamm Biol., 81: 21-30.

Suzanchi, K. and Kaur, R. 2011. Land use land cover change in National Capital Region of India: A remote sensing & GIS based two decadal spatial-temporal analyses. Procedia - Social and Behavioral Sciences, 21: 212-221. https://doi.org/10.1016/j.sbspro.2011.07.044

Zhang, Q., Xu, H., Fu, J., Yu, P. and Zhang, P. 2012. Spatial analysis of land use and land cover changes in recent 300 years in Manas River Basin. Procedia Environmental Sciences, 12: 906-916.

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Microplastic Occurrence in Marine Invertebrates Sampled from Kwazulu-Natal, South Africa in Different Seasons

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ABSTRACT

The occurrence of microplastics in the environment, together with increasing temperatures as a result of climate change, has become a global concern. However, few reports are available on microplastic ingestion in lower benthic marine animals sampled from their natural environment during different seasons of the year. This study investigated microplastic uptake in *Dotilla fenestrata* (Hilgendorf 1869), *Holothuria cinerascens* (Brandt 1835) and *Pyura stolonifera* (Heller 1878) sampled from their natural environment during winter and summer. Animals sampled were cleaned of sand and attached animals and transported on ice to the laboratory. *D. fenestrata*, *H. cinerascens* (guts), and *P. stolonifera* (soft tissues) were digested in 10% potassium hydroxide (KOH) solution for 24 hours at 60°C and analysed for microplastics. The per cent occurrences of microplastics found in sampled animals were more than 95 % in both seasons, and fibres were the most predominant microplastic types found. Season significantly affect microplastic uptake in *Dotilla fenestrata* (t-test: t-value = 2.915, df = 58, p = 0.01) while it had no effect in *H. cinerascens* and *P. stolonifera* sampled. A significantly higher number of microplastics were found in *D. fenestrata* than *H. cinerascens* (ANOVA HSD: df = 2, p = 008) and *P. stolonifera* (ANOVA HSD: df = 2, p = 000) in winter while *H. cinerascens* had a higher number of microplastics than *P. stolonifera* in summer (ANOVA HSD: df = 2, p = 002). These results show that microplastic uptake in some ectotherms may be season-dependent and that feeding method impacts the accessibility of marine invertebrates to microplastics in their environment.

INTRODUCTION

The growing concern over the exponential increase in plastic production and its prevalence in the environment worldwide has increased over the last decade (Duis & Coors 2016, Lusher et al. 2017). Improper disposal of plastics, poor management, accidental loss and degradation have resulted in the ubiquitous presence of microplastics (< 5 mm) in the entire ecosystem (Au et al. 2017, Duis & Coors 2016). Microplastics small size, varied densities and proliferation in the environment have made them readily available and accessible to a wide range of organisms (Tosetto et al. 2017). Increasing temperature due to climate change is another global concern (O'Brien & Leichenko 2000). The world oceans are experiencing net warming (Bindoff et al. 2007) and the water temperature has profound influences on physiological and metabolic activities of ectotherms (Cossins & Bowler 1987, Yukihira et al. 2000). Increased temperature will also speed up the disintegration of macroplastics to microplastics due to photochemical processes activated by ultraviolet (UV) light and result in increased microplastic concentration in the environment (Cao et al. 2017). Increased precipitation and heavy rainfall (Loo et al. 2015), extreme and frequent flood events (Woodward et al. 2010) associated with global climate change can facilitate plastics transport from land into the ocean. Increased wave action due to sea-level rise will increase the mechanical breakdown of larger plastic to microplastics (Vianello et al. 2013).

Some studies have documented microplastic uptake in marine invertebrates sampled from their natural environment. This includes decapod crustacean (*Nephrops norvegicus*) from Clyde Sea (Murray & Cowie 2011), gooseneck barnacles (*Lepas spp.*) from the North Pacific Subtropical Gyre (Goldstein & Goodwin 2013), mussels from Nova Scotia's Eastern shore (Mathalon & Hill 2014), brown shrimp (*Crangon crangon*) from the Southern North Sea and channel area (Devriese et al. 2015), blue mussels (*Mytilus edulis*) and lugworm (*Arenicola marina*) from the French, Belgian and Dutch North Sea coastlines (Cauwenberghe et al. 2015). However, microplastic uptake in marine invertebrates at different seasons has not been investigated despite the possible influence of season on microplastic availability for uptake in the environment and ectotherms metabolic activities. This study investigates microplastics

occurrence in *Dotilla fenestrata*, *Holothuria cinerascens* and *Pyura stolonifera* sampled from their natural environment at different seasons. Ecologically, benthic invertebrates such as filter and deposit feeders are vital components of food webs that support higher vertebrates (Sokolova & Lannig 2008). Suspension feeding and filter-feeding animals (*H. cinerascens* and *P. stolonifera*) are said to be an essential link between the suspended phytoplankton and higher trophic levels in aquatic food chains (Riisgard & Larsen 2010). *Dotilla fenestrata* as a basal consumer links detritus and primary producers with higher trophic levels (Vermeiren & Sheaves 2015). *Holothuria cinerascens* are important prey to a vast array of predators such as sea stars, sea otters (*Enhydra lutris*) triton snails, crustaceans such as crabs and gastropods, fishes, some birds, turtles, marine mammals, and humans also consume sea cucumbers (Purcell et al. 2016). A great variety of predators such as polychaetes, crabs, gastropods, fish and other omnivores and carnivorous invertebrates, birds, and man feed on ascidians (Manriquez et al. 2016). *Dotilla* is a food source for many bird species and other animals inhabiting mangroves (Pereira & Goncalves 2000).

MATERIALS AND METHODOS

Ethical approval: Ethical approval with protocol reference number AREC/069/016D for collection and experimentation of *D. fenestrata* was obtained from the University of KwaZulu-Natal ethics committee. Experimentation of *Pyura stolonifera* and *Holothuria cinerascens* did not require an ethics permit (AREC). However, all animals were collected according to the Department of Agriculture, Forestry, and Fisheries (DAFF) permit number (RES2017/71 and RES2018/101). Transportation and experimentation of sampled animals were done following the University of KwaZulu-Natal ethics requirements.

Sample collection: According to Durban weather chat, July and February are the coolest and warmest months in Durban (KwaZulu-Natal) (www.weather-and-climate.com). Thus, animals for winter and summer studies were collected in July and February respectively. During winter (July 2017) and summer (February 2018), 30 sea squirts and 30 sea cucumbers were collected at low tide from the intertidal zone of Park Rynie Beach (30°19′ S; 30°44′ E) located on the southern KwaZulu-Natal, on the east coast of South Africa. In the field, attached marine invertebrates and shells were carefully detached from sampled animals. Also, 30 crabs were collected at low tide by shovelling and handpicking from Durban harbour (29º52’ S; 31º02’ E) on the east coast of KwaZulu-Natal, South Africa. The sites for animals’ collection were chosen based on their abundance in these locations. The reason for the difference in sampling sites was because *H. cinerascens* and *P. stolonifera* are intertidal animals found in rocky shores attaching to rocks while *D. fenestrata* is found occupying intertidal sandy shores (Manzoor et al. 2016). To remove mud and debris, sampled animals were thereafter rinsed with seawater. They were immediately placed in ice to reduce metabolism and to prevent loss of microplastics through depuration (Lusher et al. 2017). Animals were transported to the laboratory at University of KwaZulu-Natal and kept in freezers (-20 °C) before digestion procedures.

Sample analysis: Prior to dissection and digestion, animals were rinsed several times in deionised water to remove any attached microplastics (Desforges et al. 2015). After dissection, sea cucumber (gut), sea squirt soft tissues (Cauwenberghe et al. 2015) and crab (with shell) due to their small size and ability to uptake microplastics into the gills (Watts et al. 2014) were weighed to the nearest 0.1 g (Lusher et al. 2017) and placed in clean glass honey jars. Individual animals were digested in 150 mL of 10 % KOH for 24 h at 60°C (Dehaut et al. 2016, Lusher et al. 2017). Thereafter, filtered hypersaturated (140 $g.L^{-1}$) sodium chloride (NaCl) solution was added to the digestate (Naidoo et al. 2015) to further float and separate microplastics from sand and chitin (Lusher et al. 2017). Other floatation methods reportedly used for separating microplastics from other materials in microplastics studies are sometimes expensive. Thus, the cost-effective method which involved the solution that is similar to that of the environment was used. Digestate was thoroughly stirred using a glass rod for a few minutes (Dehaut et al. 2016) and allowed to settle for a minimum of 72 h. The supernatant containing floating plastics particles were vacuum-filtered onto 1.6 µm GF/A Filters. Each filter was placed into a new clean plastic petri dish, covered and labelled, and dried in the oven at 60°C for 24 h. Petri dishes were removed from the oven and stored until further analyses.

To avoid cross-contamination of samples, glass honey jars, Petri dishes, forceps, scissors, and washable laboratory equipment used, were acid washed, rinsed with series of distilled and deionised water and kept in a covered container. Also, all dissections and sample preparation were done in a fume hood. Samples contamination with plastics fibre from clothing was avoided by wearing white laboratory safety equipment made of cotton. Also, procedural blanks were run concurrently during sample processing to identify contamination (Lusher et al. 2017). To check for potential airborne microplastics contamination, filter paper placed in a clean petri dish was exposed to the air during sample preparation and microscopic analysis (Kanhai et al. 2017).

For microplastics identification, each filter was analysed using a light microscope (Stereo Nikon® AZ100) at the University of KwaZulu-Natal Microscopy and Microanalysis Unit (MMU). Identified microplastics were counted and clas-

sified into types (microbead, fragment, fibre, film and foam) using morphological features (Hidalgo-Ruz et al. 2012). Some of the identified microplastics (some of the minute microplastics could not be picked from the filter paper) were further subjected to a hot needle test (Lusher et al. 2017).

Statistical analysis: Statistical analysis was done using SPSS software (SPSS 2017: IBM version 25), and a test of significance was conducted using independent sample t-test and Analysis of Variance (ANOVA). All values (number of microplastics) were ranked to reach normality. One-sample Kolmogorov-Smirnov test was performed to check for normality and the p-value was considered significant at a 95 % confidence interval ($p < 0.05$).

RESULTS

Microplastic abundance: The percentage (%) occurrence of microplastics were: 100 % in both summer and winter in *D. fenestrata*, 100 % and 96.7 % in summer and winter in *H. cinerascens*, and 96.7 % and 100 % in *P. stolonifera* in summer and winter, respectively (Table 1). The number of particles per individual ranged from 1-24 (7.57± 1.13) and 4-25 (12.07 ± 1.06) in *D. fenestrata*, 2-25 (10.23 ± 1.09) and 0-19 (8.13 ± 1.04) in *H. cinerascens*, and 0-19 (4.60 ± 0.67) and 1-14 (3.57 ± 0.51) in *P. stolonifera* in summer and winter respectively (Table 1). The total number of microplastics found in *D. fenestrata* in winter (362) were more than summer (227), while more microplastics were found in *H. cinerascens* and *P. stolonifera* in summer (307 and 138) than in winter (244 and 107) (Table 1). However, there was significant difference in mean microplastics found between seasons in *D. fenestrata* only (t-test: t-value = 2.915, df = 58, $p = 0.005$) while the mean microplastics found between seasons in *H. cinerascens* and *P. stolonifera* was not statistically different (t-test: df = 58, $p > 0.05$) (Fig. 1).

There was no difference between the overall total number of microplastics found in winter and summer (t-test: t-value = 0.516, df = 178, $p = 0.606$) (Fig. 2). However, there were differences between the number of microplastics found in sampled animals within each season (One-way ANOVA: winter, $F_{(2,87)} = 22.084$, $p = 0.000$; summer, $F_{(2,87)} = 8.173$, $p = 0.001$). In winter, the mean number of microplastics (12.07 ± 1.06) found in *D. fenestrata* was greater than the mean number of microplastic (8.13 ± 1.04) found in *H. cinerascens*

Table 1: Percentage occurrence and the total number of microplastics found in the animals sampled in summer and winter.

	Season	No. of sampled animals	No. of animals with MPs	% MPs occurrence	Total MPs	Range of MPs
D. fenestrata	summer	30	30	100	227	1 - 24
	winter	30	30	100	362	4 - 25
H. cinerascens	summer	30	30	100	307	2 - 25
	winter	30	29	96.7	244	0 - 19
P. stolonifera	summer	30	29	96.7	138	0 - 19
	winter	30	30	100	107	1 - 14

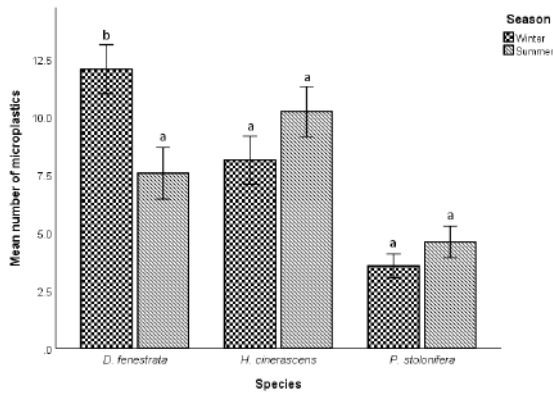


Fig.1: Mean (± SE) number of microplastics found in *D. fenestrata*, *H. cinerascens* and *P. stolonifera* sampled in summer and winter. [Means sharing the same alphabets are not significantly different at $p = 0.05$. Lower case *(a, b)* denotes Independent t-test significant difference in means within specie (n = 60). Error bars indicate ± 1 standard error (SE)].

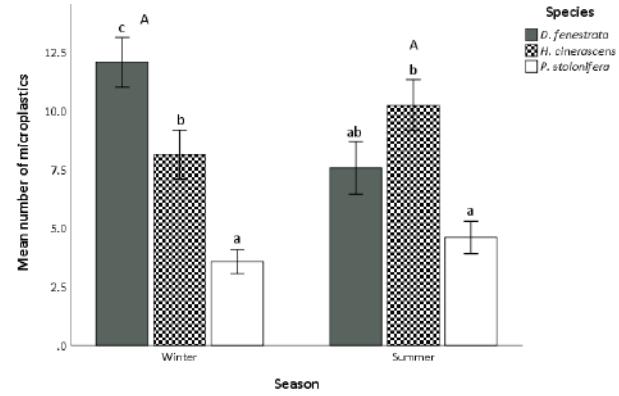


Fig. 2: Mean (± SE) number of microplastics found in animals within and between seasons. [Means sharing the same alphabets are not significantly different at $p = 0.05$. Lower case *(a, b)* denotes Tukey HSD post hoc significant differences in mean within the season (n = 90), Upper case *(A, B)* denotes Independent t-test significant difference in means between seasons (n = 180). Error bars indicate ± 1 standard error (SE)].

(ANOVA HSD: df = 2, p = 008) and *P. stolonifera* (3.57 ± 0.51) (ANOVA HSD: df = 2, p = 000); and the number of microplastic (8.13 ± 1.04) found in *H. cinerascens* was greater than the number of microplastics (3.57 ± 0.51) found in *P. stolonifera* (ANOVA HSD: df = 2, p = 002). In summer, the mean number of microplastics (10.23 ± 1.09) found in *H. cinerascens* was higher than the mean number of microplastics (4.60 ± 0.67) found in *P. stolonifera* (ANOVA HSD: df = 2, p = 000).

Microplastic characterization: The different types of microplastic identified in sampled animals included microbeads, fragments, fibres, films and foam (Fig. 3). Fibres were the most dominant microplastic type recorded in *D. fenestrata* (73.1% and 68.0%) and *H. cinerascens* (56.7% and 50%) for summer and winter respectively while in *P. stolonifera* fibres dominated in summer (97.8%) and fragments in winter (52.3%) (Fig. 4). Microplastic fragments were the second dominant microplastic type found in *D. fenestrata* (12.3% & 27.6%) and *H. cinerascens* (27.6% & 34.0%) in both seasons. Other types of microplastic found in these animals occurred in smaller quantities when compared with fibres and fragments.

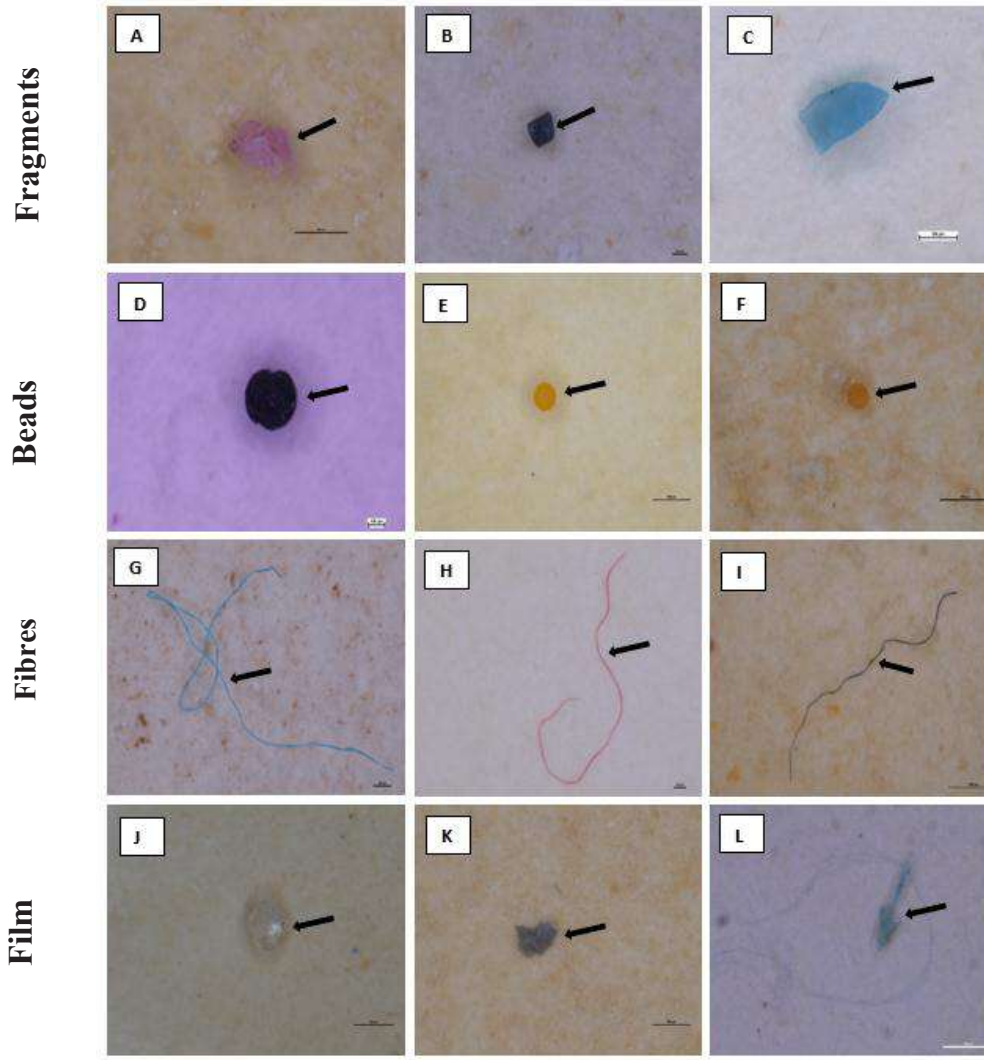


Fig. 3: Direct photographs of different microplastics (Black arrow) from the filter paper in *H. cinerascens*, *P. stolonifera*, and *D. fenestrata* collected in summer and winter from the southern and east coast of KwaZulu-Natal, South Africa. [(A) pink fragment- sc: 500 µm, (B) black fragment- sc: 100 µm, (C) blue fragment- sc: 100 µm, (D) black microbead- sc: 100 µm, (E-F) yellow microbead- sc: 500 µm, (G) blue fibre- sc: 100 µm, (H)- red fibre sc: 100 µm, (I)- black fibre - sc: 1000 µm, (J)- white film- sc: 500 µm, (K)- black film- sc: 500 µm, and (L)- blue film- sc: 500 µm. NB: *sc = scale*. (Plastics were identified and classified as described by Hidalgo-Ruz et al. 2012)].

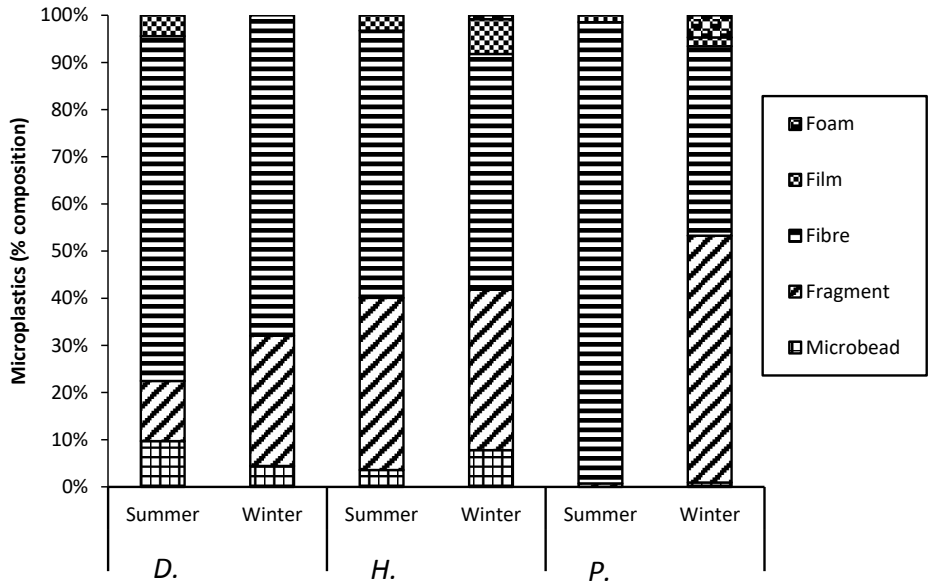


Fig. 4: Relative proportion of microplastic types (%) found in sampled animals in summer and winter.

DISCUSSION

The differences recorded in the total number of microplastics found in sampled animals (Table 1 & Fig. 2) may be due to the different feeding methods employed by the animals and sampling sites (Murphy et al. 2017). Rezania et al. (2018) reported that variation in the rates of microplastic ingestion by marine biota might be due to the difference in feeding behaviour and preferred habitat, among many other factors. Likewise, Taylor et al. (2016) in their study observed lower microplastic loads in filter feeders than deposit- feeders and it was reported that deposit feeders may be more vulnerable to microplastics ingestion (Brennecke et al. 2015) due to factors such as particle selection based on size or shape, density and abundance of microplastics in their environment, etc. *Dotilla fenestrata* is surface deposit feeders scooping sediment and sifting particulate organic matter such as algae, microbes, bacteria, diatoms, fungi, nematodes, detritus and ciliates from the inorganic matter (Gherardi et al. 2002, Khanyile 2012). Gherardi et al. (2002) identified five different genera of diatoms in the stomach of *D. fenestrata*, and Reisser et al. (2014) indicated that diatoms were the most colonizers of microplastics when compared with other groups of organisms collected from Australia inshore and offshore waters. This may be the reason why more microplastics were recorded in *D. fenestrata* since diatoms are one of the organic particulates consumed (Khanyile 2012) thereby, increasing the crabs' susceptibility to microplastics as they feed on diatoms. In addition, *D. fenestrata* sometimes uses a floatation feeding mechanism to float organic matter from the heavier inorganic matter (Davie et al. 2015). This could make *D. fenestrata* more susceptible to microplastics via ventilation mechanism process. It has been reported that shore crabs (*Carcinus maenas*) accumulated microplastic particles in their gills owing to the ventilation mechanism (Watts et al. 2014). Additionally, sediments may act as a reservoir for many microplastics, because close to 54 % of plastics produced have a greater density than water, and the rapid bacterial colonies formation (biofouling) on the less dense microplastics would increase their density and make them sink (Watts et al. 2015). Furthermore, sediments were reported to be more stable than water (Nel et al. 2018), thus increasing the chances of *D. fenestrata* accessing more microplastics during feeding due to the stable (no wave action, water current, etc.) environment, unlike *H. cinerascens* and *P. stolonifera* which feed on the suspended particles in the water column.

It was observed in this study that more microplastics were found in *H. cinerascens* than *P. stolonifera* in both seasons although both were sampled from the same environment (Fig. 2). This may be due to the different feeding mechanisms employed by these animals; suspension/indiscriminate feeding (*H. cinerascens*) versus filter/selective feeding (*P. stolonifera*) processes. *H. cinerascens* are suspension/deposit feeders spreading their tentacles to capture and feed on suspended particles found in the water column (Purcell et al. 2016) and on substratum to deposit feed (Roberts & Bryce 1982), while *P. stolonifera* is sessile ciliary-mucus filter feeders that filter their food by trapping the suspended and

re-suspended particulate matter in the water column (Shenkar et al. 2017). Our finding supports that of (Peters et al. 2017) who reported that selective (filter-feeding) invertebrates are less likely to ingest microplastics than the generalist (suspension-feeding) invertebrates. Desforges et al. (2015) also reported that indiscriminate (suspension) feeders in the water column are at risk of ingesting microplastics because they might mistake it for their natural food.

Furthermore, *D. fenestrata* were sampled from Durban harbour located in an urban centre which has increasingly been exposed to intense anthropogenic activities as a result of rapid human population growth, urbanisation and industrialization (Department of Environmental Affairs 2015), while *H. cinerascens* and *P. stolonifera* were sampled from Park Rynie located away from dense human population, urbanisation, and industrialisation. Park Rynie is located in Ugu District Municipality of KwaZulu-Natal, with the total district population of 753 336 compared to Durban in the eThekwini district Municipality of KwaZulu-Natal with a total district population of 3 702 231 (Statistics South Afrio ca 2018). The eThekwini district population is almost five times the Ugu district population. Brennecke et al. (2015) reported a spatial association between level of microplastic pollution in water and nearness to urban centres. Factors such as population densities and high anthropogenic activities have a great influence on the microplastic abundance in aquatic environments (Horton et al. 2017). Previous studies have shown that high microplastic abundance is regularly found near urban areas with high human populations and activities (Horton et al. 2017). Noren & Naustvoll (2010) also reported that industrial coastal areas are microplastic hotspots, placing organisms that inhabit urbanised coastal areas at potential risk for microplastic exposure (Au et al. 2017). This could be the reason for the higher number of microplastics recorded in *D. fenestrata* than in *H. cinerascens* and *P. stolonifera* because *D. fenestrata* were sampled from urban areas (Durban harbour), where microplastics are more abundant than the remote area from where *H. cinerascens* and *P. stolonifera* were sampled. Naidoo et al. (2015) reported that Durban harbour had the highest mean plastic particles compared to the other sites investigated. However, we suggest further studies to investigate and compare microplastic abundance in sediment and water samples from both sites.

The higher number of microplastics ingested by *D. fenestrata* in winter than summer (Fig. 1) may be because Durban receives less/no rainfall during winter. Therefore, in winter *D. fenestrata* can emerge and feed on well-drained sediment when the tide recedes (Bulcao & Hodgson 2012) whereas rainfall might interrupt their feeding activities (personal observation) in summer. Bulcao & Hodgson (2012) reported that sediment drainage is a vital factor that affects *Dotilla* activity. They further suggested the lack of sediment drainage to partly explain the inactivity of *Dotilla* in one of the days in their study. Hartnoll (1973) found that *D. fenestrata* activity was inhibited by heavy rain. Low temperatures that characterise winter will enable *D. fenestrata* to spend most of the time feeding at low tide while high temperature that characterises summer might make them feed less when the tide recedes as they tend to take cover in their burrow intermittently in avoidance of desiccation. On the contrary, a higher number of microplastics recorded (although not significant) in *H. cinerascens* and *P. stolonifera* during summer (Fig. 1) might be due to the increase in water temperature. Increased water temperature has been linked with increased metabolic activities in ectotherms (Cossins & Bowler 1987). This might cause the animals to feed more to compensate for the increasing body metabolism, therefore increasing their chances of ingesting microplastics. Rainfall and coastal flooding will facilitate the transport of macro and microplastics from land/dunes via urban runoff into marine environments, leading to higher concentrations of microplastics (Loo et al. 2015, Woodward et al. 2010) since Durban receives more rainfall during summer. According to Au et al. (2017) and Vianello et al. (2013), the increase in temperature and wave action due to rise in sea level/ storm surges will also facilitate the breakdown of macroplastics to microplastics leading to increased microplastics concentration. All the aforementioned will increase the bioavailability of microplastics to *H. cinerascens* and *P. stolonifera* during summer. The differences in the total number of microplastics recorded in sampled animals in different seasons, although insignificant in *P. stolonifera* and *H. cinerascens*, could be an indication of seasonal influence on microplastics uptake in marine invertebrates. However, studies involving sampling of animals in different seasons for a longer duration may help to further ascertain the influence of season on microplastics uptake.

Microplastics occurrence was recorded in over 95 % of the animals sampled in both seasons in this study (Table 1) which agrees with the field study conducted by Cauwenberghe et al. (2015) who found that 100 % of *M. edulis* and *A. marina* sampled had ingested microplastics. Li et al. (2015) found microplastics in all nine commercial bivalves collected from a fishery market in China. It was also reported that 83 % of *Nephrops norvegicus* collected from Clyde Sea in the United Kingdom had ingested microplastics (Murray & Cowie 2011). Goldstein & Goodwin (2013) recorded 33.5% of lepadid barnacles sampled from North Pacific Subtropical Gyre had ingested plastics particles. Ferreira et al.

(2016) reported plastic contaminants in 100 % of *Cynoscion acoupa* sampled from Goiana estuary in South America. The multiple types of microplastic identified in this study are similar to findings of Li et al. (2015), where multiple types of microplastics were found in the tissue of bivalves (*Scapharca suberenata, Tegillarca granosa*, *Meretrix lusoria*, *Cyclina sinensis*, etc).

In this study, fibres were the most dominant microplastics encountered in sampled animals, followed by fragments (Figs. 3 & 4). Previous studies also revealed fibres as the most dominant microplastics types followed by fragments (Devriese et al. 2015, Li et al. 2015, Neves et al. 2015, Woodall et al. 2014). Other types of microplastics found in this study were also reported in previous studies are fragments (Santillo et al. 2017), microbeads (Castaneda et al. 2014), and films (Santillo et al. 2017). Notably, more microplastics types were found in *D. fenestrata* during summer than winter, while more microplastic types were found in *P. stolonifera* and *H. cinerascens* in winter. This may be due to their availability in sediments and the water column during this season. The high number of fibres recorded in *D. fenestrata* in winter and *H. cinerascens* and *P. stolonifera* in summer can be linked to the overall total number of microplastics ingested by these animals in a particular season. It could be that fibres that were predominantly available in the water column to filter feeders (*P. stolonifera*) and suspension feeders (*H. cinerascens*) during summer might have settled in sediments during winter due to biofilm/ bacteria activities thus making them readily available to deposit feeders (*D. fenestrata*). The presence of microplastics in these animals is an indication of their potential to transfer microplastics to higher organisms along the food webs, including to humans. This is because they serve as prey to many higher trophic animals. Previous studies showed microplastic transfer from copepod and polychaete larvae to mysid shrimps (Setala et al. 2014), blue mussels to common shore crabs (Farrell & Nelson 2013), and fish to lobsters (Murray & Cowie 2011). According to Van Cauwenberghe & Janssen (2014) seafood consumption could serve as a possible route of human exposure to microplastics as humans are top consumer in the food web. Lastly, a recent study reported nine different microplastic types in human stool screened for microplastics (Liebmann et al. 2018); the authors opined that humans unintentionally ingest plastics.

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REFERENCES

Au, S.Y., Lee, C.M., Weinstein, J.E., Hurk, P.E. and Klaine, S.J. 2017. Trophic transfer of microplastics in aquatic ecosystems: Identifying Critical Research Needs. Integr, Environ, Asses., 13(3): 505-509

Bindoff, N., Willebrand, J., Artale, V., Cazenave, A., Gregory, J., Gulev, S., Hanawa K., Le Quere C., Levitus S., Nojiri Y., Shum C.K., Talley L. and Unnikrishnan A. 2007. Observations: oceanic climate and sea level. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and NY, USA. pp. 385-432.

Brennecke, D., Ferreira, E.C., Costa, T.M.M., Appel, D., da Gama B.A.P. and Lenz, M. 2015. Ingested microplastics (>100 µm) are translocated to organs of the tropical fiddler crab *Uca rapax*. Mar. Pollut. Bull., 96(1-2): 491-595.

Bulcao, C. and Hodgson, A.N. 2012. Activity and feeding of *Dotilla fenestrata* (Brachyura: Ocypodidae) in a warm, temperate South African estuary. Afr. J. Aquat. Sci., 37(3): 333- 338.

Cao, D., Wang, X., Luo, X., Liu, G. and Zheng, H. 2017. Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil. IOP Conf. Series: Earth Environ. Sci., 1: 2631.4.

Castaneda, R.A., Avlijas, S., Simard, M.A. and Ricciardi, A. 2014. Microplastic pollution in St. Lawrence River sediments. Can. J. Fish. Aquat. Sci., 71(12): 1767-1771.

Cauwenberghe, L.V., Claessens, M., Vandegehuchte, M.B. and Janssen, C.R. 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ Pollut., 199: 10-17.

Cossins, A.R. and Bowler, K. 1987. Temperature Biology of Animals. Pub Chapman and Hall, London. pp. 339.

Davie, P.J., Guin, T.D. and Ng, P.K.L. 2015. Anatomy and functional morphology Of Brachyura. Koninklijke Brill NV, Leiden. Crustacea, 9C(71-2): 11-163.

Dehaut, A., Cassone, A.L., Frere, L., Hermabessiere, L., Himber, C., Rinnert, E., Riviere, G., Lambert, C., Soudant, P., Huvet, A. Duflos, G. and Paul-Pont, I. 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ Pollut., 215: 223-233.

Department of Environmental Affairs 2015. Durban Bay: Estuarine Management Plan. pp.54.

Desforges, J.W., Galbraith, M. and Ross, P.S. 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. Arch. Environ. Contam. Toxicol., 69: 320- 330.

Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-Pont I., Frere, L., Robbens, J. and Vethaak, A.D. 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Mar. Pollut. Bull., 98: 179-187.

Duis, K. and Coors, A. 2016. Microplastics in the aquatic and terrestrial environment: Sources (with a specific focus on personal care products), fate and effects. (Review). Environ. Sci. Eur., 28: 2.

Farrell, P. and Nelson, K. 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ Pollut., 177: 1-3.

Ferreira, G.V.B., Barletta, M., Lima A.R.A., Dantas, D.V., Justino, A.K.S, and Costa, M.F. 2016. Plastic debris contamination in the life cycle of Acoupa weakfish (*Cynoscion acoupa*) in a tropical estuary. ICES J. Mar. Sci., 73(10): 2695-2707.

Gherardi, F., Russo, S. and Lazzara, L. 2002. The function of wandering in the sand-bubbler crab, *Dotilla fenestrata*. J. Crustacean Biol., 22(3): 521-531.

Goldstein, M.C. and Goodwin, D.S. 2013. Gooseneck barnacles (Lepas

spp.) ingest microplastic debris in the North Pacific Subtropical Gyre. Peer J., 1: e184.

Hartnoll, R.G. 1973. Factors affecting the distribution and behaviour of the Crab *Dotilla fenestrata* on East African Shores. Estuar. Coast. Mar. Sci., 1: 137-152.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M. 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol., 46(6): 3060-3075.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E. and Svendsen, C. 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ., 586: 127-141.

Kanhai, L.D., Officer, R., Lyashevska, O., Thompson, R.C. and O'Connor, I. 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. Mar. Pollut. Bull., 115(1-2): 307-314.

Khanyile, S.N. 2012. Salinity Tolerance and Osmoregulation in Several Subtropical Decapods. M.Sc. Thesis. pp. 84.

Li, J., Yang, D., Li, L., Jabeen, K. and Shi, H. 2015. Microplastics in commercial bivalves from China. Environ. Pollut., 207: 190- 195.

Liebmann, B., Koppel, S., Konigshofer, P. Bucsics, T., Reiberger, T. and Schwabl, P. 2018. Assessment of microplastic concentrations in human stool - final results of a prospective study. Available from: https://www.researchgate.net/publication/328702413_Assessment_of_Microplastic_Concentrations_in_Human_Stool_Final_Results_of_a_Prospective_Study (Accessed Dec. 23, 2018).

Loo, Y.Y., Billa, L. and Singh, A. 2015. Effect of climate change on seasonal monsoon in Asia and its impact on the variability of monsoon rainfall in Southeast Asia. Geosci. Front., 6(6): 817- 823.

Lusher, A.L., Welden, N.A., Sobral, P. and Cole, M. 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods, 9: 1346.

Manriquez, P.H., Castilla, J.C., Ortiz, V. and Jara, M.E. 2016. Empirical evidence for large-scale human impact on intertidal aggregations, larval supply and recruitment of *Pyura praeputialis* around the Bay of Antofagasta, Chile. Austral Ecol., 41: 701-714.

Manzoor, R., Haider, S., Fatima, M. and Qari, R. 2016. Study on abundance, breeding and allometric growth of the ocypodidae crab *Dotilla blanfordi* in Karachi Coast, Pakistan. Int. J. Mar. Sci., 6(22): 1-14.

Mathalon, A. and Hill, P. 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. Mar. Pollut. Bull., 81: 69-79.

Murphy, F., Russell, M., Ewins, C. and Quinn, B. 2017. The uptake of macroplastic and microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland. Mar. Pollut. Bull., 122(1-2): 353-359.

Murray, F. and Cowie, P.R. 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull., 62(6): 207-1217.

Naidoo, T., Glassom, D. and Smit, A.J. 2015. Plastic pollution in five urban estuaries of KwaZulu-Natal, South Africa. Mar. Pollut. Bull., 101: 473-480.

Nel, H.A., Dalu, T. and Wasserman, R.J. 2018. Sinks and sources: Assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system. Sci. Total Environ., 612: 950-956.

Neves, D., Sobral, P., Ferreira, J.L. and Pereira, T. 2015. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar. Pollut. Bull., 101(1): 119-126.

Noren, F. and Naustvoll, F. 2010. Survey of microscopic anthropogenic particles in Skagerrak. 551 Report commissioned by Klima-og Forurensningsdirektoratet, Norway. Oceanogr. Mar. Biol. (An Annual Review), 54: 367-386.

O'Brien, K.L. and Leichenko, R.M. 2000. Double exposure: assessing the impacts of climate change within the context of economic globalization. Global Environ. Chang., 10: 221-232.

Pereira, M.A.M. and Goncalves P.M.B. 2000. A preliminary investigation of the effects of human physical disturbance on the ecology of the soldier crab *Dotilla fenestrata* (Crustacea, Ocypodidae) at Praia da Costa do Sol, Maputo. Presented at the 2nd National Conference on Coastal Areas Research. Maputo, pp. 27-29.

Peters, C.A., Thomas, P.A., Rieper, K.B. and Bratton, S.P. 2017. Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast. Mar. Pollut. Bull., 124: 82- 88.

Purcell, S.W., Conand, C., Uthicke, S. and Byrne, M. 2016. Ecological roles of exploited sea cucumbers (An Annual Review). Oceanogr. Mar. Biol., 54: 20.

Reisser, J., Shaw, J., Hallegraeff, G., Proietti, M, Barnes, D.K.A., Thums, M., Wilcox, C., Hardesty, B.D. and Pattiaratchi, C. 2014. Millimeter-sized marine plastics: A new pelagic habitat for microorganisms and invertebrates. PLoS ONE 9(6): e100289.

Rezania, S., Park, J., Md Din, M. F., Mat Taib, S., Talaiekhozani, A., Kumar Yadav, K. and Kamyab, H. 2018. Microplastics pollution in different aquatic environments and biota: A review of recent studies. Mar. Pollut. Bull., 133: 191-208.

Riisgard, H.U. and Larsen, P.S. 2010. Particle capture mechanisms in suspension-feeding invertebrates. Mar. Ecol. Prog. Series., 418: 255-293.

Roberts, D. and Bryce, C. 1982. Further observation on tentacular feeding mechanisms in holothurians. J. Exp. Mar. Biol. Ecol., 59: 151- 163.

Santillo, D., Miller, K. and Johnston, P. 2017. Microplastics as contaminants in commercially important seafood species. Integr. Environ. Asses., 13: 516-521.

Setala, O. Fleming-Lehtinen, V. and Lehtiniemi, M. 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut., 185: 77- 83.

Shenkar, N., Gittenberger, A., Lambert, G., Rius, M., Moreira, Da Rocha, R., Swalla, B.J. and Turon, X. 2017. Ascidiacea World Database. http://www.marinespecies.org/ascidiacea (Accessed: July 25, 2017).

Sokolova, I. and Lannig, G. 2008. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. Clim. Res., 37(2-3): 181- 201.

Statistics South Africa. 2018. KwaZulu-Natal Citizen Satisfaction Survey: Analytical Report. Published by Statistics South Africa, Pretoria. pp. 124.

Taylor, M.L., Gwinnett, C., Robinson, L.F. and Woodall, L.C. 2016. Plastic microfibre ingestion by deep-sea organisms. Sci. Rep., 6(1): 33997.

Tosetto, L., Williamson, J.E. and Brown, C. 2017. Trophic transfer of microplastics does not affect fish personality. Anim. Behav., 123: 159-167.

Van Cauwenberghe, L. and Janssen, C.R. 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut., 193: 65-70.

Vermeiren, P. and Sheaves, M. 2015. Predictable habitat associations of four crab species across the low intertidal landscape of a tropical estuary over time. Estuar. Coast., 38: 285-295.

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A. and Da Ros, L. 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuar. Coast. Shelf S., 130: 54-61.

Watts, A.J.R., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler,

C.R. and Galloway, T.S. 2014. Uptake and retention of microplastics by the shore crab *Carcinus maenas*. Environ. Sci. Technol., 48 (15): 8823-8830.

Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C. and Galloway T.S. 2015. Ingestion of plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy balance. Environ. Sci. Technol., 49(24): 14597-14604.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A. Rogers, A.D., Narayanaswamy, B.E. and Thompson, R.C. 2014. The deep sea is a major sink for microplastic debris. Roy. Soc. Open Sci., 1(4): 140317-140317.

Woodward, G., Perkins, D.M. and Brown, L.E. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philos. T. R. Soc. B: Biol. Sci., 365(1549): 2093-2106.

Yukihira, H., Lucas, J.S. and Klumpp, D.W. 2000. Comparative effects of temperature on suspension-feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima*. Mar. Ecol. Prog. Ser., 195: 179-188.

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Biochemical Changes Induced by Cartap Hydrochloride (50% SP), Carbamate Insecticide in Freshwater Fish *Cirrhinus mrigala* (Hamilton, 1822)

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ABSTRACT

The freshwater fish *Cirrhinus mrigala* was exposed to Cartap hydrochloride (50% SP) for 24, 48, 72 and 96 h. The LC_{50} values were found to be 0.436, 0.419, 0.394 and 0.376 mg^{-1} in static method and 0.399, 0.371, 0.361 and 0.339 $mg.L^{-1}$ in continuous flow-through system. The static LC_{50} values are higher than the continuous flow-through method. The LC_{50} values showed a decreasing trend with an increase in time of exposure in both the methods. The decrease was more in a continuous flow-through method than in the static method. The fish were exposed to sub-lethal ($1/10^{th}$ of 96 h LC_{50} value 0.0376 $mg.L^{-1}$) and lethal (96 h LC_{50} value 0.376 $mg.L^{-1}$) concentrations of the pesticide for 24 and 96 hours to study the alterations in glycogen, total proteins and nucleic acids (DNA & RNA) contents of various tissues viz., gill, brain, liver, kidney and muscle. Glycogen, total proteins and nucleic acids (DNA & RNA) content values decreased in all the tissues of exposed fish and the per cent decrease is more apparent in lethal concentrations than in sub-lethal concentrations. From the present study, it can be concluded that Cartap hydrochloride caused a decline in the glycogen, total protein and nucleic acids (DNA, RNA) content in *Cirrhinus mrigala* and the changes are more pronounced in lethal exposure than in sub-lethal exposure.

INTRODUCTION

Indiscriminate use of pesticides is one of the main reasons for the pollution of aquatic ecosystems. These toxic pesticides are causing deleterious effects on aquatic organisms. They are causing stress to aquatic organisms which are reflected as biochemical changes in their body (Mayers 1977). Fish acts as a bioindicator species and can be used for monitoring of water pollution as they accumulate the contaminants from polluted water and diet (Chaudary & Jabeen 2011, Kafilzadeh et al. 2012). Fish accumulate these pollutants directly or indirectly from polluted waters and food chain (Jabeen et al. 2016, Chaudary & Jabeen 2011). Carbamates are extensively used water-soluble pesticides in agricultural practices. In India, Cartap hydrochloride is a Carbamate pesticide which is considered as nereistoxin analog is extensively used in rice and sugarcane crops to control pests. The present investigation is aimed to study the toxic effects of Cartap hydrochloride in sub-lethal and lethal concentrations at 24 and 96 hours of exposure period on glycogen, total protein, DNA and RNA contents of freshwater fish *Cirrhinus mrigala* (Hamilton 1822).

MATERIALS AND METHODS

The fingerlings of the test fish *Cirrhinus mrigala* size 6-8 ± ½ cm and weight 6-7 ± ½ g were procured from local fish hatcheries of Nandivelugu, Tenali Mandal, Guntur district, Andhra Pradesh. The fish were acclimated at (28 ± 2°C)) in the laboratory conditions for two weeks. All the precautions laid down by APHA et al. (1998) were followed. During the acclimation period, the fish were fed with rice bran and groundnut cake. One day before the experimentation feeding was stopped. Cartap hydrochloride (50% SP) commercial grade was purchased from Mangalagiri, Guntur District. The stock solution was prepared with water as a solvent. The acclimatized fish were exposed to static sub-lethal (0.376 $mg.L^{-1}$) and lethal concentrations (3.76 $mg.L^{-1}$) of Cartap hydrochloride (50% SP) for 24 and 96 h. The hydrographical properties of water were estimated by the modified method followed by Golterman & Claimo (1969) method. Finney's probit analysis (Finney 1971) as reported by Roberts % Boyce (1972) was followed to calculate the LC_{50} value. The 95% confidence limits of the LC_{50} values for each test were also calculated for different time periods

by using SPSS software. At the end of the exposure periods, the tissues like gill, brain, liver, kidney and muscle were taken out from exposed and control fish and processed for the estimation of glycogen, total proteins and nucleic acids (DNA & RNA). Glycogen was estimated by the method of Kemp et al. (1954), total protein by Lowry et al. (1951) and DNA and RNA by the methods of Searchy & Maclinnis (1970a & 1970b).

The data obtained in the present work were expressed as means of four observations ± SD (standard deviation) and were statistically analysed using student "t" test (Pillai & Sinha 1968) to compare means of treated data against their controls and the result was considered significant at ($P < 0.05$) level.

RESULTS AND DISCUSSION

Glycogen, total proteins and nucleic acids (DNA & RNA) decreased in various tissues, viz. gill, brain, liver, kidney and muscle of *Cirrhinus mrigala* exposed to sub-lethal and lethal concentrations of cartap hydrochloride for 24 and 96 hours were graphically represented in Fig. 1 to Fig. 8. In exposed fish per cent decrease is more apparent in lethal concentrations than at sub-lethal concentrations.

Glycogen

The changes in glycogen content observed in the various tissues of *Cirrhinus mrigala* after the Cartap hydrochloride exposure along with the control are graphically represented in Fig .1 and Fig. 2.

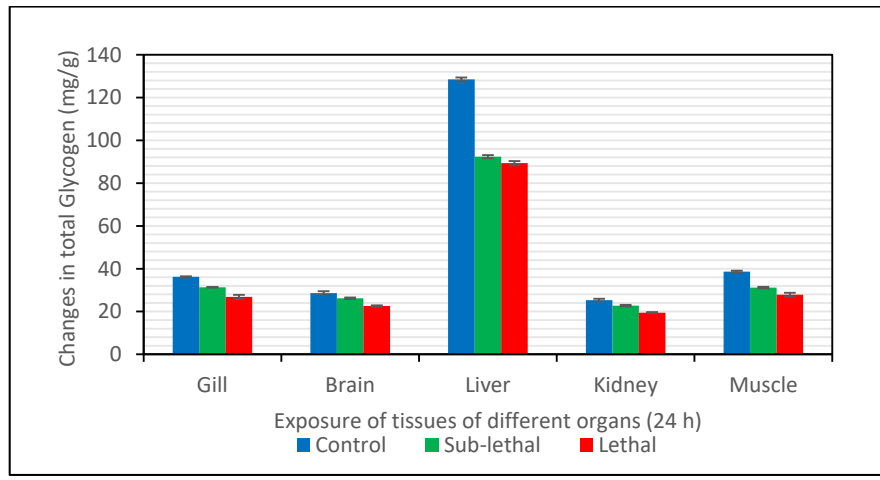


Fig.1: Changes in the glycogen content (mg/g wet weight of the tissue) in the tissue of fish *Cirrhinus mrigala* on exposure to sub-lethal and lethal concentration of Cartap hydrochloride (50%SP) for 24 hours.

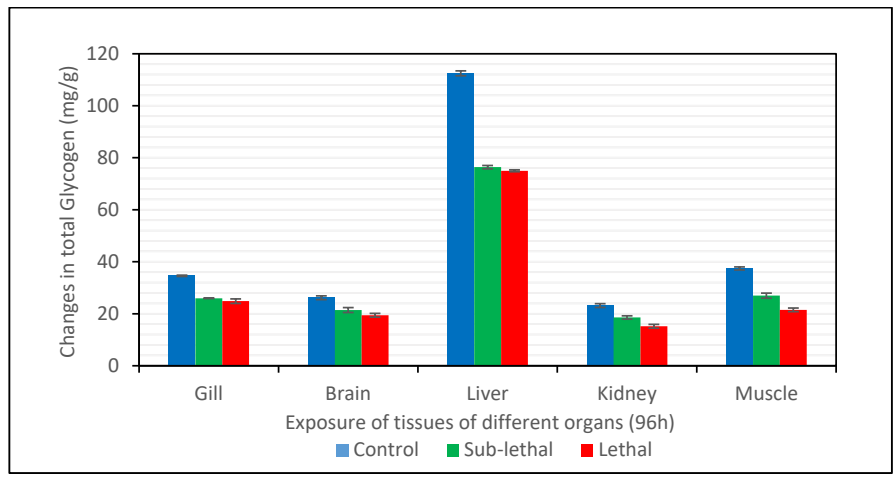


Fig. 2: Changes in the glycogen content (mg/g wet weight of the tissue) in the tissue of fish *Cirrhinus mrigala* on exposure to sub-lethal and lethal concentration of Cartap hydrochloride (50% SP) for 96 hours.

In the tissues of control fish, *Cirrhinus mrigala* glycogen content was in the order of:

Liver > Muscle > Gill > Brain > Kidney

Under exposure to sub-lethal and lethal concentrations of Cartap hydrochloride for 24 and 96 hours, the amount of glycogen was found to decrease in all the tissue of *Cirrhinus mrigala*. The lyotropic gradation series in terms of per cent decrement at 24 h and 96 h exposure was:

Sub-lethal -24 h: Liver > Muscle > Gill > Kidney > Brain

Lethal -24 h: Liver > Muscle > Gill > Kidney > Brain

Sub-lethal 96 h: Liver > Muscle > Gill > Kidney > Brain

Lethal-96 h: Muscle > Kidney > Liver > Gill > Brain

Exposure of *Cirrhinus mrigala* to Cartap hydrochloride for 24 hours caused a maximum decrease of glycogen in the liver (sub-lethal 28.117, lethal 30.453). For 96 h maximum decrease was found in the liver (sub-lethal 32.073) and muscle (lethal 42.570). In the present study under Cartap hydrochloride 24 h sub-lethal and lethal exposure minimum percentage of depletion was in the brain (8.595) and (20.96). For 96 h exposure minimum depletion was found in the brain (sub-lethal 18.056, lethal 25.707).

Srivastava & Singh (2013) observed reduction in glycogen content in different tissues of freshwater fish *Clarius batrachus* exposed to 80% of LC_{50} (22.87 $mg.L^{-1}$) of Mancozeb at different time intervals of 24 and 96 h. Sastry et al. (1982) reported decreased glycogen content of liver and muscles decreased when *Channa punctatus was exposed to* sub-lethal concentration of the carbamate pesticide, Sevin (1.05 $mg.L^{-1}$) for 15, 30 and 60 days. Veeraiah et al. (2013a) observed that exposure to sub-lethal and lethal concentrations of cadmium chloride in the fish *Cirrhinus mrigala* for 96 h caused changes in the total glycogen level which may be attributed to toxic stress, resulting in the disruption of enzymes associated with carbohydrate metabolism. Bantu & Rathnamma (2013) reported that there is a decrease in the amount of glycogen in the fish *Labeo rohita* exposed to sub-lethal and lethal concentrations of Dimethoate for 8 days. Priya et al. (2013) observed depletion of glycogen in the liver of freshwater teleost *Channa punctatus* (Bloch) when exposed to various concentrations of Imidacloprid (0.002 ppm, 0.00 ppm, 0.006 ppm, 0.008 ppm and 0.01 ppm) for 96 h suggesting the possibility of an alter from aerobic to anaerobic mode of energy metabolism of the liver. Dhanalakshmi (2013) noticed decrement in the tissue glycogen concentration in fish *Cirrhinus mrigala* when exposed to 0.25 ppm concentration of the metal chromium sulphate for 24, 48, 72 h and 10, 20 and 30 days which may be due to its enhanced utilization, since glycogen forms the immediate source of energy to meet energy demands under metallic stress caused by test toxicant.

Tataji & Kumar (2016) reported a decline in glycogen content of freshwater fish *Channa punctatus* exposed for 8 days to 1/5th of LC_{50} 96 hours of both Butachlor technical grade and Machete (50% EC), i.e. 32 ppb and 71.2 ppb for both technical and 50% EC respectively. Reduction in glycogen content was also noticed by Naik et al. (2016) in all the tissues of *Labeo rohita* when exposed to sub-lethal concentrations of cypermethrin for 1, 2 and 3 weeks. Exposure of sub-lethal doses (40% and 80 % of LC_{50} of 24 h) of glyphosate for 24 or 96 h against the freshwater non-target fish *Channa punctatus* caused significant ($P < 0.05$) alteration in biochemical parameters in liver and muscle tissues of the fish *Channa punctatus* (Bloch) was reported by Singh et al. (2017). Veeraiah et al. (2018) observed that exposure to lethal and sub-lethal concentrations of cyhalothrin 2.5% EC, in the fish *Ctenopharyngdon idellus* for 24 and 96 hours caused a decrease in glycogen content in all tissues.

According to Dezwaan & Zandee (1973) depletion of glycogen in tissues may be due to direct utilization of the compound for energy generation, a demand caused by pesticide-induced hypoxia. Under hypoxia condition, the fish derives its energy from anaerobic breakdown of glucose which is available to the cell by increased glycogenolysis (Chandravathy & Reddy 1996, Rajamannar & Manohar 1998, Rajamanickam 1992). Reduction in glycogen is probably due to its more rapid break down for energy requirement of fish (Muley et al.1996).

Liver suggested as an organ for detoxification. During exposure to Cartap hydrochloride exposure fishes came under stress condition and need more energy to cope with the toxicants. glycogen serves as reserve material. It is utilized when the body came under stress condition. Depletion of glycogen in liver and tissues may be due to increment in the glycolysis pathway. During stress conditions, the glycogen reserves depleted to meet energy demand (Rawat et al. 2002). Fall in glycogen levels indicates its rapid utilization to meet the enhanced energy demands intoxicant treated animals through glycolysis or hexose monophosphate pathway as observed by Cappon & Nicholas (1975). The above findings support the alterations of glycogen in the present study.

Proteins

The changes in protein content observed in the various tissues of *Cirrhinus mrigala* after Cartap hydrochloride exposure along with the control was graphically represented in Fig. 3 and Fig. 4.

The Protein content in different tissues in control fish *Cirrhinus mrigala* was in the order of:

Muscle > Liver > Brain > Kidney > Gill

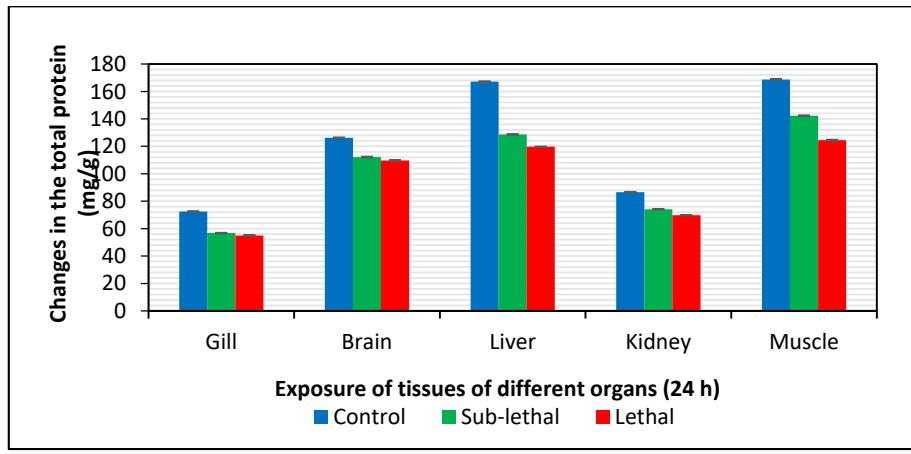


Fig. 3: *Changes* in protein content (mg/g wet wt of the tissue) in different tissues of fish *Cirrhinus mrigala* (Hamilton) on exposure to sub-lethal and lethal concentration of Cartap hydrochloride (50% SP) for 24 hours.

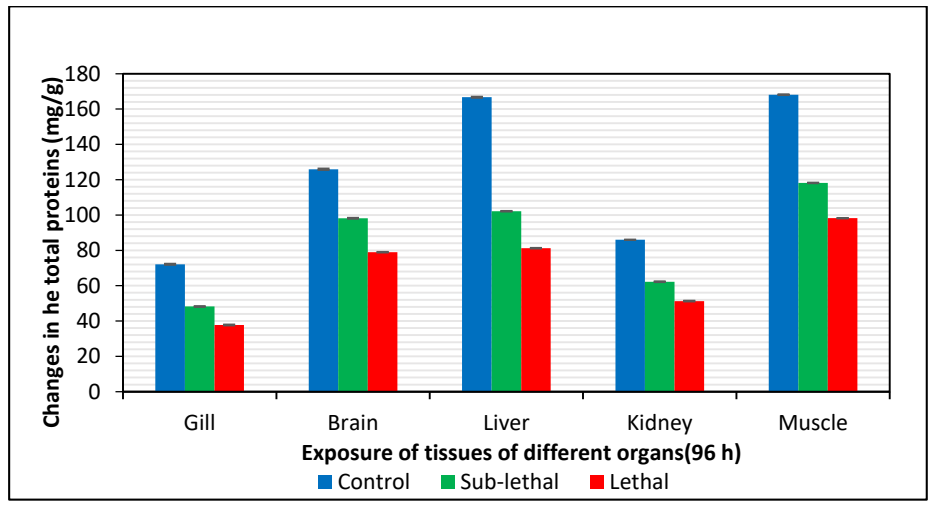


Fig. 4: Changes in protein content (mg/g wet wt of the tissue) in different tissues of fish *Cirrhinus mrigala* (Hamilton) on exposure to sub-lethal and lethal concentration of Cartap hydrochloride (50% SP) for 96hours.

Under exposure of sub-lethal and lethal concentrations of Cartap hydrochloride for 24 h, the amount of protein was found to decrease in all the tissue of *Cirrhinus mrigala.*

The lyotropic gradation series in terms of per cent decrement at 24 h and 96 h exposure was:

Sub-lethal -24 h: Liver > Gill > Muscle > Kidney > Brain

Lethal-24 h- Liver > Muscle > Gill > Kidney > Brain

Sub-lethal -96 h: Liver > Gill > Muscle > Kidney > Brain

Lethal-96 h: Liver > Gill > Muscle > Kidney > Brain

For 24 h exposure maximum per cent of the decrease in total protein was observed in the liver (sub-lethal 23.05, lethal 28.33). For 24 h exposure minimum percentage of depletion of total protein was found in the brain (sub-lethal 11.18, lethal 13.13). For 96 h the percentage of decrease was maximum in the liver (sub-lethal 38.72, lethal 51.27). Similarly, for 96 h minimum percentage of decrease was noticed in the brain (sub-lethal 22.06, lethal 37.30).

Protein is the most primary biochemical ingredient present in large quantities in the body of fish. Liver is rich in protein and centre for various metabolism of the fish. In the present study maximum decrease of total protein in the liver is due to the increased rate of proteolytic activity or repeated break down of protein to yield energy due to stress caused due to pesticide exposure. Anitha & Rathnamma (2016) noticed decreased protein levels in all the tissues like liver, kidney, brain, gill and muscle of *Labeo rohita* exposed to lethal and sub-lethal concentrations of Pyraclostrobin 20%

WG (carbamate) for 24 h and sub-lethal concentrations for 5 and 10 days. A decline in the protein content was noticed by Kumari et al. (2014) in the liver of *Clarias batrachus* when exposed to sub-lethal concentrations (2 and 4 $mg.L^{-1}$) of the Carbaryl for 96 h. A decrease in protein may be due to the impairment of protein synthesis or an increase in the rate of its degradation to amino acids. Exposure to Carbryl for 4 and 24 days, decreased protein content in liver and muscle of fish *Mugil cephalus*, when exposed to the lethal and sub-lethal concentration of Carbaryl for 4 days and 21 days respectively was reported by Shivanagouda et al. (2013). Kumar et al. (2017) reported a reduction in proteins in the liver and kidney of freshwater fish, *Channa punctatus* exposed to different sub-lethal concentrations of pesticide Carbaryl for a period of 15, 30, 45, 60, 75 and up to 90 days. Muddassir (2015) observed significant decrease value in Total protein in the liver of *Channa punctatus* was treated with 0.1 mL Carbofuran and 0.09 mL Malathion pesticides at different time intervals 7, 14, 21 and 28 days. The decrement of total protein may be due to the inhibition of RNA synthesis disturbing the protein metabolism or this may be due to liver damage where most protein synthesis usually occurs, these results agreed with that of Singh & Sharma (1998). The depletion of protein might also be attributed to spontaneous utilization of amino acids in various catabolic reactions inside the organism to combat the stress condition (Borah & Yadav 1996). Wankhedkar & Bhavsar (2015) found that total protein content significantly decreased in foot and hepatopancreas in land snail *C. moussonianus* when treated with Cartap hydrochloride and Imidacloprid at lowest concentration i.e. LC_{50} 0.41ppm and LC_{50} 0.54 ppm respectively. Veeraiah et al. (2018) observed a decrease in protein content in all tissues exposed to lethal and sub-lethal concentrations of cyhalothrin 2.5% EC, in the fish *Ctenopharyngdon idellus* for 24 and 96 h.

Proteins are important organic constituents of the animal cells. Understanding the protein components of the cell becomes necessary in the light of the radical changes taking place in protein profiles during pesticide intoxication (Anitha & Rathnamma 2016). The decreased trend of the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids through gluconeogenesis pathway for the synthesis of glucose or due to the directing of free amino acids for the synthesis of necessary proteins, or for the maintenance of osmotic and ionic regulation (Schmidt Neilson 1975).

Nucleic Acids (DNA & RNA)

The changes in DNA content observed in the various tissues of *Cirrhinus mrigala* after the Cartap hydrochloride exposure along with the control was graphically represented in Fig. 5 and Fig. 6.

In control fish, DNA content present in different organs was in the order of:

Muscle > Gill > Brain > Liver > Kidney.

Under exposure to sub-lethal and lethal concentrations of Cartap hydrochloride, for 24 h the amount of DNA was found to decrease in all the tissue of *Cirrhinus mrigala.* The lyotropic gradation series in terms of per cent decrement at 24 h and 96 h exposure was:

Sub-lethal -24 h: Gill > Liver > Muscle > Brain > Kidney

Lethal-24 h-Gill > Liver > Muscle > Brain > Kidney

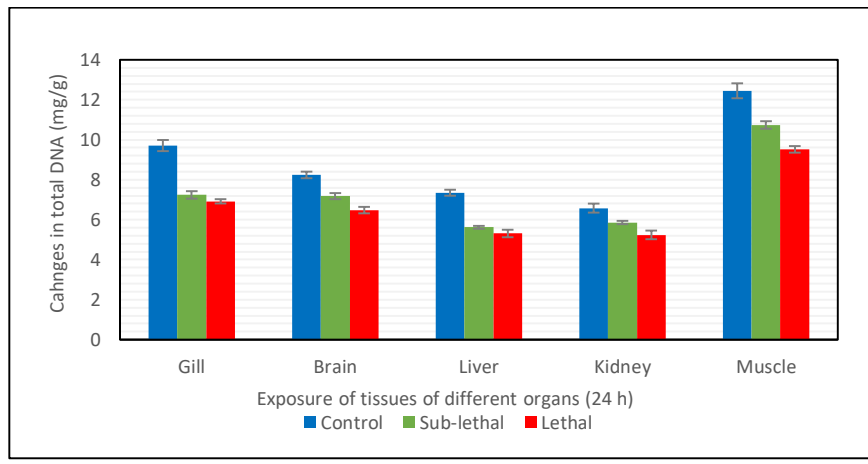


Fig. 5: Changes in the amount of deoxyribonucleic acid (DNA) (mg/g wet weight of the tissue) in different tissues of the fish, *Cirrhinus mrigala* on exposure to sub-lethal and lethal concentration of Cartap hydrochloride (50% SP) for 24 h.

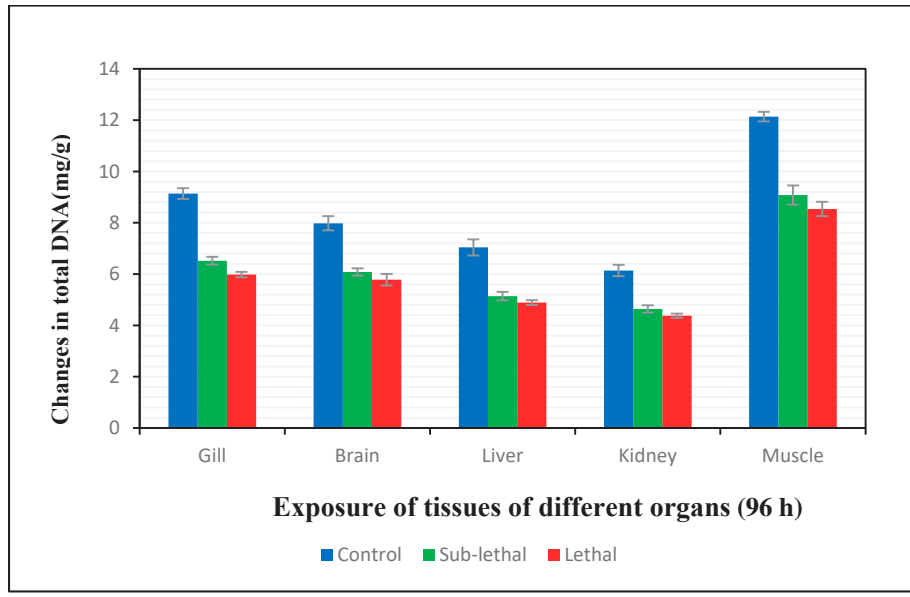


Fig. 6: Changes in the amount of deoxyribonucleic acid (DNA) (mg/g wet wt of the tissue) in different tissues of the fish *Cirrhinus mrigala* on exposure to sub-lethal and lethal concentration of Cartap hydrochloride (50% SP) for 96 h.

Sub-lethal-96 h: Gill > Liver > Muscle > Kidney > Brain

Lethal-96 h: Gill > Liver > Muscle > Kidney > Brain

In the present study under Cartap hydrochloride exposure for 24 h, maximum percentage of depletion in amount of DNA was noticed in Gill (sub-lethal 25.44, lethal 28.74). Minimum percentage of depletion was exhibited in the Kidney (sub-lethal 10.95, lethal 20.37). Under exposure to sub-lethal and lethal concentrations of Cartap hydrochloride for 96 h, maximum percentage of depletion was seen in Gill (sub-lethal 28.67, lethal 34.58) and minimum percentage of depletion was noticed in the brain (sub-lethal 23.81, lethal 27.57).

The changes in RNA content observed in the various tissues of *Cirrhinus mrigala* after Cartap hydrochloride exposure along with the control was graphically represented in Fig. 7 and Fig. 8.

The RNA content in different tissues in control fish *Cirrhinus mrigala* was in the order of:

Muscle > Brain > Gill > Liver > Kidney.

Under exposure to lethal and sub-lethal concentrations of Cartap hydrochloride, for 24 and 96 h, the amount of RNA was found to decrease in all the tissues. The lyotropic gradation series in terms of per cent decrement at 24 h and 96 h exposure was:

Sub-lethal -24h: Muscle > Gill > Kidney > Brain > Liver

Lethal-24h: Muscle > Gill > Kidney > Liver > Brain

Sub-lethal -96h: Gill > Muscle > Kidney > Liver > Brain

Lethal-96h: Gil l > Muscle > Liver > Kidney > Brain

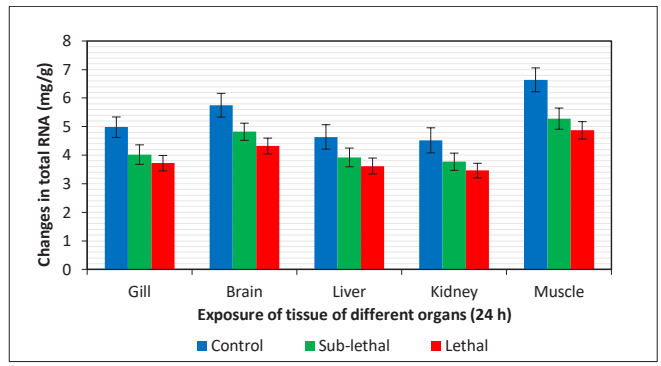


Fig. 7: Changes in the amount of ribonucleic acid (RNA) (mg/g wet wt of the tissue) in different tissues of the fish, *Cirrhinus mrigala* on exposure to sub-lethal and lethal concentration of Cartap hydrochloride (50% SP) for 24 h.

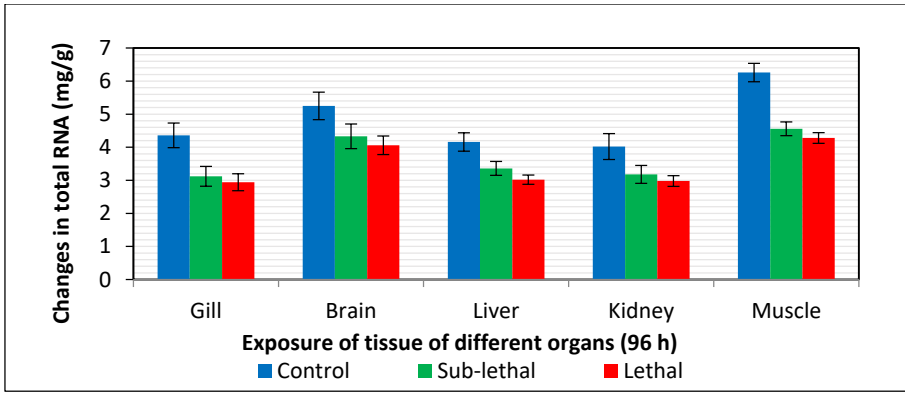


Fig. 8: Changes in the amount of ribonucleic acid (RNA) (mg/gm wet wt of the tissue) in different tissues of the fish, *Cirrhinus mrigala* on exposure to sub-lethal and lethal concentration of Cartap hydrochloride (50% SP) for 96 h.

After 24 h of exposure maximum percentage of decrease in the amount of RNA was found in muscle (sub-lethal 20.48, lethal 26.65). Minimum per cent of the decrease in RNA was found in the liver (15.51) in sub-lethal concentration and brain (19.65) in lethal concentration. After 96h of exposure maximum percentage of decrease in the amount of RNA was found in Gill (sub-lethal 28.44, lethal 32.56). Minimum percentage of decrease in RNA was found in brain(sub-lethal 17.52, lethal 22.66).

The results indicated that the DNA and RNA content in all the tissues of test fish were decreased compared to controls and the decreasing trend was more pronounced in lethal concentrations than in sub-lethal concentrations. In maintaining the physiological configuration of the fish Nucleic acids play a vital role. As Nucleic acid and protein play the main role in regulating different activities of cells they are regarded as important biomarkers of the metabolic potential of cells (Veeriah et al 2013a).

The decrease in nucleic acid content in the present study was in accordance with Vivek (2015) in fingerlings of *Labeo rohita* exposed to sub-lethal concentrations of Cartap hydrochloride for 24, 48, 72 and 96 h. Similar results were also found by Dasu (2014) in fingerlings of *Labeo rohita* were exposed to Thiocarb (Larvin 75% WP) a thiocarbamate pesticide. Anitha & Rathnamma (2016) noticed decreased DNA and RNA levels in all the tissues like liver, kidney, brain, gill and muscle of *Labeo rohita* exposed to lethal and sub-lethal concentrations of Pyraclostrobin 20%WG (carbamate) for 24 h and sub-lethal concentrations for 5 and 10 days.

Tilak et al. (2009) noticed a decreased level of DNA and RNA content in Alachlor treated freshwater fish, *Channa punctatus* (Bloch). The decrease of RNA may be due to inhibiting the function of RNA polymerase or due to interference in the incorporation of precursor in the nucleic acid synthesis. The alterations in DNA levels may be due to disturbances in DNA synthesis and its turnover rate besides degenerative changes caused by pesticides.

From the present study, it can be concluded that exposure of *Cirrhinus mrigala* to Cartap hydrochloride caused a decline in the glycogen, total protein and Nucleic acids (DNA, RNA) content which is more pronounced in lethal exposure than in sub-lethal exposure. The alterations caused during pesticide exposure may be due to the decreased catabolism of the biomolecules to meet the energy demand of test organism under stress or their reduced synthesis due to impaired tissue function. Therefore, the results of this study suggest a serious concern towards the potential danger of Cartap hydrochloride for the aquatic environment and organisms suggesting judicious and careful use of this pesticide in the agricultural area.

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REFERENCES

Anitha, A. and Rathnamma, V.V. 2016. Toxicity Evaluation and Protein Levels of Fish Labeo rohita Exposed to Pyraclostrobin 20% Wg (Carbamate). International Journal of Advanced Research, 4(3): 967-974.

APHA 1998. Standard Method for the Examination of Water and Wastewater, A.D. Eaton, L.S. Clesceri and A.E. Greenberg (Eds.), 20th edition. American Public Health Association, AWWA and WEF, Washington D.C.

Bantu, N. and Rathnamma Vakita 2013. Effect of dimethoate on mortality and biochemical changes of freshwater fish *Labeo rohita* (Hamilton). Journal of Biology and Today's World, 2(10): 456-470.

Borah, S. and Yadav, R.N.S. 1996. Effect of Rogor (30% w/w dimethoate) on the activity of lactate dehydrogenase, acid and alkaline phosphatase in muscle and gill of a freshwater fish, *Heteropneustes fossilis*. Journal of Environmental Biology, 17(4): 279-283.

Cappon, I.D. and Nicholls, D.M. 1975. Factors involved in increased protein synthesis in liver Microsomes after administration of DDT. Pestic. Biochem. Physiol., 5: 109-118.

Chandravathy, V.M. and Reddy, S.L.N. 1996. Lead nitrate exposure changes in carbohydrate metabolism of freshwater fish. Journal of Environmental Biology, 17: 75-79.

Chaudhry, A.S. and Jabeen, F. 2011. Assessing metal, protein, and DNA profiles in *Labeo rohita* from the Indus River in Mianwali, Pakistan. Environ. Monit. Assess., 174(1-4): 665-679.

Dasu, P.M. 2014. Thiocarb 75 % wp a carbamate insecticide induced toxicity biochemical and histopathological changes in the freshwater Indian major carp *Labeo rohita* Hamilton, Ph.D. Thesis, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, A.P, India

Dezwaan, A. and Zandee, D.I. 1973. Body distribution and seasonal changes in glycogen content of the common sea mussel, *Mytilus edulis*. Comp. Biochem. Physiol., 43: 53-55.

Dhanalakshmi, B. 2013. Acute and chronic toxicity of chromium on biochemical composition of the freshwater major carp *Cirrhinus mrigala* (Hamilton). Asian J. Sci. Technol., 4: 021-026.

Finney, D.J. 1971. Probit Analysis, 3rd edition. Cambridge University Press.

Golterman, H. and Claimo, R.S. 1969. Methods for Chemical Analysis of Freshwater. Blackwell Sci. Pub., 166 pp.

Jabeen, F., Chaudhry, A.S., Manzoor, S. and Shaheen, T. 2016. Carbamates and neonicotenoids in fish, water and sediments from the Indus River for potential health risks. Environ. Monit. Assess., 187(2): 29.

Kafilzadeh, F., Shiva, A.H., Malekpour, R. and Azad, H.N. 2012. Determination of organochlorine pesticide residues in water, sediments and fish from Lake Parishan, Iran. World J. Fish. Mar. Sci., 4(2): 150-154.

Kemp, A. and and Van Heijningen, A.J.K. 1954. A colorimetric method for the determination of glycogen in tissues. Bio. Chem. J., 56: 646-648.

Kumar, A., Singh, S. and Sharma, H.N. 2017. Changes in total protein in Liver and Kidney of freshwater fish, *Channa punctatus* (Bloch.) after intoxication of Carbaryl. Journal of Advanced Laboratory Research in Biology, 8(2): 41-43.

Kumari, A., Srivastava, A. and Jha, M.M. 2014. Carbaryl Induced alteration in histology and certain biochemical parameter in liver of *Clarias batrachus*. Global Journal of Bioscience and Biotechnology, 3(3): 259-263.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.1951. Protein measurement with the Folin Phenol Reagent. J. boil. Chem., 193: 265-275

Mayers, P. A. 1977. In: Review of Physiological Chemistry, 16th edition. Eds: Harper H A. Rodwell U V and Mayers P.A. Large Medical Publications, California.

Muddassir, A.T. 2015. Comparative study of biochemical alterations induced by carbofuran and malathion on *Channa punctatus* (Bloch.). International Research Journal of Biological Sciences, 4(9): 61-65.

Muley, D.V., Kamble, G.B. and Gaikwad, P.T. 1996. Endosulfan toxicity in the freshwater fish *Tilapia mossambica*. Journal of Aquatic Biology, 11: 61-66.

Naik, B. R., Rao, G. N. and Neelima, P. 2016. Sub-lethal toxicity of cyperkill (a synthetic pyrethroid pesticide) on glycogen content in the tissues of *Labeo rohita.* Int. J. Curr. Res. Aca. Rev., 4(12): 127-134.

Pillai, S.K. and Sinha, H.C.1968. Statistical Methods for Biological Works. Ramprasad and Sons, Agra.

Prasada Rao, G. D. V., Veeraiah, K., Krishna, Ch., Rajeswari, A. and Sindhoori, E. 2018. Cyhalothrin induced bio-chemical alterations in the grass carp *Ctenopharyngodon idellus* (Valenciennes). European Journal of Biomedical and Pharmaceutical Sciences, 5(9): 525-536.

Priya, B.P. and Maruthi, Y.A. 2013. Imidacloprid toxicity on biochemical constituents in liver tissue of fresh water teleost *Channa punctatus*. International Journal of Pharma and Bio Sciences, 4(4b): 50-54.

Rajamanickam, C. 1992. Effects of Heavy Metal Copper on the Biochemical Contents, Bioaccumulation and Histology of the Selected Organs in the Freshwater Fish, *Mystus Vittatus* (Bloch). Ph.D. Thesis, Annamalai University, India.

Rajamannar, K. and Manohar, L. 1998. Sublethal toxicity of certain pesticides on carbohydrates, proteins and amino acids in *Labeo rohita*. J. Ecobiol., 10(3): 185-191.

Rawat, D.K., Bais, V.S. and Agrawal, N.C. 2002. A correlative study on liver glycogen and endosulfan toxicity in *Heterpneustes fossilis*. J. Environ. Biol., 23: 205-207.

Roberts, M. and Boyce, C.B.C. 1972. In Methods in Microbiology. 7-A Edition. (eds: J.R. Norris and D.W. Ribbows). Academic Press, New York, pp 479.

Sastry, K.V. and Abad A Siddiqui. 1982. Chronic toxic effects of the carbamate pesticide Sevin on carbohydrate metabolism in a freshwater snakehead fish, *Channa punctatus*. Toxicology Letters, 14(1-2): 123-130.

Schmidt Nielson, B. 1975. Osmoregulation: Effect of salinity and heavy metal. Fed. Proc., 33: 2137-2146.

Searchy, D.G. and MacLennis, A.J. 1970a. Determination of DNA by the Barton Diphenylanine technique. In: Experiments and Techniques in Parasitology. (eds: A.J. Mac Lnnis and M. Voge) W.H. Freeman and Co, San-Franscisco, 190-191 pp.

Searchy, D.G. and MacLennis, A.J. 1970b. Determination of RNA by Dische orcinol technique. In: Experiments and Techniques in Parasitology. (eds: A.J. Mac Lennis and M. Voge). W.H. Freeman and Co, San-Francisco, pp. 189-190.

Shivanagouda, N., Sanagoudra and Bhat, U.G. 2013. Carbaryl induced changes in the protein and cholesterol contents in the liver and muscle of marine benthic fish, *Mugil cephalus*. American Journal of Biochemistry, 3(2): 29-33.

Singh, A. and Singh, A. 2017. Studies on toxicity stress, behavioural alterations and biochemical changes induced by glyphosate herbicide on the freshwater fish, *Channa punctatus* (Bloch). Int. J. Food Agricul. Veter. Sci., 7(3): 39-48.

Singh, R.K. and Sharma B. 1998. Carbofuran induced biochemical changes in *Clarias batrachus*. J. Pestic. Sci., 53: 285-290.

Srivastava, P. and Singh, A. 2013. In vivo study of effects of dithiocarbamates fungicide (mancozeb) and its metabolite ethylene thiourea (ETU) on freshwater fish *Clarius batrachus*. Journal of Biology and Earth Sciences, 3(2): B228-B235.

Tataji, P.B. and Kumar, M.V. 2016. Biochemical changes induced by Butachlor and Machete 50% EC to the freshwater fish *Channa punctata* (Bloch). International Journal of Science and Research, 5: 2048-2052.

Tilak, K.S., Raju, P.W. and Butchiram, M.S. 2009. Effects of alachlor on biochemical parameters of the freshwater fish, *Channa punctatus* (Bloch). J. Environ. Biol., 30(3): 421-426.

Veeraiah, K., Venkatrao, G., Vivek, Ch. and Hymaranjani, G. 2013a. Heavy metal, cadmium chloride induced biochemical changes in the Indian major *Cirrhinus mrigala* (Hamilton). Int. J. Bioassays, 2(07): 1028-1033.

Veeraiah, K., Vivek, Ch., Srinivas Rao, P. and Venkatrao, G. 2013b. Biochemical changes induced by Cypermethrin (10% EC), a pyrethroid compound in sub-lethal and lethal concentrations to the freshwater fish

Cirrhinus mrigala (Hamilton). J. Atoms and Molecules, 3(6): 625-634.
Vivek, Ch. 2015. Impact of Cartap Hydrochloride (50% SP) on Biochemical, Haematological and Enzymatic Activities of the Freshwater Fish, *Labeo Rohita* (Hamilton). Ph.D. Thesis submitted to Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, A.P, India.
Wankhedkar, P. T. and Bhavsar, S.S. 2015. Effect of Cartap hydrochloride and Imidacloprid on biochemical parameters of *Cerastus moussonianus*. Biolife, 3(1): 125-131.

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Synergistic Effect of Fungal Consortia and C/N Ratio Variation on Rice Straw Degradation

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ABSTRACT

In this study, the efficiency of fungal consortia was evaluated on the degradation of rice straw by varying the initial carbon/nitrogen ratio of the compost piles. Consortia of three potent cellulose-degrading fungal strains: *Aspergillus fumigatus, Aspergillus terreus* and *Aspergillus flavus* were used as an inoculant to degrade rice straw in a 90-day composting process. The carbon/nitrogen ratio of the composting piles was varied by varying the proportion of bedding material in different treatments. The composts thus achieved were subjected to physic-chemical analysis and phytotoxicity assay using *Solanum lycopersicum* as test seeds. After 90 days of composting, compost from treatment 4 with initial carbon/nitrogen ratio 34 depicted maximum stability by achieving a final carbon/nitrogen ratio of 16.25. Compost from treatment 4 showed the highest Germination Index (%) followed by treatment 3 and treatment 2 as 94.32%, 88.88% and 79%, respectively on the growth of *Solanum lycopersicum* seeds. Results concluded that fungal consortia derived agro-waste compost with an initial carbon/nitrogen ratio 34 depicted the earliest maturity which is suggestive of its suitability for agricultural application.

INTRODUCTION

Crop residue management is currently a global challenge. The quantity of agricultural waste generated throughout the world is enormous. Also, the problem of pollution associated with the conventional waste disposal methods demands exploration of alternative and environment-friendly methods of dealing with agro-wastes. The demand for food has been consistently increasing with the increasing population and has, therefore, created a need for upgrading of agricultural by-products. Disposal of such a huge quantity of lignocellulosic wastes is a big challenge to our environment. Wood and crop residue burning for energy leads to a release of toxic gases that cause environmental pollution (Tuomela et al. 2000). The accumulation of agro-waste raises health, safety, environmental, and aesthetic concern. Therefore, safe disposal techniques need to be explored. Crop residue management is currently a global challenge. The Ministry of New and Renewable Energy (MNRE) has reported that about 500 Mt of agro-wastes are produced every year wherein cereals are found to produce most of the residues (352 Mt). Fibres (66 Mt), oilseeds (29 Mt), legumes (13 Mt) and sugarcane (12 Mt) are also produced subsequently. 70% of agro-waste is contributed by rice, wheat, maize and millets collectively whereas 34% is generated from rice crop alone (Jain et al. 2014).

Rice (*Oryza sativa L.*) is a major crop, grown worldwide producing a large amount of rice straw. Rice straw is a stringy, lingo-cellulosic rice crop residue that remains after the harvest. It is not considered a good substrate owing to its complex lingo-cellulosic composition that renders it hard to degrade. Wastes obtained from plants, like rice straw, are unmanageable due to their lignin content which interferes with biodegradation (Van Soest 2006). Rice straw constitutes a considerable amount of silica which exhibit various repercussions related to animal health problems and grants this crop residue a gruff appearance thereby weakening its suitability for animal intake due to its reduced digestibility. The direct incorporation of straw in the soil is coupled with problems like immobilization of plant nutrients especially nitrogen and reduces the germination of crop seeds. It can hamper seedbed preparation and add to disease and weed problems. Therefore, at present, there are limited options for rice straw because of its pitiable quality for forage, bioconversion, and engineering applications. Rice straw is usually not integrated in the crop field due to its protracted rate of decomposition and susceptibility to pest invasion (Nigam & Pandey 2009).

Farmers generally opt for open field burning to get rid of this crop residue thereby contributing to greenhouse emissions into the atmosphere. A significant amount of rice straw is burned on-farm to make way for the succeeding wheat crop. Stubble burning results in air pollution which has dire consequences on the ecosystem due to the deteriorating soil quality, soil erosion, intensification of air pollutants and greenhouse gases (Gadde et al. 2009). 80% of rice straw is burnt in Punjab, Himachal Pradesh and Haryana while 50% is burnt in Karnataka and 25% in the state of Uttar Pradesh (Gupta et al. 2003). This *in situ* burning of rice straw can be attributed to the modern method of harvesting done with the aid of combine harvesters. To tackle this issue, composting has emerged as an eco-friendly and economic treatment technology and as an effective approach to achieve enhanced and sustainable output from agriculture (Sarkar & Chourasia 2017). It aids in the reduction of waste volume, weight, moisture content, odour, pathogens and spread of diseases, thereby, increasing potential nutrients in the compost rendering it suitable for agricultural applications (Gautam et al. 2011). It is seen as a key process where the final product is safer to use and microbes such as fungi have proved to be beneficial in accelerating the degradation of lignocellulosic waste. Compost properties differ significantly depending on the composting feedstock and composting procedures. Carbon and nitrogen are imperative elements essential for microbial breakdown (Ain et al. 2017). Carbon provides energy and structural support and constitutes half of the cell biomass in microbes whereas nitrogen being a chief constituent of enzymes and nucleic acids helps in growth and performance of cells. It is important to consider the carbon and nitrogen (C/N) ratio of the compost components to establish the optimal amounts of carbon and nitrogen. Narrowing of C/N ratio of agro-wastes by using urea as a supplement has been in practice earlier (Gaind & Gaur 2000). But developments in agriculture and an increasing inclination towards organic farming has catered to the use of organic wastes rich in nitrogen. Chicken litter or poultry manure can be considered as one such alternative (Ogunwande et al. 2008). It is also recognized that when the rice straw is combined with poultry manure, it reduces the C/N ratio of rice straw and even impedes the loss of nitrogen thereby developing an end-product which is equipped to provide required nourishment to the plants (Gaind et al. 2010).

Naturally, very few microorganisms are potent enough to break the intricate structure of lignin during composting. Fungi, owing to their potential to conceive prolific spores and filamentous morphology, are quite effective in degrading lingo-cellulosic crop residues. Also, fungi are the most influential and dominant groups present in soil which impact the structure and functioning of the ecosystem and therefore play a major role in many ecological services. Fungi produce an array of hydrolytic enzymes and hence subsist in nature in saprophytic mode and are more effective than bacteria in acidic soils and decomposing cellulose rooted in lignin (Goyal et al. 2011). They can stand a wide range of pH as compared to bacteria and hence are more preferred than bacteria for the decomposition of complex organic wastes. Composting of agro residues with high C/N ratio can be efficiently achieved by inoculating cellulolytic fungi such as *Aspergillus sp* (Ashraf et al. 2007). Also, these fungi have been reported to slash down the duration of the composting process by one month.

Though microbes amended composting has been studied earlier but rice straw composting aided with fungal inoculation has less explored literature. In the present research, the synergistic effect of fungal consortia on the degradation of rice straw by varying initial C/N ratio was studied and the compost thus achieved were tested for their phytotoxic effects on *Solanum lycopersicum* (tomato) seeds.

MATERIALS AND METHODS

For composting, paddy straw was obtained after the harvest of rice from the rice field of Kanheli village, Rohtak city (Haryana). It was then pre-treated by soaking in 0.1% urea solution and stacked along with green leaves and poultry droppings to make composting piles. Pre-treatment of rice straw with urea helped in delignification of rice straw and made it susceptible to degradation by cellulase enzyme. The proportion of green leaves and poultry droppings (Fig. 1) was varied to achieve the desired C/N ratios in the composting piles.

Three cellulolytic fungal strains cultured at Department of Environmental Science, MDU, Rohtak were used as inoculants for composting purpose. Fungal spore suspension was prepared as per the method described by Samsudin et al. (2013). 5-day old culture slants were washed with 0.9% NaCl saline solution and shaken rapidly for one minute. Haemocytometer was used to count fungal spores and the number of spores was adjusted to contain approximately 10^7 spores/mL of each fungal isolate. A broth inoculum containing 10^7 spores/mL of each isolate was used for inoculation.

A 90-day experiment was conducted to degrade rice straw. Bins were set in triplicate with circular holes of 100 mm diameter to provide aeration to the compost material. Control bin was also set in which no inoculants were added. Prepared compost treatments have been described in Table 1.

Table 1: Various compost treatments prepared for the study.

Treatment	Initial C/N ratio	Composition
Treatment 1	Control	No inoculants added
Treatment 2	30:1	Rice straw = 2000 g, Green Leaves = 1000 g, Poultry droppings = 1000 g + Fungal Inoculants
Treatment 3	26:1	Rice straw = 2000g, Green Leaves = 1000 g, Poultry droppings = 1300 g + Fungal Inoculants
Treatment 4	34:1	Rice straw = 2000 g, Green Leaves = 1000 g, Poultry droppings = 750 g + Fungal Inoculants



Fig.1 (A-D): Setting of compost bins.



Fig. 2: Compost piles.

The compost bins were kept inverted to form heaps (Fig. 2). The components of the compost bins were turned at regular intervals to provide aeration (Jusoh et al. 2013). Compost samples were taken at 30, 60 and 90-days intervals and were subjected to physic-chemical analysis as per standard protocols mentioned in Table 2. The mature compost obtained after 90 days was also subjected to phytotoxicity analysis to check the effectiveness of compost on the growth of test seeds *Solanum lycopersicum*. For this, seeds of *Solanum lycopersicum* were disinfected by dipping in 7% alcohol for about 3 minutes. Later it was left in a suspension of 0.001 $HgCl_2$ for about 2 minutes where it was stirred intermittently. It was repeatedly washed with distilled water to ensure that no toxins are present. 10 mL volume of compost extract was poured on filter paper spread in a Petri plate. 20 sterilized seeds of *Solanum lycopersicum* were then set on filter paper and Petri plates were carefully sealed using tape to reduce the loss of water. They were then incubated for 72 hours at room temperature. All tests were carried out in triplicate. The percentage of root elongation, seed germination and germination index (GI) was deduced using equations 1 to 3 (Zucconi et al. 1981).

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated in compost extract}}{\text{Number of seeds germinated in control}} \times 100 \quad \ldots(1)$$

$$\text{Root elongation (\%)} = \frac{\text{Mean root length in compost extract}}{\text{Mean root length in control}} \times 100 \quad \text{...(2)}$$

$$\text{Root elongation (\%)} = \frac{\text{Mean root length in compost extract}}{\text{Mean root length in control}} \times 100 \quad \text{...(3)}$$

RESULTS AND DISCUSSION

After 90 days of composting, the compost obtained had a uniform and granular texture, was relatively dry, earthy, and dark brown (Fig. 3). It looked crumbly and had a pleasant aroma, like freshly turned earth. Similar results were obtained by Pandharipande et al. (2004) who stated that finished compost must be black or dark brown in colour, granular and spongy with normal smell.

Results of the physic-chemical analysis of compost samples have been served in Table 3. pH alteration is crucial to plant health. Results reveal that all treatments were recorded with an alkaline pH to some extent in the early stages of decomposition. With due course of composting, pH values show signs of gradual decrease which might be due to the production of organic acids and loss of ammonia to the atmosphere. pH in all treatments stabilized at the end of 90 days with pH 7.5, 7.1, 7.2 and 6.7 for treatments 1, 2, 3 and 4 respectively (Fig. 4). Electrical Conductivity (EC) showed an increasing trend in all treatments during composting (Fig. 5). Treatment 4 (C/N 34) showed maximum electrical conductivity (3.6 dS/m) followed by treatment 3 (3.4 dS/m) and treatment 2 (3.1 dS/m). The increase in EC can be attributed to an increase of potassium (K^+) and other ions during composting and also due to the release of inorganic salts during decomposition (Bernal et al. 2009). EC was found to be higher in treatment 4 which might be due to the release of soluble salts (Huang et al. 2004, Wang et al. 2004). Total organic carbon (TOC) depicts a decreasing trend (Fig. 6) with value of treatment 4 (18.2%) > treatment 2 (18%) > treatment 3 (17.4%) after 90 days of composting. This might be due to the loss of carbon in the form of CO_2 as decomposition proceeds. This is in accordance with results indicated by Getahun et al. (2012). Also, the maximum value for Total Nitrogen content was observed in treatment 4 (1.12%) followed by treatment 3 (0.94%) and treatment 2 (0.91%) at 90 days of composting (Fig. 7).

Table 2: Methods used to detect parameters in compost samples.

Parameters	Methods used
pH	pH meter
Electrical conductivity	Conductivity meter
Total organic carbon	Walkley and Black method (Khiari et al. 2005)
Total nitrogen	Kjeldahl method (Katyal et al. 1987, Bremner 1996)

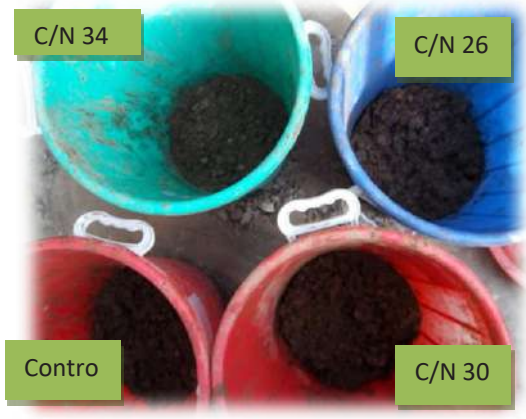


Fig. 3: Compost after 90 days of composting.

After 90 days of composting, treatment 4 with initial C/N ratio 34 depicted maximum stability by achieving a final C/N of 16.25 (Fig. 8) which shows that the compost from treatment 4 has attained earliest maturity and is suitable for agricultural application (Owis et al. 2016). On the contrary, a high C/N in compost obtained from control treatment depicts that carbon has been left unused in the compost mixture (Dobermann & Fairhurst 2002). This was supported by the fact that in treatment 4, the compost showed highest Germination Index (%) followed by treatment 3 and treatment 2 as 94.32%, 88.88% and 79%, respectively on the growth of *Solanum lycopersicum* seeds (Fig. 9 & Fig. 10) which implies that for most treatments phytotoxicity has been eliminated. Similar results were observed by Azim et al. (2014) and Abdelhamid et al. (2004). It can therefore be inferred from the study that rice straw can be efficiently degraded by lowering its initial C/N ratio to 34 using poultry droppings as an amendment. Similar results were recorded by Kausar et al. (2014).

Table 3: Physico-chemical analysis of composts during the 90-day study.

Parameters	Initial C/N Ratio	30 days of composting	60 days of composting	90 days of composting
pH	C/N 34	7.8 ± 0.02	7.1 ± 0.17	6.7 ± 0.26
	C/N 30	8.1 ± 0.13	7.9 ± 0.16	7.1 ± 0.29
	C/N 26	7.9 ± 0.32	7.6 ± 0.11	7.2 ± 0.16
	Control	8.3 ± 0.11	7.5 ± 0.31	7.5 ± 0.26
Total Organic Carbon (%)	C/N 34	21 ± 0.25	19.7 ± 0.19	18.2 ± 0.09
	C/N 30	20.2 ± 0.18	19 ± 0.27	18 ± 0.27
	C/N 26	20 ± 0.22	18.9 ± 0.05	17.4 ± 0.16
	Control	21 ± 0.15	20.3 ± 0.01	19.8 ± 0.22
Total Nitrogen (%)	C/N 34	0.65 ± 0.06	0.78 ± 0.16	1.12 ± 0.07
	C/N 30	0.79 ± 0.37	0.88 ± 0.25	0.91 ± 0.13
	C/N 26	0.78 ± 0.23	0.86 ± 0.18	0.94 ± 0.27
	Control	0.71 ± 0.19	0.73 ± 0.16	0.73 ± 0.21
Electrical Conductivity (dS/m)	C/N 34	2.7 ± 0.04	3.1 ± 0.07	3.6 ± 0.15
	C/N 30	2.3 ± 0.12	2.7 ± 0.16	3.1 ± 0.19
	C/N 26	2.4 ± 0.26	2.9 ± 0.11	3.4 ± 0.34
	Control	2.3 ± 0.08	2.5 ± 0.13	2.8 ± 0.28
C/N ratio	C/N 34	32.3 ± 0.05	24.8 ± 0.18	16.25 ± 0.17
	C/N 30	25.5 ± 0.14	21.5 ± 0.04	19.7 ± 0.04
	C/N 26	25.6 ± 0.12	21.9 ± 0.25	18.5 ± 0.16
	Control	29.5 ± 0.18	27.8 ± 0.07	27.1 ± 0.11

(n = 3; Mean ± SD)

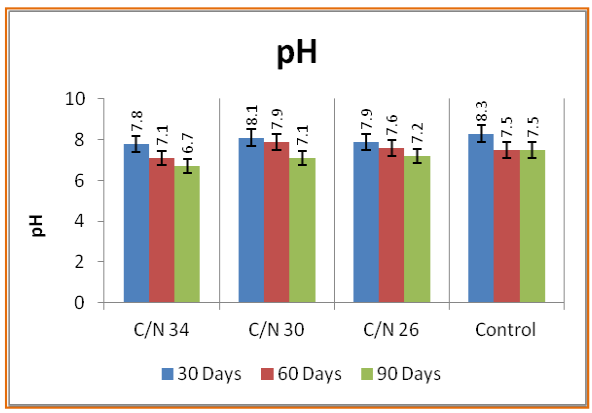


Fig. 4: pH values during composting.

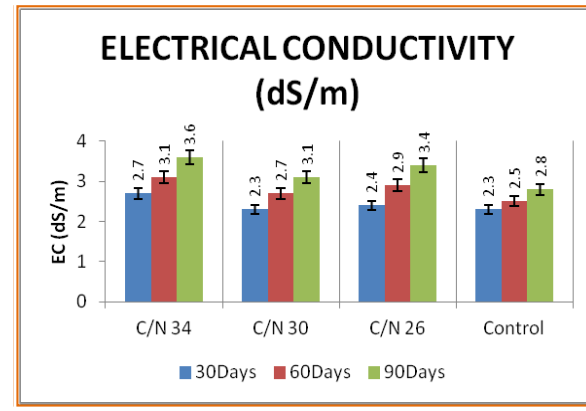


Fig. 5: Electrical conductivity (dS/m) of compost.

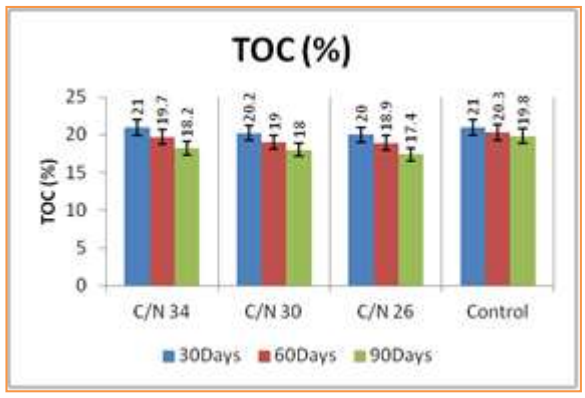


Fig. 6: Total organic carbon in compost samples.

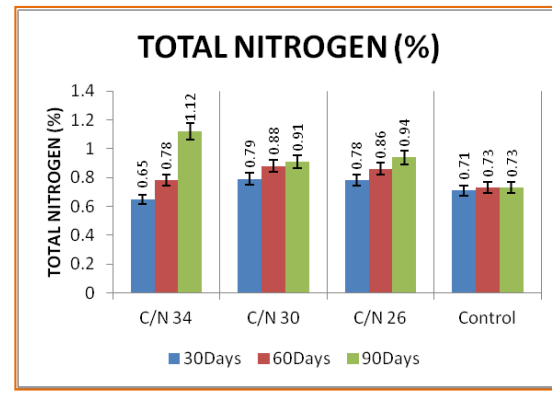


Fig. 7: Total nitrogen content in compost samples.

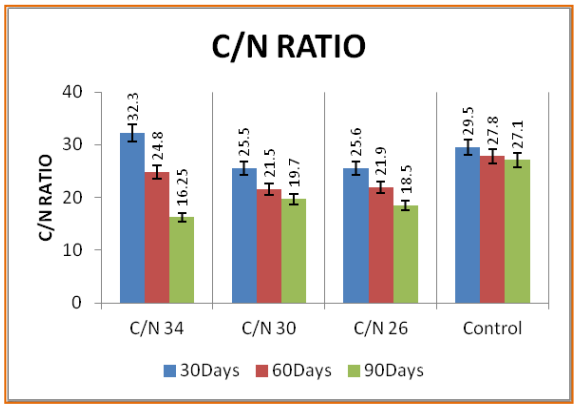


Fig. 8: C/N ratio of compost samples.

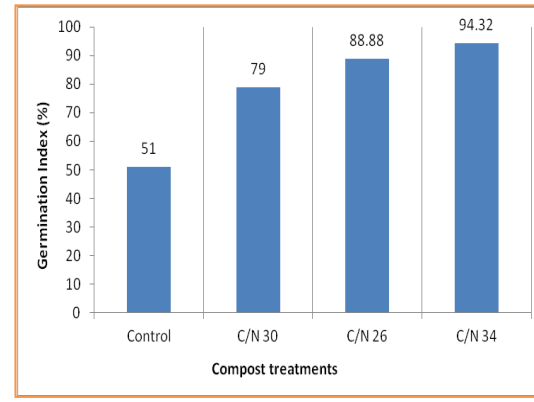


Fig. 9: Germination index (%) of final composts at the end of 90 days of composting.



(a) Seeds before Incubation



(b) Seeds after 72 hours of incubation

Fig.10 (a-b): Germination Index of mature compost.

Table 4: ANOVA: Two-factor without replication.

Source of variation	SS	df	MS	F	P-value	F crit
Rows	5237.736173	13	402.9027826	1.987737096	1.02707E-28	1.78052833
Columns	21.83360536	3	7.277868452	2.595262191	0.046159652	2.845067805
Error	109.3673196	39	2.804290247			
Total	5368.937098	55				

*null hypothesis is rejected and significant difference exists between the treatments

Statistical Analysis

To deduce the variables involved in inferring the compost quality, through physic-chemical characteristics and their relationships with each other, Two-way ANOVA (Table 4) and Pearson correlation coefficient (Table 5) were applied using SPSS 23.0 (0.05 levels).

CONCLUSION

The consortia of *Aspergillus fumigatus, Aspergillus terreus and Aspergillus flavus* efficiently degraded rice straw, thereby reducing the decomposition time. The final values of C/N observed in treatments 2, 3 and 4 are considered as acceptable levels for compost maturity. It is evident from the study

Table 5: Correlation between physico-chemical parameters of compost samples during 90 days of composting.

	pH 30	pH 60	pH 90	TOC 30	TOC 60	TOC 90	TN 30	TN 60	TN 90	EC 30	EC 60	EC 90	C/N 30	C/N 60	C/N 90
pH 30	1.000														
pH 60	0.489*	1.000													
pH 90	0.853*	0.511*	1.000												
TOC 30	0.185	-0.719	-0.105	1.000											
TOC 60	0.487*	-0.520	0.281	0.923*	1.000										
TOC 90	0.770*	-0.172	0.536*	0.760*	0.931*	1.000									
TN 30	0.246	0.935*	0.442*	-0.904	-0.713	-0.425	1.000								
TN 60	-0.349	0.645*	-0.220	-0.920	-0.980	-0.853	0.776*	1.000							
TN 90	-0.946	-0.521	-0.976	-0.012	-0.378	-0.654	-0.378	0.282	1.000						
EC 30	-0.814	-0.866	-0.866	0.418*	0.087	-0.266	-0.759	-0.208	0.876*	1.000					
EC 60	-0.990	-0.586	-0.899	-0.049	-0.374	-0.680	-0.374	0.239	0.970*	0.886*	1.000				
EC 90	-0.999	-0.526	-0.872	-0.136	-0.447	-0.739	-0.294	0.309	0.957*	0.842*	0.995*	1.000			
C/N 30	-0.202	-0.914	-0.423	0.924*	0.737*	0.462*	-0.998	-0.790	0.346	0.731*	0.333	0.250	1.000		
C/N 60	0.482*	-0.527	0.340	0.887*	0.991*	0.917*	-0.686	-0.989	-0.413	0.066	-0.379	-0.445	0.705*	1.000	
C/N 90	0.947*	0.255	0.891*	0.328	0.651*	0.857*	0.066	-0.563	-0.948	-0.691	-0.927	-0.942	-0.032	0.675*	1.000

* Values greater than p-value of 0.355 are significant

that composts attained maturity in 90 days and possessed a good amount of nutrients and organic matter. Moreover, no signs of phytotoxicity were detected in the final composts, except in control treatment. This study illustrates that supplementing rice straw with poultry droppings helped enhance the quantity of organic compounds in the final product. The study also indicates that reducing the initial C/N of rice straw to 34 by combining it with poultry droppings and inoculating with *Aspergillus* strains produced good quality mature compost as compared to the treatment done without using any inoculants. It can, therefore, be concluded that rice straw can be efficiently degraded by reducing its initial C/N ratio to 34 by amending it with poultry droppings and using a consortium of *Aspergillus fumigatus, Aspergillus terreus and Aspergillus flavus* as an inoculant. The compost thus formed can be deemed suitable for agricultural applications.

Thus we need to resort to composting rather than burning rice straw. Composting of crop residues is a promising avenue to create employment for people and it will also help to deal with the problem of increasing air pollution caused due to stubble burning.

REFERENCES

Abdel-Hamid, M.T., Horuchi, T. and Oba, S. 2004. Composting of rice straw with oilseed rape cake and poultry manure and its effects on faba bean (Vicia faba L.) growth and soil properties. Bioresour. Technol., 93: 183-189.

Ain, A.N., Hariz, A.M., Fariza, M.N. and Alyani, S.N. 2017. Physico-chemical and microbiological study during conventional composting using different rates of rice straw and cattle manure mixture. J. Trop. Agric. And Fd. Sci., 45(2): 145-154.

Ashraf, R., Shahid, F. and Ali, T.A. 2007. Association of fungi, bacteria and actinomycetes with different composts. Pak. J. Bot., 39 (6): 2141-2151.

Azim, K., Ouyihya, K., Amellouk, A., Perissol, C., Thami Alami, I. and Soudi, B. 2014. Dynamic composting optimization through C/N ratio variation as a startup parameter. Building Organic Bridges, 3: 787-790.

Bernal, M.P., Alburquerque, J.A. and Moral, R. 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresour. Technol., 5453-5444 :(22)100.

Bremner, J.M. 1996. Nitrogen-Total. DL Sparks (ed.) Methods of Soil Analysis. Part 3, SSSA Book Ser. 5. SSSA, Madison, WI p. 1085-1121.

Dobermann, A. and Fairhurst, T.H. 2002. Rice straw management. BCI, 16 (1): 7-11.

Gadde, B., Bonnet, S., Menke, C. and Garivait, S. 2009. Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. Environ. Pollut., 157 (5): 1554-1558.

Gaind, S. and Gaur, A.C. 2000. Effect of microbial inoculants and levels of Mussoorie rock phosphate on the quality of organo-biofertilizer. Geobios., 27 (1): 21-24.

Gaind, S. and Nain, L. 2010. Exploration of composted cereal waste and poultry manure for soil restoration. Bioresour. Technol., 101 (9): 2996-3003.

Gautam, S.P., Bundela, P.S., Pandey, A.K., Awasthi, M.K. and Sarsaiya, S. 2011. Isolation, identification and cultural optimization of indigenous fungal isolates as a potential bioconversion agent of municipal solid waste. Annals of Environmental Science, 5 (1): 4.

Getahun, T., Mengistie, E., Haddis, A., Wasie, F., Alemayehu, E., Dadi, D., Gerven, T.V. and Van der Bruggen, B. 2012. Municipal solid waste generation in growing urban areas in Africa: current practices and relation to socioeconomic factors in Jimma, Ethiopia. Environ. Monit. Assess., 184 (10): 6337-6345.

Goyal, S. and Sindhu, S.S. 2011. Composting of rice straw using different inocula and analysis of compost quality. J. Microbiol., 1(4): 126-138.

Gupta, R.K., Naresh, R.K., Hobbs, P.R., Jiaguo, Z. and Ladha, J.K. 2003. Sustainability of post-green revolution agriculture: the rice-wheat cropping systems of the Indo-Gangetic Plains and China. Improving the Productivity and Sustainability of Rice-wheat Systems: Issues and Impacts, 65: 1-25.

Huang, G.F., Wong, J.W.C., Wu, Q.T. and Nagar, B.B. 2004. Effect of C/N on composting of pig manure with sawdust. *Waste Manage.,* (Oxford), 24 (8): 805-813.

Jahromi, M.F., Liang, J.B., Rosfarizan, M., Goh, Y.M., Shokryazdan, P. and Ho, Y.W. 2011. Efficiency of rice straw lignocelluloses degradability by Aspergillus terreus ATCC 74135 in solid state fermentation. African Journal of Biotechnology, 10(21): 4428-4435.

Jain, N., Bhatia, A. and Pathak, H. 2014. Emission of air pollutants from crop residue burning in India. Aerosol Air Qual Res., 14 (1): 422-430.

Jusoh, M.L.C., Manaf, L.A. and Latiff, P.A. 2013. Composting of rice straw with effective microorganisms (EM) and its influence on compost quality. *J. Environ*. Health Sci., 10(1): 17.

Katyal, J.C., Singh, B., Vlek, P.L.G. and Buresh, R.J. 1987. Efficient nitrogen use as affected by urea application and irrigation sequence. Soil Sci. Soc. Am. J., 51(2): 366-370.

Kausar, H., Ismail, M.R., Saud, H.M., Habib, S.H., Othman, R. and Bhuiyan, M.S.H. 2014. Changes of physical and chemical characteristics during microbial composting of rice straw at various pH levels. *Compost* Sci. Util., 163-153 :(3)22.

Khiari, L. and Parent, L.E. 2005. Phosphorus transformations in acid light-textured soils treated with dry swine manure. Can. J. Soil Sci., 87-75 :(1)85.

Nigam, P.S.N. and Pandey, A. eds., 2009. Biotechnology For Agro-Industrial Residues Utilisation: Utilisation of Agro-Residues. Springer Science & Business Media.

Ogunwande, G.A., Osunade, J.A., Adekalu, K.O. and Ogunjimi, L.A.O. 2008. Nitrogen loss in chicken litter compost as affected by carbon to nitrogen ratio and turning frequency. Bioresour. Technol., 99 (16): 7495-7503.

Owis, A.S., El-Etr, W.M., Badawi, F.S.F., El-soud, A.A. and Abdel-Wahab, A.F.M. 2016. Bioprocessing the crop residues with different amendments for producing high quality compost. Int. *J. Chemtech* Res., 9: 43-54.

Pandharipande, S.L., Gadekar, S. and Agrawal, R. 2004. Bio conservation of press mud (a waste from sugar industry). Asian. Jr. of Microbiol. Biotech. Env., 6(1): 87-88.

Sarkar, P. and Chourasia, R. 2017. Bioconversion of organic solid wastes into biofortified compost using a microbial consortium. *Int. J*. Recycl. Org. Waste Agric., 4)6): 321-334.

Tuomela, M., Vikman, M., Hatakka, A. and Itävaara, M. 2000. Biodeg-

radation of lignin in a compost environment: a review. Bioresour. Technol., 183-169 :(2) 72.

Van Soest, P.J. 2006. Rice straw, the role of silica and treatments to improve quality. Animal Feed Science and Technology, 130 (3-4): 137-171.

Wang, P., Changa, C.M., Watson, M.E., Dick, W.A., Chen, Y. and Hoitink, H.A.J. 2004. Maturity indices for composted dairy and pig manures. Soil Biol. Biochem., 776-767 :(5)36.

Zucconi, F. 1981. Evaluating toxicity of immature compost. Biocycle, 22(2): 54-57.

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Isolation and Characterization of Bacterial Isolates from *Psidium guajava* Obtained from Local Markets of Patna and Their Antibiotic Sensitivity Test

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Microbiological test
Bacterial isolates

ABSTRACT

The study was conducted to investigate the presence of antibiotic resistant bacteria in guava, sold in the local markets of Patna. A total of twenty five fresh samples of guava were collected from five different market places in Patna city. Several microbiological tests were performed to assess the growth and type of bacteria. The colonies were isolated and identified as isolate 1 and 3 (identical to *Staphylococcus* spp.), isolate 2 (identical to *Escherichia* spp.) and isolate 4 and 5 (identical to *Bacillus* spp.) based on their cultural, morphological, Gram's staining and biochemical characteristics. Antibiotic susceptibility tests were conducted to detect their actual resistance capability. In the present study, S1 and S3 were found resistant to ampicillin, chloramphenicol, ofloxacin and intermediate to gentamycin while S2 was found resistant to ampicillin, chloramphenicol, gentamycin, ciprofloxacin, cephalexin and intermediate to ofloxacin. The isolates S4 and S5 were found sensitive to gentamycin, intermediate to chloramphenicol and resistant to ciprofloxacin. Results of this study showed that the guava samples obtained from different markets of Patna possess multidrug resistant bacteria.

INTRODUCTION

Guava (*Psidium guajava*) is widely distributed throughout the regions of India and also considered as popular indigenous fruit. It is most often called the "apple of the tropics" belonging to the family Myrtaceae (Shruthi et al. 2013). It possesses excellent medicinal properties with a pleasant aroma. Guava is a popular and important fruit consumed all over India for its easy availability, low cost and high nutritive value (Kaneria & Chanda 2011). Fruits like guava, which are mostly eaten raw and are highly perishable, and can also be a source of several bacterial diseases due to their improper handling during storage and transportation. It exhibits antioxidant properties, recognized for its anti-allergic, antimicrobial, antidiabetic, anti-inflammatory significance, and is a rich source of vitamin C (Shruthi et al. 2013). It can also be used for the treatment of gastroenteritis, diarrhoea and dysentery as well as green mature guava is a rich source of pectin, calcium and phosphorus (Morton et al. 1987). India, due to its diverse climatic conditions and rich biodiversity is the natural habitat for varieties of fruit. Extending from the Himalayan belt in the north to the southern tropical belt, India offers much in terms of fruit diversity. Among Indian states, Bihar is one of the leading producers of guava (Dinesh & Vasugi et al. 2010).

Improper manipulation and exposure to the environment such as soil, water and air can harbour a heterogeneous range of microorganisms (Sarker et al. 2018). Microorganisms, especially bacteria, thrive on the fresh guava fruits making them prone to several diseases. Consumption of such guava by humans can result in varieties of infection and associated health risks. Most microbes which are initially observed on whole fruit or vegetables are soil inhabitants (Andrews & Harris 2000, Janisiewicz & Korsten 2002). The worldwide use of antibiotics in different fields has created pressure to select resistance among infectious bacterial pathogen (Balotescu et al. 2003, Sharma et al. 2005). Public organization illustrates resistant bacteria as a disaster or a nightmare scenario that could have deleterious effects on human health (Viswanathan 2014).

The emergence of antibiotic resistant bacteria is a major concern of public health nowadays. The widespread misuse of antibiotics has led to an alarming increase of antibiotic resistant bacteria such as *Staphylococcus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Escherichia coli*.

It is evident that not much attention has been paid to guava fruit safety, especially, in context of Patna (Bihar). Therefore, the main objective of the present study was to isolate bacterial spp. from different guava samples collected from different

areas of Patna and to investigate their antibiotic resistant properties which are a major cause of several health issues.

MATERIALS AND METHODS

Sample Collection

Guava samples were collected from different regions of Patna (Table 1). The selected market places for sample collection were Patliputra colony, Kankarbagh, Boring road, Agamkuan and Bazar Samiti. The samples were collected in a sterilized zip lock bag with appropriate labelling.

Isolation of Bacteria

For the isolation of bacteria, samples were washed thoroughly with 10mL of sterile PBS (phosphate buffer saline) solution. Inoculation through spreading was done on a nutrient agar plate (Sanders 2012) with different serially diluted sample (up to 10^{-4} dilution) and the plates were incubated at 37°C for 24 hours. The strains that were obtained on nutrient agar plates were again streaked on eosin methylene blue (EMB) selective media and incubated at 37°C for 24-48 hours (Islam et al. 2014). The obtained colonies were pured by streaking on nutrient agar plates for further analysis.

Bacterial Enumeration

Colonies were enumerated using a colony counter by placing the plates on the colony counter and visualizing it as well as Total Viable Count (TVC) was calculated (Brugger et al. 2012).

Cultural and Morphological Characterisation

Plates were observed and colony morphology was recorded. The morphological characteristics like shape, colour, texture, margin etc. were qualitatively measured (Bai et al. 2010). Further, identification was done using Gram's staining reaction.

Biochemical Analysis of the Isolated Strains

Various biochemical tests such as catalase, coagulase, mannitol motility, citrate utilization, indole and methyl red were done to identify the bacterial strains (Sarker et al. 2018)

Antibiotic Sensitivity Test

Three isolates selected from three different genera were subjected to antibiotic sensitivity test using the disk diffusion method (Poonia et al. 2014). After the incubation at 37°C for 24 hours, plates were examined and zones of inhibition were measured with the help of millimetre scale from the edge to the disc.

RESULTS

Total Viable Count (TVC) of Different Samples of Guava

The bacterial load found to be highest in Patliputra colony sample (8.6×10^3) followed by Agamkuan (6.9×10^3), Boring road (6.3×10^3) and Kankarbagh (5.9×10^3) while Bazar Samiti sample (5.3×10^3) recorded the lowest count. The bacterial load in the guava samples (Fig. 1) sold at different market places of Patna noted varied TVC (Table 2 & Fig. 2).

Isolation of Bacteria from Guava Surface Samples and Their Morphological Characterization

The isolated microorganisms were visualized under the

Table 1: Details of samples taken for the isolation of bacteria.

Name of market	Number of guava samples collected	Total
Patliputra colony	5	25
Kankarbagh	5	
Boring road	5	
Bazar Samiti	5	
Agamkuan	5	

Table 2: TVC of different guava samples.

Markets name	TVC (CFU/mL)
Patliputra colony	8.6×10^3
Kankarbagh	5.9×10^3
Boring road	6.3×10^3
Bazar samiti	5.3×10^3
Agamkuan	6.9×10^3



Fig. 1: Guava samples collected from local markets of Patna for bacterial isolation.

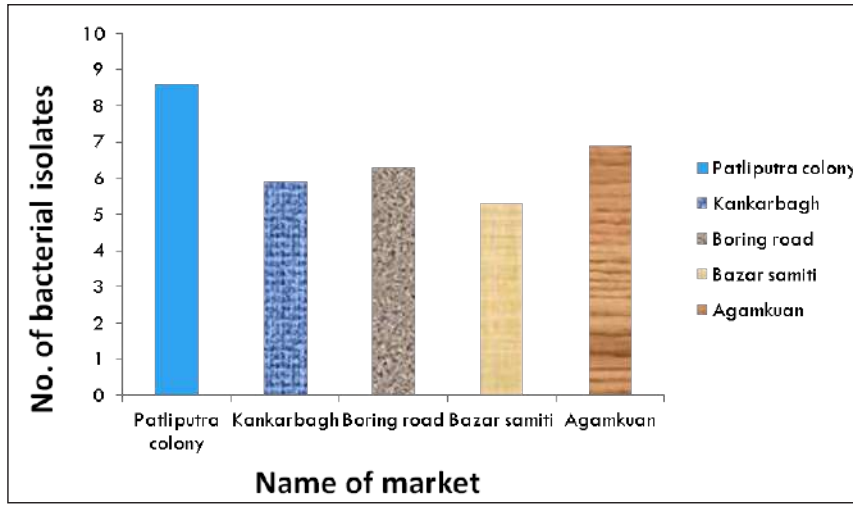


Fig. 2: Number of bacteria isolated from different guava samples.

light microscope for morphological characterization. Five different bacterial isolates were identified and further characterization was done. The bacteria isolate S1 and S3 were Gram-positive and coccus in shape while the bacteria S2 was Gram-negative, rod-shaped. The bacterial isolates S4 and S5 were Gram-positive rod-shaped (Table 3).

Biochemical Characterization

The biochemical test results of the isolated bacteria from different guava samples are given in Table 4.

Antibiotic Sensitivity Tests

All the five isolates identical to *Staphylococcus* spp. (S1 and S3), *Escherichia coli* (S2), and *Bacillus* spp. (S4 and S5) were subjected to antibiotic sensitivity test. To detect the sensitivity pattern of all the isolates, the CLSI (Clinical and Laboratory Standard Institute) standards have been used. The present study revealed, S1 and S3 resistant to ampicillin, chloramphenicol, ofloxacin and intermediate to gentamycin while S2 resistant to ampicillin, chloramphenicol, gentamy-

Table 3: Morphological characteristics of different isolates.

Bacterial isolates	Colour	Texture	Elevation	Margin	Shape
S1	White	Creamy	Flat	Circular	Coccus
S2	Greyish White	Shiny	Concave	Circular	Rod-shaped
S3	White	Creamy	Flat	Circular	Coccus
S4	White	Creamy	Concave	Circular	Rod-shaped
S5	White	Creamy	Concave	Irregular	Rod-shaped

Table 4: Biochemical characteristics of different isolates.

Basic characteristics	S1	S2	S3	S4	S5
Gram staining	G+ve, coccus	G-ve, rods	G+ve, coccus	G+ve, rods	G+ve, rods
Catalase	+ve	+ve	+ve	+ve	+ve
Coagulase	+ve	-ve	+ve	-ve	-ve
Motility	+ve	+ve	+ve	-ve	-ve
Citrate	+ve	-ve	+ve	+ve	+ve
Indole	-ve	+ve	-ve	+ve	+ve
MR (Methyl Red)	+ve	+ve	+ve	-ve	-ve
Interpretation	*Staphylococcus* spp.	*Escherichia coli*	*Staphylococcus* spp.	*Bacillus* spp.	*Bacillus* spp.

cin, ciprofloxacin, cephalexin and intermediate to ofloxacin while the isolates S4 and S5 were sensitive to gentamycin, intermediate to chloramphenicol and resistant to ciprofloxacin as shown in Table 5 and Figs. 3 & 4.

DISCUSSION

Guava is the fourth largest selling or consuming fruits in India after mangoes, bananas and citrus fruits. In the present

Table 5: Antibiotic sensitivity results of (a) S1 and S3, (b) S2 and (c) S4 and S5.

(a)

Antibiotics	Disc amount (µg)	Zone of resistant (mm)	Zone of susceptible (mm)	Isolate 1	Inference
Ampicillin (AM)	10	< or = 28	= 29	9	Resistant
Gentamycin (GM)	30	< or = 12	= 15	14	Intermediate
Chloramphenicol (C)	30	< or = 12	= 18	8	Resistant
Ciprofloxacin (CIP 5)	5	< or = 15	= 21	6	Resistant
Ofloxacin (OF)	5	< or = 14	= 18	0	-
Cephalexin (CLX)	30	< or = 14	= 18	5	Resistant

(b)

Antibiotics	Disc amount (µg)	Zone of resistant (mm)	Zone of susceptible (mm)	Isolate 2	Inference
Ampicillin (AM)	10	< or = 11	= 14	9	Resistant
Gentamycin (GM)	30	< or = 12	= 15	10	Resistant
Chloramphenicol (C)	30	< or = 12	= 18	9	Resistant
Ciprofloxacin (CIP-5)	5	< or = 15	= 21	12	Resistant
Ofloxacin (OF)	5	< or = 14	= 18	15	Intermediate
Cephalexin (CLX)	30	< or = 14	= 18	10	Resistant

(c)

Antibiotic	Disc amount (µg)	Zone of resistant (mm)	Zone of susceptible (mm)	Isolate 3	Inference
Ampicillin (AM)	10	< or = 11	= 14	12	Intermediate
Gentamycin (GM)	30	< or = 12	= 15	20	Susceptible
Chloramphenicol (C)	30	< or = 12	= 18	15	Intermediate
Ciprofloxacin (CIP 5)	5	< or = 15	= 21	14	Resistant
Ofloxacin (OF)	5	< or = 14	= 18	13	Intermediate
Cephalexin (CLX)	30	< or = 14	= 18	4	Resistant

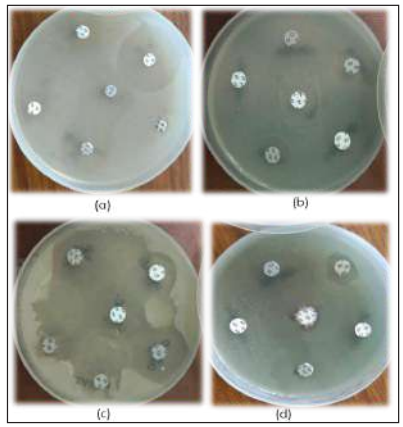


Fig. 3: Antibiotic sensitivity test of (a) (b) S1 and S3, (c) S2 and (d) S4 and S5.

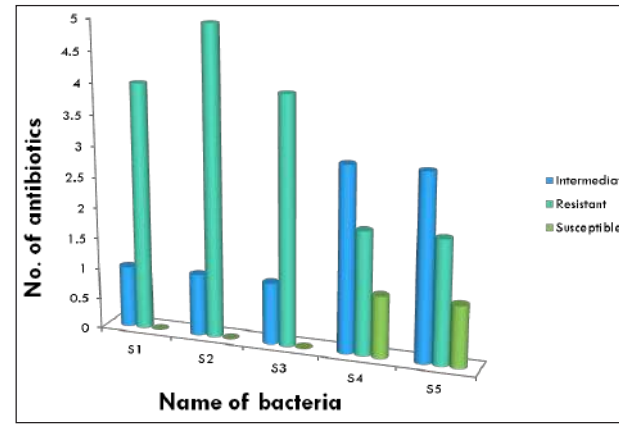


Fig. 4: Summary of antibiogram profile of isolates similar to *Staphylococcus* spp. (S1 and S3), *E. coli* (S2) and *Bacillus* spp. (S4 and S5).

study, twenty five samples of guava, all procured from different market places of Patna, were used and isolation, characterization and identification of microbes (bacteria) were made. The identified bacterial isolates were found identical to three genera such as *Staphylococcus* spp., *Escherichia coli*, *Bacillus* spp. Most of the guava samples were found contaminated with foodborne bacteria because of the poor hygiene and handling practices performed by the vendors (Gultie et al. 2013). This might have resulted in the occurrence of antibiotic resistant bacteria which could cause diseases among its consumers.

Three bacterial isolates of guava in this study were found resistant to different antibiotics that were used during the antibiotic sensitivity test. S1 and S3 resistant to ampicillin, chloramphenicol, ofloxacin and intermediate to gentamycin while S2 resistant to ampicillin, chloramphenicol, gentamycin, ciprofloxacin, cephalexin and intermediate to ofloxacin and the isolates S4 and S5 were sensitive to gentamycin, intermediate to chloramphenicol and resistant to ciprofloxacin.

The present investigation further revealed that the bacterial isolates that had been isolated from guava were found almost multidrug resistant. The occurrence of these multidrug resistant bacteria is the consequence of antibiotics either used in agricultural practises (Falkiner 1998) or used as animal manure in the agricultural fields (Boehme et al. 2004). Therefore, it can be suggested that implementation of proper hygienic conditions during pre- and post-harvest stages of production, transportation, storage and selling of the food stuffs for the safeguard of public health (Sarker et al.2018).

CONCLUSION

This study has clearly shown the presence of antibiotic resistant bacteria in the guava samples procured from local markets of Patna, Bihar. These multidrug resistant bacterial isolates could lead to health hazards for the consumers and their presence is of major health concern.

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REFERENCES

Andrews, J.H. and Harris, F.R. 2000. The ecology and biogeography of microorganisms on plant surfaces. Annual Review of Phytopathology, 38: 145-180.

Bai, N., Bae, E., Bhunia, A., Robinson, J. and Hirleman, E. 2010. Morphology characterization of bacterial colonies for predicting forward scattering patterns. Advanced Photonics and Renewable Energy, OSA Technical Digest (CD) (Optical Society of America,), paper STuC4.

Balotescu, C., Israil, A., Radu, R., Alexandru, I. and Dobre, G. 2000. Aspects of constitutive and required antibiotic resistance in *Aeromonas hydrophilia* strains isolated from water sources. Roum. Arch. Microbio. Immunol., 62: 179-89.

Boehme, S., Werner, G., Klare, I., Reissbrodt, R. and Witte, W. 2004. Occurrence of antibiotic-resistant *Enterobacteria* in agricultural foodstuffs. Mol. Nutr. Food Res., 48: 522-531.

Brugger, S.D., Baumberger, C., Jost, M., Jenni, W., Brugger, U. and Muhlemann, K. 2012. Automated counting of bacterial colony forming units on agar plates. PloS One, 7(3): e33695.

Dinesh, M.R. and Vasugi, C. 2010. Guava improvement in India and future needs. Journal of Horticultural Sciences, 5(2): 94-108.

Falkiner, F.R. 1998. The consequence of antibiotics used in horticulture. J. Antimicrob. Chemother., 41: 429-431.

Gultie, A. and Sahile, S. 2013. Microbial spectrum of fruit in Gondar town markets, North Western Ethiopia. J. Microbiol. Res., 3: 1-10.

Islam, N.N., Nur S., Farzana, Z., Uddin, I., Kamaruddin, K.M. and Siddiki, A.M.A.M.Z. 2014. Rapid identification of eosin methylene blue positive *Escherichia coli* by specific PCR from frozen chicken rinse in southern Chittagong city of Bangladesh: Prevalence and antibiotic susceptibility. Microbiology Journal, 4(2): 27-40.

Janisiewicz, W. and Korsten, L. 2002. Biological control of postharvest diseases of fruits. Annual Review of Phytopathology, 40(1): 411-41.

Kaneria, M. and Chanda, S. 2011. Phytochemical and pharmacognostic evaluation of leaves of *Psidium guajava (*Myrtaceae). Pharmacognosy Journal, 3(23): 41-45.

Poonia, S., Singh, T.S. and Tsering, D.C. 2014. Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. Indian Journal of Community Medicine, 11(8): 1145-1149.

Sarker, M.A.R., Haque, M.M., Rifa, R.A., Ema, F.A., Islam, M.A. and Khatun, M.M. 2018 Isolation and identification of bacteria from fresh guava (*Psidium guajava*) sold at local markets in Mymensingh and their antibiogram profile. Vet World, 11(8): 1145-1149.

Sanders, E.R. 2012. Aseptic laboratory techniques: Plating method. Journal of Visualized Experiments, 63: 30-64.

Sharma, R., Sharma, C.L. and Kapoor, B. 2005. Antibacterial resistance: Current problems and possible solution. Indian J. Med. Science, 59: 120-129.

Shruthi, S.D., Roshan, A., Timilsina, S.S. and Sunita, S. 2013. A review of the medicinal plant *Psidium guajava* Linn. (Myrtaceae). J. Drug Deliv., 3(2): 162-168.

Viswanathan, V.K. 2014. Off label abuse of antibiotics by bacteria. Gut Microbes, 5(1): 3-4.

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Kinetic and Adsorption Study for Removal of Arsenic from Aqueous Medium by Low Cost Bentonite of Rajmahal Hills and Hazaribagh, Jharkhand

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ABSTRACT

The paper deals with the feasibility of arsenite removal by the adsorption from bentonite mineral. Groundwater arsenic contamination has been reported in different parts of the world including Jharkhand, Bihar and Uttar Pradesh. Tube wells in Holocene Newer Alluvium are characterized by grey to black coloured organic-rich argillaceous sediments which have arsenic-contaminated groundwater. The majority of arsenic present in the groundwater is in the form of As(III) which exists as uncharged species arsenic tri hydroxide at pH value of less than 9.2. Arsenite is removed by various techniques like coagulation microfiltration, fixed bed adsorption, bioremoval, ion exchange, membrane filtration, etc. Our studies have shown that locally available bentonites containing a unit of montmorillonites can remove the arsenic from an aqueous medium. On the treatment of 100 mL arsenite solution with 300 mesh sieves bentonites up to different intervals of time, it has been found that bentonites are good adsorbent of arsenite. The percentage removal of arsenite is up to 99 per cent with 3 g sodium derivative of bentonite for 1 hour. The removal efficiency, adsorption isotherm and kinetic studies show the suitability of bentonite minerals for arsenic removal following first-order kinetics. Freundlich and Langmuir isotherms are obeyed in the adsorption of arsenite by bentonite minerals. Adsorption of arsenic by bentonite minerals has proved to be a low-cost eco-friendly method. Sodium derivative of bentonite minerals has been found more efficient for removal of arsenite.

INTRODUCTION

Arsenic contamination of drinking water has become a matter of worldwide concern. Main causes of occurrence of arsenic in groundwater may be attributed to pyrite bearing shale, As-Cu mineralization and Gold belt of the Son valley with arsenic content. Arsenic problem is not only local and national but also global. The danger of arsenic contaminating the water reserves in the entire world is on the rise. Most water enters from natural deposits in the earth. Arsenic occurs as arsenic sulphide minerals like arsenopyrites (Jha 2016, Jha 2018). Some of the arsenic impurities exist in calcium carbonate and phosphate minerals where it substitutes for carbonate and phosphate. In oxidizing soils arsenate is bound to ferric hydroxide minerals such as ferrihydrite and haematite (Mermut 1994, Brindley 1980). Organic matter converts ferric to more soluble ferrous, in effect dissolving arsenic and causing an increase in arsenic in ground level. Use of arsenical pesticides, herbicides, industrial and agricultural pollution increases the arsenic level in groundwater (Jha et al. 2011, Jha et al. 2010). Inorganic arsenicals are more toxic than organic arsenicals. As(III) is more toxic than As(V) (Jha & Mishra 2012, Lagaly 1995) Arsenite exists in aqueous medium as H_2AsO_3 and H_3AsO_3 whereas arsenate is as $HAsO_4^{2-}$ and $H_2AsO_4^{2-}$. An alarming aspect of arsenic-contaminated groundwater is in its use of irrigating crops and vegetables to which arsenic passes and then consumed by humans.

Bentonites in different colours and grades are available in many states of the country. Rajmahal hill bentonites have been found as good quality bentonites due to their cation exchange capacity, swelling power and potential for adsorption. Hazaribagh bentonites have not been analysed for physicochemical characteristics and removal capacity of arsenic and heavy metals till date. The bentonites minerals containing montmorillonite unit having a negative charge which is balanced by the cations held on the surface. It has been established that the structure of Bentonite is 2:1 which implies that one octahedral sheet is sandwiched between two tetrahedral sheets (Guyonnet et al. 2005, Bailey 1982). The bentonites have a cation exchange capacity varying from 70 to 110 meq/100 g of clay due to exchangeable cations such as $Na^{+,}$ Ca^{+2} and Mg^{+2}.

The cation exchange capacity may be represented as:

$$BH + M^+ = BM + H^+$$

Where, M^+ represents a cation, H^+ is a cation. M^+ exchange H^+ cation and BH is an exchanger.

$$K = [BM][H^+]/[BH][M^+]$$

Where, K is the thermodynamic equilibrium constant.

The molecular formula of Bentonite is $(Na, Ca)_{0.33}$, $(Al, Mg)_2$, $Si_4O_{10}(OH)_2.n\ H_2O$. The main constituent of this smectite clay mineral is silica and alumina (Brindley et al. 1986). In addition to this calcium oxide, sodium oxide, magnesium oxide, ferrous oxide/ferric oxide and traces of titanium oxide are present. Arsenic removal of water or prepared synthetic samples takes place both by ion exchange and the surface area of bentonites available for adsorption (Calvert 1981). This is the explanation commonly offered by research workers on arsenic. The pure sample, as well as derivatives of the collected bentonites from different places of Hazaribagh and Rajmahal Hills, have been analysed for arsenic removal capacity. The effect of contact time with a fixed mass of montmorillonite in 100 mL solution of sodium arsenite has been studied. The adsorption mechanism has been explained by Freundlich Adsorption Isotherm, given as

$$q_c = K.C^{1/n} \text{ or } x/m = K.C^{1/n}$$

Where, x/m is the mass of adsorbate per unit adsorbent.

$$\log x/m = \log K + 1/n \log C$$

The value of K and 1/n are obtained from the intercept and slope respectively (Karthikeyan et al. 2005, Jha 2012).

The value of x/m or q_c is obtained from the formula given below.

$$q_c = [(C_0\text{-}Ce) \times V]/w$$

Where, C_0 and Ce are the initial and equilibrium concentrations of arsenite respectively. W is the mass of adsorbent and V, the volume of solution being used. The percentage removal is given by:

$$\%\ \text{removal} = [(C_0\text{-}Ct)/C_0] \times 100$$

If a straight line is obtained in the plot of q_t/ct vs. c_t, Langmuir model is followed and a straight line obtained between a graph of log x/m and log C confirms Freundlich isotherm.

MATERIALS AND METHODS

The bentonite minerals were collected from the different sampling sites of Hazaribagh districts mainly located at lat. 23.99°N and long. 85.37°E. The places are Barhi (Kundba), Barkagaon (Debgarh), Chauparan (Chandpur), Chalkusha (Sudan), Barkatha (Shiladhi), Keredari (Masuria), Churchu (Sandi), Karbekla (Kesu) and Oriya (Rolagaon). The samples were finely powdered up to 300 mesh sieve. The sample which gave blue colour with benzidine solution indicates the presence of montmorillonite unit. The stock solution of 100 ppm arsenite solution was prepared by dissolving 173.5 mg of anhydrous sodium arsenite of analytical grade in 1 L distilled water. Two ppm solution of arsenite was prepared by dilution of the stock solution. All the glassware were washed with dilute acid and then washed with double distilled water. 100 mL 2 ppm solution was taken in 250 mL conical flask and 1 g bentonite sample was added to the conical flask and suspension was shaken in a mechanical shaker up to a different interval of time (ranging from 1 hour to 3 hours). This process was repeated with different masses of bentonite samples viz. 1 g, 2 g, 3 g up to 1 hr. After the shaking was over the solution was filtered with filter paper Whatman filter paper No. 42. The residual concentration of the arsenite ion was estimated by Mercko quant Arsenic Kit available in the laboratory. 5 mL test solution is taken in which reagent I is added and when this dissolves completely, reagent II is added. The strip supplied by the company is inserted into the tube up to 20 minutes. Special care is taken so that the strip might not come in contact with the test solution. After 20 minutes the strip is taken out and matched with the intensity of the colour. This gives the range of arsenic from 0.02 ppm to 2 ppm and more. Arsenic content was analysed by atomic absorption spectrophotometer also. The results obtained by the kit agree with the results obtained from the atomic absorption spectrophotometer (Perkin Elmer). The experiments were carried out in a neutral medium. The rate constant was calculated using the rate equation (Ramesh et al. 2007). The adsorbate per unit adsorbent values was put in the equation and tested for Freundlich and Langmuir isotherms (Sharma et al. 1995, J. Md. et al. 2008, Mondal et al. 2006, Dai et al. 1993)

RESULTS AND DISCUSSION

From Table 1, it is clear that the maximum removal of arsenite takes place with sample S_6 (2540) after treatment of 100 mL 2 ppm arsenic (III) solution (Haque et al. 2008, Maiti et al. 2007)

The Effect of contact time and adsorbent dosage has been studied with different grades of pure bentonite samples and their derivatives (Deliyanni et al. 2007, Ersoy et al. 2003). The initial concentration of arsenic decreases up to 0.2 ppm when 100 mL 2 ppm sodium arsenite solution is treated with either 2 g, 3 g bentonite up to 1 hour, the residual concentrations after 1 hour are 0.3 ppm, 1.2 ppm and 0.6 ppm respectively, when treated with pure samples of bentonite S_6 (2540), S_7 (2541) and S_{20} (2542) (Tables 1 & 2). After 2 hours of treatment, the concentration decreases up to 0.2 ppm (Tables 1 & 4) but the maximum removal takes place after 3 hours with sodium derivative of S_6 (2540) (Table 5). When

Table 1: Concentration of arsenic (III) ion after treatment with 1 g of bentonite mineral.

Sl. No.	Bentonite Sample No.	Initial concentration of arsenic(III)ion (in ppm)	Residual concentration after 1 hour. (in ppm)	Residual concentration after 2 hours (in ppm)	Residual concentration after 3 hours (in ppm)
1	S_6 (2540)	2	0.3	0.2	0.2
2	S_7 (2541)	2	1.2	0.8	0.6
3	S_{20} (2542)	2	0.6	0.4	0.3
4	Na Derivative of S_6 (2540)	2	0.825	0.737	0.4
5	Na Derivative of S_7 (2541)	2	0.612	0.523	0.413

Table 2: Concentration of arsenic (III) ion after treatment with different masses of bentonite mineral.

Sl.No	Bentonite Sample No.	Initial concentration of arsenic(III) ion (ppm)	Residual concentration with 1 g bentonite treatment after 1 hour (ppm)	Residual concentration with 2 g bentonite treatment after 1 hour (ppm)	Residual concentration with 3 g bentonite treatment after 1hour (ppm)
1	S_6 (2540)	2	0.3	0.2	0.2
2	S_7 (2541)	2	1.2	0.6	0.05
3	S_{20} (2542)	2	0.6	0.4	0.2
4	Na Derivative of S_6 (2540)	2	0.825	0.4	0.02
5	Na Derivative of S_7 (2541)	2	0.612	0.571	0.327

Table 3: Percentage arsenic removal after treatment with 1 g of bentonite mineral for 1 hour and values of q_t, C_t, log qt and log Ct.

Sl.No	Bentonite Sample No.	% Removal	q_t	C_t	log q_t	log C_t
1	S_6 (2540)	85	170	0.3	2.230	-0.5228
2	S_7 (2541)	40	80	1.2	1.903	-0.0791
3	S_{20} (2542)	70	140	0.6	2.146	-0.2218
4	Na Derivative of S_6 (2540)	58.75	117.5	0.825	2.070	-0.0835
5	Na Derivative of S_7 (2541)	69.4	138.8	0.612	1.841	-0.213

Table 4: Percentage arsenite removal after treatment with 1 g of bentonite mineral for 2 hour and values of q_t, C_t, log qt and log Ct.

Sl.No	Bentonite Sample No.	% Removal	q_t	C_t	log q_t	log C_t
1	S_6 (2540)	90	180	0.2	2.2550	-0.6989
2	S_7 (2541)	60	120	0.8	1.7780	-0.0969
3	S_{20} (2542)	80	160	0.4	1.9030	-0.3979
4	Na Derivative of S_6 (2540)	63.15	126.3	0.737	1.8003	-0.1325
5	Na Derivative of S_7 (2541)	73.85	147.7	0.523	2.1694	-0.2814

Table 5: Percentage arsenite removal after treatment with 1 g of bentonite mineral for 3 hours and values of q_t, C_t, log qt and log Ct.

Sl.No	Bentonite Sample No.	% Removal	q_t	C_t	log q_t	log C_t
1	S_6 (2540)	90	180	0.2	2.2552	-0.6989
2	S_7 (2541)	70	140	0.6	2.1461	-0.2218
3	S_{20} (2542)	85	170	0.3	2.2304	-0.5228
4	Na Derivative of S_6 (2540)	80	160	0.4	2.204	-0.3979
5	Na Derivative of S_7 (2541)	79.35	158.70	0.413	1.8995	-0.3840

Table 6: Per cent arsenic removal after treatment with 2 g of bentonite mineral for 1 hour and values of q_t, C_t, log qt and log Ct.

Sl.No.	BentoniteSample No.	%Removal	q_t	C_t	log q_t	log C_t
1	S_6 (2540)	90	90	0.2	1.9542	-0.6989
2	S_7 (2541)	70	70	0.6	1.8450	-0.2218
3	S_{20}(2542)	80	80	0.4	1.9030	-0.3979
4	Na Derivative of S_6 (2540)	80	80	0.4	1.9030	-0.3979
5	Na Derivative of S_7 (2541)	71.45	71.45	0.571	1.8540	-0.2433

Table 7: Percentage arsenic removal after treatment with 3 g of bentonite mineral for 1 hour and values of q_t, C_t, log qt and log Ct.

Sl.No.	BentoniteSample No.	%Removal	q_t	C_t	log q_t	log C_t
1	S_6 (2540)	90	60	0.2	1.778	-0.6989
2	S_7 (2541)	97.5	65	0.05	1.8129	-1.3010
3	S_{20} (2542)	90	60	0.2	1.7781	-0.6989
4	Na Derivative of S_6 (2540)	99	66	0.02	1.8195	-1.6989
5	Na Derivative of S_7 (2541)	83.65	55.77	0.327	1.7464	-0.4854

Table 8: Percent removal of arsenite with different time intervals.

Sl. No.	Bentonite Sample No.	% Removal	q_t	C_t	log q_t	log C_t
1	S_6 (2540)	90	60	0.2	1.778	-0.6989
2	S_7 (2541)	97.5	65	0.05	1.8129	-1.3010
3	S_{20} (2542)	90	60	0.2	1.7781	-0.6989
4	Na Derivative of S_6 (2540)	99	66	0.02	1.8195	-1.6989
5	Na Derivative of S_7 (2541)	83.65	55.77	0.327	1.7464	-0.4854

Table 9: Percent removal of arsenite with different dosage of bentonite for 1 hour.

Sample no	% Removal for 1 g sample	% Removal in 2 g sample	% Removal in 3 g sample
S_6 (2540)	85	90	90
S_7 (2541)	40	70	97.5
S_{20} (2542)	70	80	90
Na Derivative of S_6 (2540)	58.75	80	99
Na Derivative of S_7 (2541)	69.4	71.45	83.65

different masses of bentonite, e.g. 1 g, 2 g and 3 g were added to 100 mL 2 ppm sodium arsenite solution, it was clear that maximum removal took place when treated with 3 g bentonite up to 1 hour. The concentration changes to 0.02 ppm from 2 ppm (Table 2). When the efficiency of adsorption of arsenite ion by bentonite minerals was compared with the derivative of the mineral, it had been found that sodium derivative of S_6 (2540) had more adsorption capacity in comparison to pure bentonite samples. There were very similar findings in the adsorption capacity of 1 g bentonite mineral S_7 (2541) and its sodium derivative. 3 g of bentonite sample S_7 (2541) removed the arsenite ion from 2 ppm to 0.05 ppm in 1 hour, whereas three grams of its sodium derivative removed the arsenite ion concentration from 2 ppm to 0.327 ppm. Bentonite sample S_6 (2540) has greater efficiency for arsenite removal compared to its sodium derivatives, whereas pure bentonite sample S_7 (2541) had lesser efficiency than its sodium derivatives for the removal of arsenite ion from aqueous medium up to different time intervals. The percentage removal vs. time with different doses of bentonite shown in Tables 6, 7, 8, 9 and Figs. 1, 3 indicate that the minimum percentage removal is 40 for S_7 (2541) and maximum removal is about 99 per cent which takes place with sodium derivative of S_6 (2540). Effect of contact time and adsorbed amount (Table 10, Fig. 4) has been studied with different grades of pure bentonite samples and their derivatives. A graph of log q_t vs. log c_t values in Fig. 2 (Table 3) showed linearity. The linearity of the graph confirmed the Freundlich isotherm. Fig. 5 (Table 11) show that plot of c_t/q_t vs. c_t is a straight line for the bentonite sample S_6 (2540), S_7 (2541), S_{20} (2542) and sodium derivatives of S_6 (2540) and S_7 (2541). Fig. 4 showed that first order rate reaction is followed. The straight lines obtained in the graph between ct/qt vs. ct indicate that Langmuir adsorption isotherm is also followed in the case of adsorption in arsenite by bentonites (Fig. 5). The adsorption process may be explained as the arsenite ion on the active sites of the adsorbent and intraparticle diffusion (Li & Bowman 2001). The adsorption depends on the surface morphology once the surface is covered with the arsenite ions; there is no further scope of adsorption of arsenite from aqueous medium (Chakroborti et al. 2003). The particle size of 300 mesh sieve of bentonites makes available more surface area for adsorption with 3 g of bentonite.

CONCLUSION

From different Government reports and tests performed, it is obvious that there is a rapid expansion of arsenic concentration in groundwater in Hazaribagh District of Jharkhand which is a matter of great concern for the inhabitant of that region. Although the impact of arsenic in the human body is slow its effect is dangerous. Government is continuously taking appropriate steps to control the effect of this slow poison. Arsenic removal plants are established in different areas. From the results obtained by different experiments

Table 10: Variation of the adsorbed amount of arsenite with time.

Sample no	Amount adsorbed in 1 hour	Amount adsorbed in 2 hour	Amount adsorbed in 3 hour
S_6 (2540)	1.7	1.8	1.8
S_7 (2541)	0.8	1.2	1.4
S_{20} (2542)	1.4	1.6	1.7
Na Derivative of S_6 (2540)	1.175	1.263	1.6
Na Derivative of S_7 (2541)	1.388	1.477	1.587

Table 11: Values of (Ct/q_t) Vs C_t for different samples of bentonites.

Sample No.	(Ct/q_t)	C_t
S_6 (2540)	0.001764	0.3
S_7 (2541)	0.015	1.2
S_{20} (2542)	0.00428	0.6
Na Derivative of S_6 (2540)	0.00702	0.825
Na Derivative of S_7 (2541)	0.0044	0.612

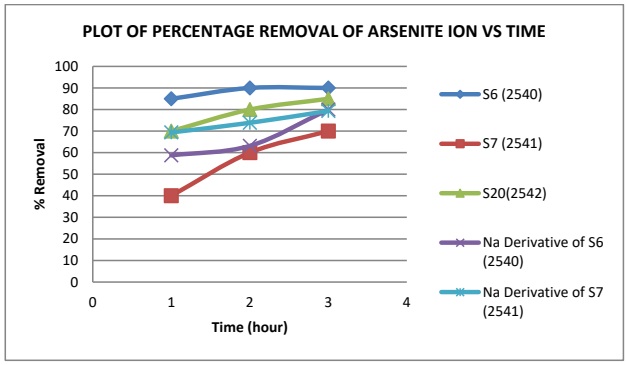


Fig. 1: Effect of contact time on percentage removal.

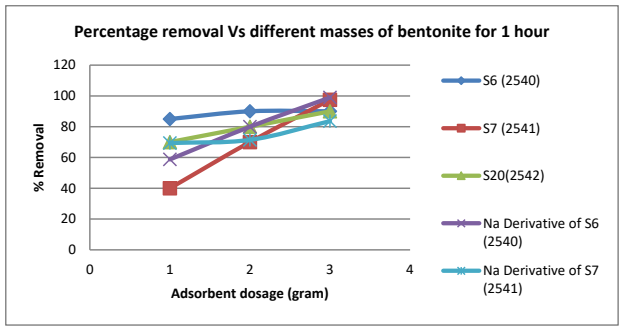


Fig. 3: Effect of dosage on percentage removal.

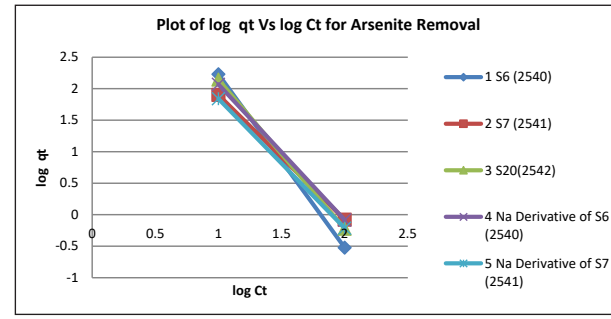


Fig. 2: Freundlich isotherm.

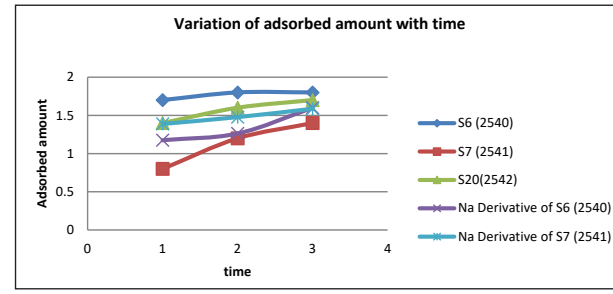


Fig. 4: Plot of variation of the adsorbed amount of arsenite ion with time.

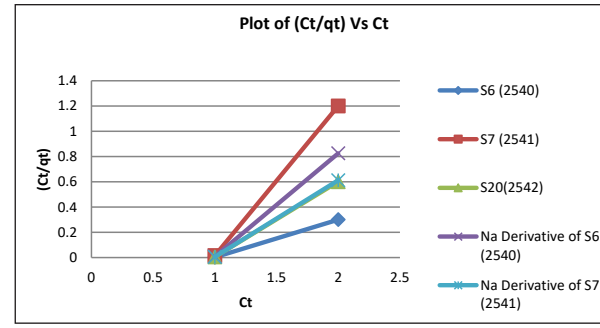


Fig. 5: Langmuir Isotherm.

performed, it is quite clear that bentonite clay and its derivatives especially sodium derivatives are essentially capable for removal of arsenic from water to make it safe for drinking. Bentonites of Hazaribagh may be exploited for removal of arsenite on large by industries also. Both Freundlich and Langmuir adsorption isotherms are followed in case of the removal of arsenite by bentonites.

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REFERENCES

Bailey, S.W. 1982. Crystal structures of clay minerals and their X ray

identification. In: (Brown, G. ed.) Crystal Structures of Clay Minerals and Their X Ray Identification, pp. 1-123

Brindley, G.W. and Brown 1980. Crystal structure of clay minerals and their x ray identification. Journal Mineralogical Society, Monographs.

Brindley, G.W., Kao, C.C., Harrison, J.L., Lipsicas, M. and Raythatha, R. 1986. Relation between structural disorder and other characteristics of kaolinites and dickites. Clays and Clay Minerals, 34(3): 239-249.

Calvert, C.S. 1981. Chemistry and mineralogy of iron-substituted kaolinite in natural and synthetic systems (Doctoral dissertation, Texas A&M University. Libraries), pp 224.

Chakraborti, D., Mukherjee, S.C., Pati, S., Sengupta, M.K., Rahman, M.M., Chowdhury, U.K., Lodh, D., Chanda, C.R., Chakraborti, A.K. and Basu, G.K. 2003. Arsenic groundwater contamination in Middle Ganga Plain, Bihar, India: a future danger?. Environmental Health Perspectives, 111(9): 1194-1201.

Dai, X., Nekrassova, O., Hyde, M.E. and Compton, R.G. 2004. Anodic stripping voltammetry of arsenic (III) using gold nanoparticle-modified electrodes. Analytical Chemistry, 76(19): 5924-5929.

Deliyanni, E.A., Peleka, E.N. and Matis, K.A. 2007. Effect of cationic surfactant on the adsorption of arsenites onto akaganeite nanocrystals. Separation science and Technology, 42(5): 993-1012. Doi: 10.1080/01496390701206306

Ersoy, B. and Çelik, M.S. 2003. Effect of hydrocarbon chain length on adsorption of cationic surfactants onto clinoptilolite. Clays and Clay Minerals, 51(2): 172-180. doi:10.1346/CCMN.2003.0510207

Guyonnet, D., Gaucher, E., Gaboriau, H., Pons, C.H., Clinard, C., Norotte, V. and Didier, G. 2005. Geosynthetic clay liner interaction with leachate: correlation between permeability, microstructure, and surface chemistry. Journal of Geotechnical and Geoenvironmental Engineering, 131(6): 740-749.

Haque, N., Morrison, G., Cano-Aguilera, I. and Gardea-Torresdey, J.L. 2008. Iron-modified light expanded clay aggregates for the removal of arsenic (V) from groundwater. Microchemical Journal, 88(1): 7-13. doi:10.1016/j.microc.2007.08.004

Haron, M.J., Ab Rahim, F., Abdullah, A.H., Hussein, M.Z. and Kassim, A. 2008. Sorption removal of arsenic by cerium-exchanged zeolite P. Materials Science and Engineering: B, 149(2): 204-208. doi:10.1016/j.mseb.2007.11.028

Jha, A.K and Mishra, B. 2012. Removal of fluoride by bentonite minerals of Rajmahal hills. Journal of Indian Chem. Soc., 89: 519.

Jha, A.K. 2012. The Problem of Fluoride, Arsenic and Heavy Metal Contamination in Drinking Water in Ganga Plain, p12, Novelty Company, Patna, ISBN 978-93-81785-14-0

Jha, A.K. 2016. Bentonite minerals and its TGA, DTA, DSC and PXRD studies. Journal Indian Chem. Soc. 93: 437.

Jha, A.K. 2018. Bentonite for chemical industries. Journal J. Indian. Chem. Soc., 95: 35.

Jha, A.K., Jha, A.K., Mishra, A.K., Kumari, V. and Mishra, B. 2011. Softening of hard water by bentonite mineral. Asian Journal of Water, Environment and Pollution, 8(4): 93-96.

Jha, D., Mishra, B.K., Mishra, S.S. and Jha, A.K 2010. Bentonite clays minerals of Rajmahal Hills. J. Haematol. and Ecotoxical., 5(1): 1-7.

Karthikeyan, G., Pius, A. and Alagumuthu, G. 2005. Fluoride adsorption studies of montmorillonite clay. Indian Journal of Chemical Technology, 12: 263-272.

Lagaly, G. 1995. Surface and interlayer reactions: bentonites as adsorbents. In: Churchman, G.J., Fitzpatrick, R.W., Eggleton, R.A. (eds) Clays Controlling the Environment. Proceedings of the 10th international clay conference, Adelaide, Australia. CSIRO Publishing, Melbourne, pp. 137-144

Li, Z. and Bowman, R.S. 2001. Retention of inorganic oxyanions by organo-kaolinite. Water Research, 35(16): 3771-3776.

Maiti, A., Das Gupta, S., Basu, J.K. and De, S. 2007. Adsorption of arsenite using natural laterite as adsorbent. Separation and Purification Technology, 55(3): 350-359. doi:10.1016/j.seppur.2007.01.003.

Mermut, A.R. 1994. Layer charge characteristics of 2: 1 silicate clay minerals. CMS workshop lectures. Clay Minerals Society.

Mondal, P., Majumder, C.B. and Mohanty, B. 2006. Laboratory based approaches for arsenic remediation from contaminated water: recent developments. Journal of Hazardous materials, 137(1): 464-479. doi:10.1016/j.jhazmat.2006.02.023

Ramesh, A., Hasegawa, H., Maki, T. and Ueda, K. 2007. Adsorption of inorganic and organic arsenic from aqueous solutions by polymeric Al/Fe modified montmorillonite. Separation and Purification Technology, 56(1): 90-100.

Sharma, P. 1995. Sequential trace determination of arsenic (III) and arsenic (V) by differential pulse polarography. Analytical Sciences, 11(2): 261-262. doi:10.2116/analsci.11.261.

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Poisonous Effects of Carbamate Pesticide Sevin on Histopathological Changes of *Channa striata* (Bloch, 1793)

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ABSTRACT

The study was conducted to assess the histopathological impairment of gill and liver of freshwater snakehead murrel, *Channa striata*. The fish were exposed to sublethal concentrations (1.1 ppm) of insecticide Sevin for 30 days and a parallel control was run simultaneously. No histopathological effects were observed in control. Gill and liver of the exposed fish exhibited some remarkable alterations in their histology. Prominent changes include vacuolation, necrosis, epithelial lifting, shortening of lamellae, the fusion of adjacent lamellae, blood congestion, architectural distortion and degeneration of gills, lamellar fusion, hypertrophy, clubbing, few lamellar missing and shrinkage of blood vessels were observed in treated fishes. Hypertrophy of hepatocytes, necrosis, blood congestion, vacuolation, cellular degeneration, damage of nuclei was observed in the liver of exposed fishes. Duration of exposure of Sevin appears to have a reflective effect on gill and liver as with the increasing duration of exposure histopathological damages become more severe.

INTRODUCTION

The aquatic ecosystems are continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. Pesticides are one of the major classes of toxic substances used for management of pests in agricultural lands. It controls pests in crops, but when it reaches the aquatic habitat, affect the nontarget organisms especially fishes. Fishes are particularly sensitive to the pesticidal contamination of water. Hence, these chemical pollutants, when entering into the body of fishes may significantly damage certain physiological and biochemical process and produce serious abnormalities in its organ systems and leads to the extinction of species (Banaee et al. 2011). The carbamate pesticide Sevin is also toxic to non-target aquatic organisms, especially fishes. Carbamates are reversible inhibitors of the enzyme acetylcholinesterase and they interfere with the cholinergic nervous system and cause death because the effects of the neurotransmitter acetylcholine cannot be terminated by carbamylated acetylcholinesterase (Wang et al. 2012). It is also found to be a neurotoxin (Branch & Jacqz 1986). The carbamate pesticide, Sevin is known to affect growth, metabolism and development of fish. According to Rani et al. (1990), Sevin is extremely toxic to aquatic and estuarine invertebrates.

The evaluation of pollution effects on various organisms including fish can be studied by using biomarker response. But most biomarkers are narrow in their expression whereas histopathology is broad in its evaluation (Medja & Golemi 2011). Histopathological assessments are a suitable tool to evaluate fish stress since they can provide information about chronic and sub-lethal effects of xenobiotics (Raskovic et al. 2013, Traversi et al. 2014). Determination of adverse effects of chemical environmental stressors can be performed by using histopathological analysis in fish, as shown by previous studies (Nunes et al. 2015a,b, Rodrigues et al. 2017a, Limbu et al. 2018). Several types of histological changes have already been reported in fish, due to exposure to various environmental stressors (Lauriano et al. 2012, Mekkawy et al. 2012). Histopathology not only gives an early indication of pollution hazard but also provide useful data on nature and degree of damage to cells and tissues (Shaikh et al. 2010, Malik et al. 2012).

Toxicants are taken up through different organs of fishes because of the affinity between them. Chemical pollutants adversely affect different organs and organ systems. Gills and liver are the common target organs in fish. Gills are the first organ to respond to environmental changes and are involved in respiration, osmoregulation, acid-base regulation, and excretion functions (Raskovic et al. 2010, Rodrigues et al. 2017a). The gills are among the most vulnerable structure of the teleost fish because of their external location and close

contact with the water. So, they are liable to be damaged by any irritant materials whether dissolved or suspended in the water. According to Takashima & Hibiya (1995), gills and gastrointestinal tract in fishes considered the main passage for the entrance of pollutants to the internal body.

The liver is a detoxification organ and it is essential for both, the metabolism and the excretion of toxic substances from the fish body (Salamat & Zarie 2012). According to Mohamed (2009), the liver is also a target organ due to its large blood supply, which causes noticeable toxicant exposure. Moreover, the liver is reported to be the primary organ for bioaccumulation and thus, has been extensively studied in regards to the toxic effects of different xenobiotics (van Dyk et al. 2007, Simonato et al. 2008). The liver is a key organ involved in uptake and accumulation and the defence against toxicants, through metabolism and excretion of toxic substances (Casarett et al. 2008). Also, the incidence of lesions in gills and liver can lead to functional damage, interfering thus directly with fundamental processes for the maintenance of homeostasis in fish. Gills and liver are among the most sensitive organs frequently evaluated as indicators in assessing the health status of fish (Raskovic et al. 2010, Yancheva et al. 2016).

Fishes play significant roles in assessing the potentially toxic effects of aquatic environmental contaminants (Yancheva et al. 2016). A commercially and medicinal important freshwater fish *Channa striatus* was selected for the present study. *Channa striatus* (Bloch, 1793), is also known as snakehead murrel. This fish is well- known for its palate, high nutrition, curative and medicinal qualities. According to Mat Jais (1991), murrel flesh has high levels of arachidonic acid which is a precursor for prostaglandin and thromboxin, chemicals that affect blood clotting and the fusion of endothelial tissue in the process of wound healing. In the present study, an effort was made to assess the exposure effect of carbamate pesticide Sevin on the histology of gills and liver of the freshwater fish *Channa striatus*.

MATERIALS AND METHODS

The healthy freshwater fish *Channa striatus* of the weight (42.26 ± 0.93 g) and length (17 ± 2 cm) were selected for the experiment. These fish were acclimatized under the laboratory conditions for 20 days in an FRP tank filled with dechlorinated water. Water quality characteristics were determined by water quality analyser EUTEC (Cyberscan series 600, Singapore), and are as follows: temperature 27.5 ± 2.37°C, pH 7.1 ± 0.02, dissolved oxygen 6.2 ± 0.2 mg/L, alkalinity 252 ± 2.5 mg/L as $CaCO_3$, total hardness 453 ± 4.1 mg/L. The fishes were fed daily with minced fish. The test solution was prepared from Sevin, Manufactured by Parikh Enterprises Ltd. The Sevin itself was used as a stock solution. After the preparation of the stock solution, the different concentrations of the test solution were prepared by serially diluting the stock solution. The toxicity tests to calculate LC50 for 96 hours is carried out by the desired concentration of Sevin, prepared by adding the distilled water (1000 mL). Series of different concentrations (2, 4, 6, 8, 10 and 12 ppm) were prepared. The healthy fishes were selected and tested for 96 hrs. The experiment was started in the morning and behavioural changes were noted. The mortality of fishes was recorded 96 hr LC 50. The LC50 values for different periods were calculated by the method of probit analysis (Finney 1964). Then, fishes exposed in a sub-lethal concentration of Sevin is 1.1 ppm. At the end of the experiment, the gill and liver tissue was isolated from control and experimental fishes. The tissue was fixed in aqueous Bouin's solution, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Sections were cut at 3-5 µ thickness by using Yorco Rotary Microtome, stained with Ehrlich Haematoxylin and Eosin. Slides were observed under the light microscope (Labomed, CXR111) at varying magnifications (10, 40 and 100X) and photographs were taken under a research microscope supported with Q-win software (Leica).

RESULTS AND DISCUSSION

Gills under Control Condition

Histological observation of gills from control fish showed normal architecture. The structural particulars of the gill of control *C. Striatus* are shown in Figs. 1A & 1B. Structurally, the gills are situated in the branchial chamber on either side of the body in fishes. The gill has a gill arch with a double row of elongated laterally projecting gill filaments. These filaments are flat and leaf-like and join at the base on gill rackers by a gill septum. Numerous semi-circular, leaf-like projections are lined up along both sides of the primary gill lamellae called as secondary gill lamellae, which consist of centrally placed rod-like supporting axis with blood vessels on either side. Each of these primary lamellae was flat leaf-like structure and has a series of secondary lamellae located to the primary lamellae with the central supporting axis. The gill is composed of many gill filaments which have primary gill lamellae and secondary gill lamellae that run perpendicular to each filament (Fig. 1A). The secondary lamellae also term as respiratory lamellae are highly vascularized and covered with a thin layer of epithelial cells. Blood vessels are extended into each of the secondary gill filaments provided with pillar and chloride cells. The secondary lamella is supplied with marginal blood sinus lined by endothelium. In between the secondary gill

lamellae and the primary filament, lined by a thick stratified epithelium. This region between the two adjacent secondary gill lamellae is known as the interlamellar region. The gill arch is a bony structure from which radiate double rows of paired filaments. The lamellae are lined by a squamous epithelium composed by pavement and non-differentiated epithelial cells. Each lamella is made up of two sheets of epithelium bordered by many pillar cells which are contractile and separate the blood channels in which erythrocytes are usually recognized within each capillary lumen. Between the lamellae, the filament is lined by a thick stratified epithelium constituted by several cellular forms, such as chloride cell, mucous or goblet cells.

Mucous cells and chloride cells are also existing in the epithelium of the filament and at the base of lamellae. Gills have an extensive surface area and minimal diffusion distance between dissolved oxygen and blood capillaries for efficient gaseous exchange. However, fish gills are equipped with a defence mechanism working against the environmental irritants which essentially is the mucus cell. The mucus cells react instantaneously to head is covered over by a thick tissue composed of mucus glands, taste buds, and connective tissue. The gill head contains bony part of gill arch and gill rackers. The epithelium surface of the gill arch showing the parallel arrangement of epithelial cells having mucus pores (Fig. 1B)

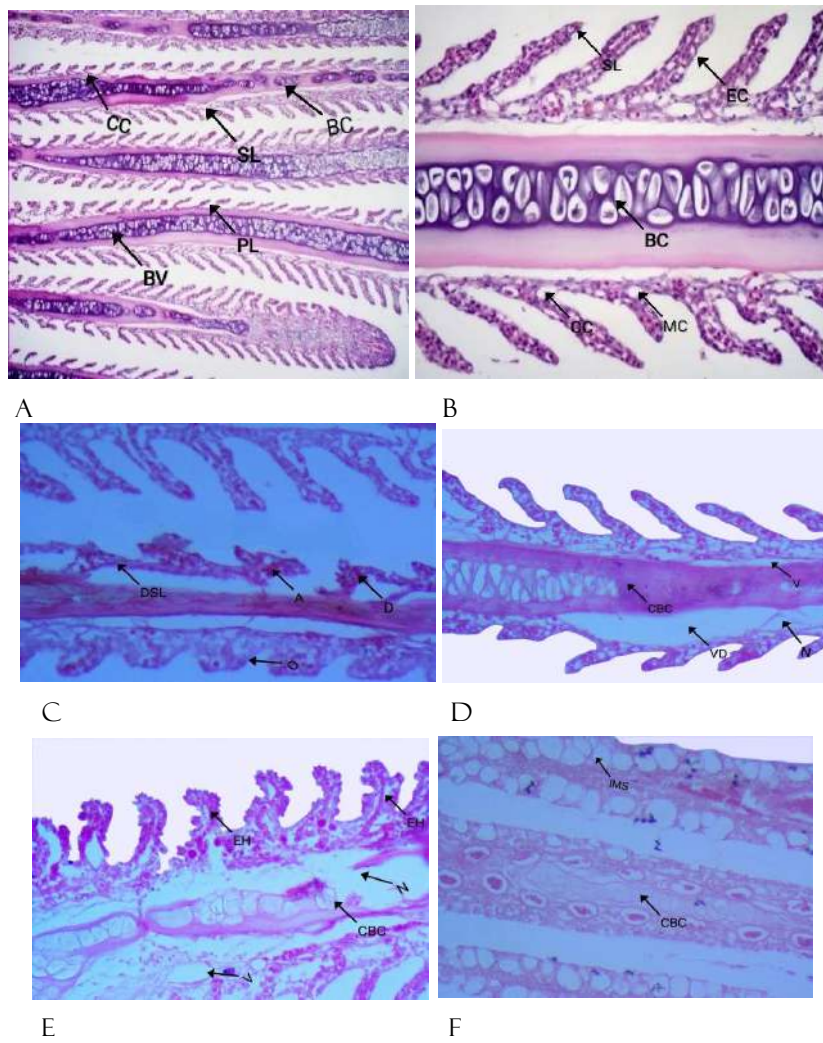


Fig. 1: Gill tissue of *C. striata*: A & B Control- PL- Primary Lamellae, SL- Secondary Lamellae, Chloride Cell, Mucous Cell, Blood Cells, Blood Vessels, EEpithelial Cells (H&E, 10 and 40X). (B, C, D, E and F) exposed to 1.1 ppm of Sevin V- Vacoules, H- Hypertrophy, DSL- Distorted Secondary Lamellae, Aneurism, O- Oedema, CBC- Congested Blood Cells, VD- Vasodialation, D- Desquamation, EH- Epithelial Hypertrophy, IMC- Increased Mucous Cells (H&E, 40X).

Histopathology of Gills Treated with Sevin

At a sublethal concentration of Sevin 1.1 ppm for 30 days, the gill tissues of *Channa striatus* revealed large inter-lamellar space, necrosis, lamellar fusions enlarged secondary gill lamellae, curved secondary gill filaments resulting in distension of the lamellae. Distorted, swelling of the tip of secondary gill lamellae and the erosion was observed (Fig. 1C and D). A large number of blood cells accumulated in secondary gill lamellae and resulted in the enlargement of gill lamellae, the fusion of secondary gill lamellae was also observed in some areas, epithelial hyperplasia, swelling of epithelial cells and bronchial epithelium disorganization were evident. Furthermore, the epithelial layer detached completely from the central portion of the gill lamellae. epithelial lifting, cell swellings congestions, the bending of secondary lamellae, the formation of edematous space between the layers of epithelium which may become infiltrated with red blood cells. Gill hyperplasia was observed in the primary epithelial cells. Then, the whole lamellar epithelium was found to be degeneration (Fig.1. E and F).

Sevin is considered as a highly toxic pesticide to fresh-water organisms. The present study was in agreement with similar observations reported under Sevin toxicity at 96 hr in the freshwater fish, *Channa punctatus* (Deepasree & Nair 2015). Degenerative epithelium of gill filaments and secondary lamellae accompanied by separation of their epithelium from the lamellar supporting cells was also demonstrated in the experiment of Bhuvaneshwari et al. (2015) on Zebrafish exposed to organochlorine pesticide and heavy metals. Dilation of blood capillaries, abnormal swellings epithelium was observed by Banae et al. (2013) in Rainbow trout exposed to pesticide diazinon.

There are several reports on various histological changes in fish gills, acute or chronic exposure with sublethal toxicant concentrations (Velcheva et al. 2010a&b, Muthukumaravel et al. 2013, Sousa et al. 2013 a&b, Khatun et al. 2016). According to Camargo & Martinez (2007), Ayandiran et al. (2009) and Vigario & Saboia-Morais (2014) the toxicants could induce gill histological alterations such as epithelium degeneration and necrosis, which means fish can develop numerous defence mechanisms, which could prevent the toxicant negative effects. These mechanisms are expressed in different morphological changes including oedema, the proliferation of epithelium and fusion.

Epithelium lifting was observed in the Sevin treated gills. Epithelial lifting increases the distance between the blood and epithelial layer and thus decrease the velocity of penetration of the molecules of Sevin into the blood. So, the epithelial lifting can be considered as a defence mechanism adopted by fishes to slow down the penetration of pesticide through the lamellar epithelium. The epithelial lifting in gills decelerate oxygen uptake and produce dysfunctional gills, and eventually asphyxiate the fish (Kumar et al. 2010).

Gill lesions associated with the lamellar aneurysm were observed in prevalence in all treatment groups. The lesions may be due to the disturbance of blood flow in the blood channels. Similar histopathological lesions have been reported by (Devi & Mishra 2013) chlorpyrifos exposed in *Channa punctatus*.

Liver under Control Condition

The liver is the organ which is most associated with the detoxification and biomarker process. Due to its function, position and blood supply, it is also one of the most affected organs by contaminants in water (Camargo & Martinez 2007). Histological observation of liver from control fish in the present study showed normal architecture. The liver of untreated fish was exhibited parenchymatous appearance and mainly consisted of polygonal-shaped hepatocytes with their central nuclei. Sinusoids are irregularly distributed between the polygonal hepatocytes. Hepatopancreatic alveoli of the exocrine pancreas were seen to be placed in the parenchyma and the melanomacrophages centre stained light brown were located close to the hepatopancreas. Some of the hepatocyte nearby the hepatopancreas was slightly vacuolated it could be due to storage of glycogen and lipid (Fig. 2A & B).

Histopathology of the Liver Treated with Sevin

In sublethal concentration, 1.1 ppm of Sevin to *Channa striatus*, showed congestion of central vein, degeneration of hepatocytes, cytoplasmic vacuolization and a large number of distorted hepatocytes. The liver treated with a high dose of Sevin showed centrilobular necrosis characterized by necrosis of hepatocytes around the congested central vein. Hepatocytes showed increased granularity of cytoplasm with nuclear pyknosis leading to necrosis and complete loss of hepatic parenchyma (Fig. 2C). The liver of fishes treated with a low concentration of Sevin was reflected by disorganization of hepatic cords, degeneration, hepatic hypertrophy, focal necrosis with complete loss of hepatocytes at many places (Fig. 2D).

In fish, the liver is the main organ that plays an important role in the detoxification of chemicals and pesticides. During metabolism, the liver helps to break down the harmful substances, but beyond a certain limit, these toxic compounds distract the regulating mechanism of the liver and cause morphological alteration (Brusle et al. 1996). The fatty infiltration, hemosiderosis and congested central vein, se-

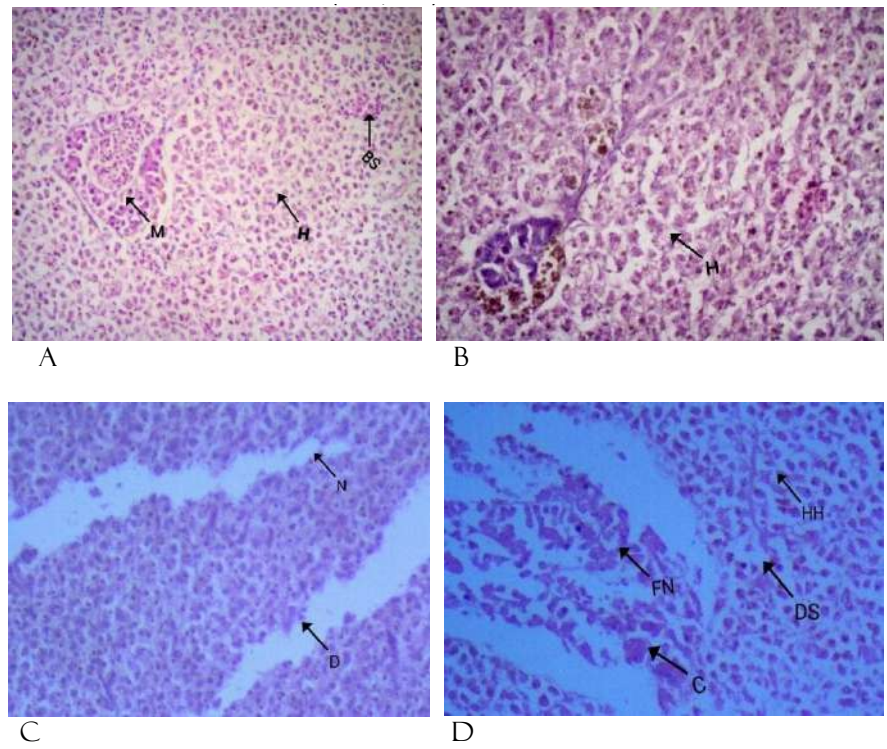


Fig. 2: Photomicrograph of liver control. H– Hepatocytes, N- Nucleus (5µm thickness; H&E staining 10 &40 X) C and D: Photomicrograph of liver of fish exposed to Sevin. DH- Degradation of cellular hepatocytes, DS-Distortion, H- hypertrophy, N-Necrosis, C- Congestion, FN- Focal necrosis, HH- Hypertrophy of Hepatocytes (5µm thick; H&E staining; 10X and 40X).

vere necrosis, haemorrhage, vacuolation, severe infiltration of leukocytes, pyknotic and hepatic necrosis in the liver of the fish *Clarias gariepinus* after exposing to lethal concentrations of cypermethrin were observed by Ayoola & Ajani (2008); present result agrees with this result. The infiltration of fatty molecules and vacuolization in the liver specify the accumulation of fat and disparity between the rate of synthesis and release of a substance in hepatocytes (Gingerich 1982), whereas necrosis in some part of liver may appear due to extra workload on hepatocyte during detoxification (Petal & Bahadur 2011). Focal necrosis is probably due to the involvement of liver cells in the metabolic transformation of the insecticide, causing functional and structural changes to the cells (De Melo et al. 2008). Histological changes associated with different pesticides in freshwater fish have been studied by many authors *Clarius gariepinus* (Ayoola & Ajani 2008); *Oreochromis mossambicus* (Navaraj & Yasmin 2012); *Channa punctatus* (Magar & Shaikh 2013); *Labeo rohita* (Nagaraju & Rathnamma 2014); *Cyprinus carpio* (Chamarthi et al. 2014); *Channa gachua* (Rai & Mishra 2014, Kadam & Patil 2018); *Heteropneustes fossilis* (Pandey & Dubey, 2015); *Cyprinus carpio* (Lakshmaiah 2016) and *Trichogaster fasciata* (Mukti 2018).

Thus, the histological changes that were taking place in the present study, as the initial period of exposure in the organs of the fish on exposure to Sevin toxicity might be a part of the defence mechanism. The further accumulation of Sevin in the organs of the fish on continued exposure destroyed the organ structures. The slight structural reorganization of the gill and liver of the fish observed at day 30 of exposure to Sevin toxicity gives support to some extent that the ability of the fish to resist the sublethal stress and in the repair of the damage caused to the organ by enhancing the protein synthetic potentials and other associated activities of the cell.

CONCLUSION

Pesticide toxicant leads to many pathological changes in different tissues of fish exposed to Sevin. The increasing amounts of carbamate pesticide Sevin incoming to aquatic bodies can result in the amount of accumulation of contaminants increased in the fish and their consumers, which create a serious hazard to ecosystems. In the last decades, the fast growth of industry and agriculture has resulted in increased pesticides pollution, which is a significant environmental hazard for invertebrates, fish, and humans.

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REFERENCES

Ayandiran, T.A., Fawole, O.O., Adewoye, S.O. and Ogundiran, M.A. 2009. Bioconcentration of metals in the body muscle and gut of *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluent. Journal of Cell and Animal Biology, 3: 113-118.

Ayoola, S.O. and Ajani, E.K. 2008. Histopathological effects of cypermethrin on juvenile African catfish (*Clarias gariepinus*). World Journal of Biological Research, 1(2): 1-14.

Banaee, M., Mirvaghefei, A.R., Majazi-Amiri B., Rafei, G.R. and Nematdost, B. 2011. Hematological and histopathological study of experimental diazinon poisoning in common carp fish (*Cyprinus carpio*). J. Fish. Iran. J. Nat. Res., 64: 1-14.

Banaee, M., Sureda, A., Mirvagefei, A.R. and Ahmadi, K. 2013. Histopathological alterations induced by diazinon in rainbow trout (*Oncorhynchus mykiss*). International Journal of Environmental Research, 7(3): 735-744.

Bhuvaneshwari, R., Padmanabhan, K. and Babu Rajendran, R. 2015. Histopathological alterations in muscle, liver and gill tissues of Zebra fish *Danio Rerio* due to environmentally relevant concentrations of organochlorine pesticides and heavy metals. Int. J. Environ. Res., 9(4): 1365-1372.

Bloch, M. E. 1793. Naturgeschichte der auslandischen fische. 7, Berlin, Germany, Morino and Co. 7, 1-xiv+1. 44: 325-360.

Branch, R.A and Jacqz. E. 1986. Subacute neurotoxicity following long-term exposure to carbaryl. Am. J. Med., 80: 741-745.

Brusle, J., Gonzalez, I. and Anadon, G. 1996. The structure and function of fish liver. In: Munshi, J.S.D. and Dutta (Ed.) Fish Morphology. Science Publishers, New York, USA.

Camargo, M.M.P. and Martinez, C.B.R. 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotropical Ichthyology, 5(3): 327-336.

Casarett, L.J., Klaassen, C.D., Amdur, M.O and Doull, J. 2008. Toxicology: The Basic Science of Poisons, 7th Edn. McGraw-Hill, Health Professions Division, New York, pp. 557-551.

Chamarthi, R.R., Bangeppagari, M., Gooty, J. M., Mandala, S., Tirado, J. O. and Marigoudar, S.R. 2014. Histopathological alterations in the gill, liver and brain of *Cyprinus carpio* on exposure to quinalphos. American Journal of Life Sciences, 2(4): 211-216.

Costa, P.M., Diniz, M.S., Caeiro, S., Lobo, J., Martins, M., Ferreira, A.M, Caetano, M., Vale, C., DelValls, T.A and Costa, M.H. 2009. Histological biomarkers in liver and gills of juvenile *Solea senegalensis* exposed to contaminated estuarine sediments: A weighted indices approach. Aquat. Toxicol., 92: 202-212.

De Melo, G., Donattib, L., Rudnikia, C. and Fanta, E. 2008. Hepatic alterations in the fish Rhamdia quelen contaminated with Folidol 600®. Ecotoxicol. Environ. Saf., 71: 821-829.

Deepasree, M.I and Rajendran Nair, M. S. 2013. Histological and ultra-structural alterations of gills of freshwater fish *Channa punctatus* (Bloch), on exposure to pesticide, Sevin. International Journal of Science and Research, 5(6): 991-995.

Devi, Y. and Mishra, A. 2013. Histopathological Alterations in gill and liver anatomy of freshwater, air breathing fish *Channa punctatus* after pesticide Hilban (Chlorpyrifos) Treatment. Adv Biores., 2: 57- 62.

Finney, D.J. 1971. Probit Analysis, University Press, Cambridge, pp. 333.

Gingerich, W.H. 1982. Hepatic toxicology of fishes. In: Aquatic Toxicology, pp. 55-105.

Kadam, P.L. and Patil, R.N.2018. Histopathological alterations produced in the liver of snake head fish, *Channa gachua* (F. Hamilton) exposed to dichlorvos. Bioscience Discovery, 9(1): 08-14.

Khatun, N., Rahman, T. and Mahanta, R. 2016. Histopathological studies of chlorpyrifos toxicity in catfish. Global Journal of Medical Research and Pathology, 16(3): 49-54.

Kumar, M., Prasad, M.R., Srivastava, K., Tripathi, S. and Srivastav, A.K. 2010. Branchial histopathological study of Catfish *Heteropneustes fossilis* following exposure to purified neem extract, Azadirachtin. World Journal of Zoology, 5(4): 239-243.

Kunjamma, A., Philip, B., Bhanu, S. and Jose, J. 2008. Histopathological effects on *Oreochromis mossambicus* (Tilapia) exposed to chlorpyrifos. Journal of Environmental Research and Development, 2(4): 553-559.

Lakshmaiah, G. 2016. A histopathological study on the liver of common carp *Cyprinus carpio* exposed to sublethal concentrations of phorate. International Journal of Applied Research, 2(6): 96-100.

Lauriano, E.R., Calo, M., Silvestri, G., Zaccone, D., Pergolizzi, S. and Cascio, P.L. 2012. Mast cells in the intestine and gills of the sea bream, *Sparus aurata*, exposed to a polychlorinated biphenyl, 126. Acta. Histochem., 114(2): 166-171.

Limbu, S. M., Zhou, L., Sun, S. X., Zhang, M. L and Du, Z. Y. 2018. Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk. Environ. Int., 115: 205-219.

Magar, R.S. and Afsar Shaikh 2013. Effect of malathion toxicity on detoxifying organ of freshwater fish *Channa punctatus*. International Journal of Pharmaceutical, Chemical and Biological Sciences. 3(3): 723-728.

Malik, G. M., Raval, H. V. and Ahmed Khali, H.K. 2012. Toxic effects of effluent on mortality and behavior changes on freshwater fish *Poecilia reticulate*. Environ. Res. Develop., 7(2A): 1036-1039.

Mat Jais, A.M. 1991. Haruan, *Channa striatus*, farming in backyard. Proceedings of the Third Asian Conference of Technology for Rural Development. 91: 230-232.

Medja, N. and Golemi, S. 2011. Histopathology of larva *Carassius gebelio* biomarker of pollution with cyanobacteria of Shkodra lake water, Albania. Journal of Environmental Research and Development, 6(2): 281-285.

Mekkawy, I.A.A., Mahmoud, U.M., Wassif, E.T and Naguib, M. 2012. Protective roles of tomato paste and vitamin E on cadmium-induced histological and histochemical changes of liver of Oreoc*hromis niloticus* (Linnaeus, 1758). J. Fisher. Aqua.Sci. 7: 240-265.

Mohamed, F.A.S. 2009. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World J. Fish Mar. Sci., 1(1): 29-32.

Mukti, S.S., Ahmed, G.U., Ahmed, Z.F., Sumon, K.A. and Fatema, M.K. 2018. Histopathological study of female striped Gourami (*Trichogaster fasciata*), Bloch and Schneider, 1801) gill exposed to chlorpyrifos. International Journal of Aquaculture, 8(14): 104-111.

Muthukumaravel, K., Sathick, O. and Raveendran, S. 2013. Lambda cyhalothrin induced biochemical and histological changes in the liver of *Oreochromis mossambicus* (Peters). Int. J. Pure Appl. Zool., 1(1): 80-85.

Nagaraju, B. and Rathnamma, V. 2014. Effect of profenofos and organophosphate on protein levels in some tissues of freshwater fish *Labeo rohita* (Hamilton). International Journal of Pharmacy and Pharmaceutical Sciences, 5(1): 276-279.

Navaraj, P.S. and Yasmin, J. 2012. Histopathological impact of industrial wastewater on the vital organs of *Oreochromis mossambicus*. Toxicological and Environmental Chemistry, 94(3): 574-582.

Nunes, B., Antunes, S.C., Gomes, R., Campos, J.C., Braga, M.R., Ramos, A.S and Correia, A.T. 2015a. Acute effects of tetracycline exposure in the freshwater fish *Gambusia holbrooki*: Antioxidant effects, neurotoxicity and histological alterations. Arch. Environ. Contam. Toxicol., 68: 371-381.

Nunes, B., Campos, J.C., Gomes, R., Braga, M.R., Ramos, A.S., Antunes, S.C and Correia, A.T. 2015b. Ecotoxicological effects of salicylic acid in the freshwater fish *Salmo truttafario*: Antioxidant mechanisms and histological alterations. Environ. Sci. Pollut. Res., 22(1): 667-678.

Pandey, A.K and Dubey, S. 2015. Histological changes in liver and kidney of catfish, *Heteropneustes fossilis*, exposed to pentachlorophenol (PCP). Plant Archives, 15(2): 1117-1120.

Petal, J.M. and Bahadur, A. 2011. Histopathological alterations in *Catla catla* induced by chronic exposure of copper ions. J. Cell. Tissue.Res., 10: 2365-2370.

Rai, M.K. and Mishra, A.P. 2014. Histopathological alteration in gill *of Channa gachua* exposed to an organophosphate. The Asian Journal of Animal Science, 9(2): 169-173.

Rani, S., Shaik Dawood, A. and Ramasamy, M. 1990. Toxicity of a carbamate fungicide, cuman to an edible, freshwater fish, *Sarotherodon mossambicus* (Peters). J. Aquor., 3(1): 29-36.

Raskovic, B., Jaric, I., Koko, V., Spasic, M., Dulic, Z., Markovic, Z. and Poleksic, V. 2013. Histopathological indicators: A useful fish health monitoring tool in common carp (*Cyprinus carpio*: Linnaeus, 1758) culture. Cent. Eur. J. Biol., 8(10): 975-985.

Raskovic, B., Poleksic, V., Zivic, I. and Spasic, M. 2010. Histology of carp (*Cyprinus carpio* L.) gills and pond water quality in semi intensive production. Bulg. J. Agric. Sci., 16(3): 253-262.

Raskovic, B., Poleksic, V., Zivic, I. and Space, M. 2010. Histology of carp (*Cyprinus carpio*, L.) gills and pond water quality in semi intensive production. Bulg. J. Agric. Sci., 16: 253-262.

Rodrigues, S., Antunes, S.C., Correia, A.T. and Nunes, B. 2017b. Rainbow trout (*Oncorhynchus mykiss*) pro-oxidant and genotoxic responses following acute and chronic exposure to the antibiotic oxytetracycline. Ecotoxicology, 26: 104-117.

Rodrigues, S., Antunes, S.C., Nunes, B. and Correia, A.T. 2017a. Histological alterations in gills and liver of rainbow trout (*Oncorhynchus mykiss*) after exposure to the antibiotic oxytetracycline. Environ. Toxicol. Pharmacol., 53: 164-176.

Salamat, N. and Zarie, M. 2012. Using of fish pathological alterations to assess aquatic pollution- A review. World Journal of Fish and Marine Sciences, 4(3): 223-231.

Shaikh, F. I., Ustad, I. R. and Ansari, N.T. 2010. Effect of heavy metal on the ovary of freshwater crab *Barylelphusa cunicularis*. The Bioscan, 5(2): 335-338.

Simonato, J.D., Guedes, C.L.B., Martinez, C.B.R. 2008. Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. Ecotoxicology and Environmental Safety, 69(1): 112-120.

Sousa, D.B.P., Almeida, Z.S. and Carvalho-Neta, R.N.F. 2013b. Integrated analysis of two biomarkers in *Sciades herzbergii* (Ariidae, Siluriformes), to assess the environmental impact at São Marcos. Latin American Journal of Aquatic Research, 45: 305-312.

Sousa, D.B.P., Almeida, Z.S. and Carvalho-Neta, R.N.F. 2013a. Histology biomarkers in two estuarine catfish species from the Maranhense coast. Arquivo Brasileiro de Medicina Veterinaria Zootecnia, 65(2): 369-376.

Takashima, F. and Hibiya, T. 1995. An Atlas of Fish Histology. Normal and Pathological Features. 2nd ed. Tokyo, Kodansha Ltd., pp. 51.

Traversi, I., Gioacchini, G., Scorolli, A., Mita, D.G., Carnevali, O. and Mandich, A. 2014. Alkyl phenolic contaminants in the diet *Sparus aurata* juvenile's hepatic response. Gen. Comp. Endocrinol., 205: 185-196.

van Dyk, J.C., Pieterse, G.M., van Vuren, J.H.J. 2007. Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. Ecotoxicol. Environ. Saf., 66: 432-440.

Velcheva, I., Arnaudov, A. and Georgieva, E. 2010a. Influence of zinc on gill morphology of Gibelio carp *Carassius gibelio*. Ecologia Balkanica, 2: 19-23.

Velcheva, I., Tomova, E., Arnaudova, D. and Arnaudov, A. 2010b. Morphological investigation on gills and liver of freshwater fish from dam lake. Bulgarian Journal of Agricultural Science, 16: 364-368.

Vigario, A.F. and Saboia-Morais, S.M.T. 2014. Effects of the 2,4-D herbicide on gills epithelia and a liver of the fish *Poecilia vivipara*. Pesqisa Veterinaria Brasiliera, 34(6): 523-528.

Wang, J., Chen, F., Zheng, P., W.J., Yuan, J., Peng, B., Wang, R.C., Liu, W.J., Zhao, H. and Wang, Y.Q. 2012. Huperzine a ameliorates experimental autoimmune encephalomyelitis via the suppression of t cell-mediated neuronal inflammation in mice. Exp. Neurol., 236: 79-87.

Yancheva, V., Velcheva, I., Stoyanova,s and Georgieva, E. 2016. Histological biomarkers in fish as a tool in ecological risk assessment and monitoring programs: A review. Applied Ecology and Environmental Research, 14(1): 47-75.

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Reduction of Greenhouse Gas Emissions of *Azolla pinnata* Inclusion in Backyard Chicken Production

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ABSTRACT

Backyard chicken production is an integral part among rural families in the tropics like the Philippines. However, it has been declining as it continues to suffer low productivity with its small-scale operations. Among its production inputs, feeds remain to be the top cost driver, as well as the top contributor to greenhouse gas (GHG) emissions that result in global warming potential (GWP). In a prior experiment, 50% inclusion of Azolla Pinna was recommended in feed ration due to its favourable and comparable growth performance of Dominant DZ backyard chickens. Hence, this study further evaluated the inclusion in terms of environmental performance. The 50% inclusion resulted in reductions of CO_2 by 35%, N_2O by 22.32%, and CH_4 by 4.74%. The gross effect of this reduction of conventional feeds is a climate change mitigation equivalent 28.47% of GWP kg CO_2 -eq./1,000 birds. The potential impacts indicate that Azolla Pinnata can be a cost-effective and sustainable feedstuff in backyard chicken rearing system especially that it requires simple propagation method. The environmental impacts and savings can encourage more livelihood activities in rural.

INTRODUCTION

Backyard Chicken Production

Despite the soaring modernization of commercial poultry production in the global arena, backyard chicken production continues to be a significant activity in the rural and peri-urban areas (Padhi 2016). In this set-up, the backyard chickens, which are usually indigenous, native or improved breed, are typically housed in simple night shelters with limited management and disease prevention measures. They are fed a mixture of household food waste and second-grade crops and are supplemented scavenging for opportunistic food sources such as insects and food scraps. This backyard production is traditionally family-based in small scale and is estimated to contribute 4% of total poultry meat production and 14% of total eggs production worldwide (MacLeod et al. 2013). For small-scale farmers in developing countries, the family-based poultry represents one of the few opportunities for saving, investment and security against risk. Though it is rarely the sole means of livelihood, it is integrated and complementary to farming activities. It can provide additional income from the sale of birds and eggs, as well as, accessibility to valuable source of protein in the diet (Thieme et al. 2014). However, backyard chicken production has been declining in most countries. Its ability to sustain and achieve the optimal solution to food and nutrition security can only be achieved if constraints, such as inherent low production, diseases, fluctuation in feed prices and access to veterinary services, are properly addressed (Wong et al. 2017).

Just like most rural areas, backyard chicken production in the Philippines is popular. It has been an integral part of the farming systems as a source of chicken meat and eggs. By definition, backyard production comprises of 100 birds of native or improved breeds that are raised primarily for their own consumption in a free-range system of management, which allow birds to forage and look for their food (Sison 2014). The produce of indigenous chicken has been perceived and preferred over the commercial broiler chicken because of taste, leanness and colour, which are more suitable for popular Filipino dishes. With their limited supply, both meat and eggs are priced more than twice the commercial production.

The top producing provinces in the country are Negros Occidental, Iloilo, Bukidnon, Davao del Sur, and Cebu. As of 2016, the backyard operation with native chicken inventory registered at 78.4M in 2016, native breed accounts 44.7% of total chicken, a significant decline from 54.7% in 2001 (PSA 2018). The approximately 20% decline for backyard production can be attributed to the lack of attention and focus given there is little to gain from a very small production base. The decline may continue to progress as the backyard production continue to suffer low productivity and high mortality rates with the limited of technical know-how and usage of optimal resources and inputs. Moreover, technology improvements are not that welcomed with the limited capital and other resources, and adoption disposition (Chang 2007).

Backyard chickens need essential nutrients such as carbohydrates, proteins, fats and oils, minerals, vitamins, and water to survive, grow, and reproduce. To have a consistent and stable marketable weight, some farms used commercial feeds for feeding to complement the forage and free-range activities of the birds. However, the use of commercial feeds increase the cost of production, thereby, supplemental feeding is more economical (Mananghaya 2017).

In terms of duration, feeding is around 90 days to achieve the taste and leanness of native chicken. This means longer feeding period for backyard chicken to achieve 0.7-1.1 kilo weight in ninety days, versus broiler chicken of 1-1.5 kilo over 28 days (Jaturasitha et al. 2002, Roxas 2000).

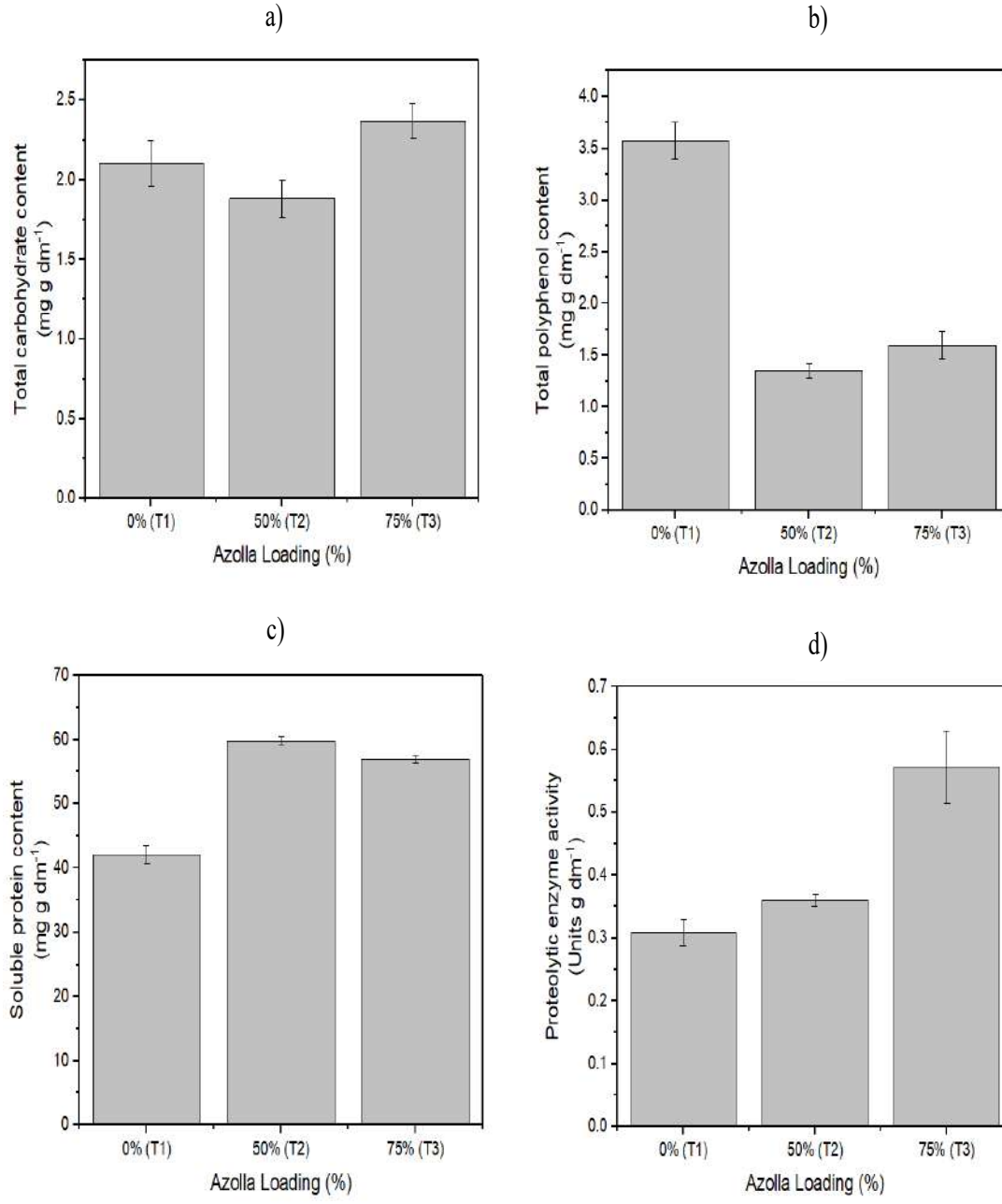
Azolla Pinnata Feedstuff

In any poultry production system, the highest cost driver is feeds, which accounts for about approximately 50 to 70%. The feed can go as high as 75% of the total cost of production in view of commercial feeds, being the major portion of the variable costs. (Dozier et al. 2008) In the Philippines, the price of commercial feeds will continue to rise. Most of the ingredients are imported such as soybean meal, which is the primary protein source. Various studies have explored the different alternative protein feedstuffs that are abundant in the tropics. One of the promising alternative forages is Azolla Pinnata. It is a floating fern belonging to the family of Azollaceae. The fixation and assimilation of atmospheric nitrogen are generated when it hosts the blue-green algae, Anabaena azollae symbiotically. Hence, it provides the carbon source and favourable environment for the growth and development of the algae (Rascio & La Rocca 2008). This unique symbiotic relationship makes Azolla, an effective and economical plant with high protein content. It has rich nutritive value, which includes proteins, essential amino acids, vitamins (vitamin A, vitamin B12 and beta-carotene), growth promoter intermediaries and minerals like calcium, phosphorous, potassium, ferrous, copper, magnesium etc. It contains 25 to 35 % protein, 10 to 15 % minerals and 7-10% amino acids, bio-active substances and bio-polymers. Its nutrient composition makes it a highly efficient and effective feed for livestock, which can be easily digested, with its high protein and low lignin content (Pillai et al. 2005). In Nigeria, the effect of incorporating graded levels of Azolla meal (AZM) in diets of growing pullets was investigated. Particular reference was given to growth, haematology and subsequent laying performance, among 2-week old Nera brown pullets. The results demonstrated a benefit from AZM at a low level of supplementation and up to 15% AZM can be incorporated in diets of growing pullets without jeopardizing the health and subsequent laying performance (Alalade et al. 2007). In another research, the impact of feeding restricted diets supplemented with free fresh Azolla (as a good source of crude protein and metabolizable energy, and extra essential nutrients as vitamins and minerals) on performance and economic efficiency of Fayoumi growing chicks was evaluated in Egypt. It was concluded that free fresh Azolla could be compensated the restriction diet up to 15% without any counterproductive effects on the performance and edible parts (carcass and internal organs) of Fayoumi growing chicks until marketing age. Moreover, the Fayoumi chicks on the 45% restricted diet, plus free fresh azolla achieved the best feed conversion rate and economic efficiency (Namra et al. 2010).

Case Study in the Philippines

In an experiment of the authors of the growth performance, a Dominant CZ Chicken supplemented with AZM was very promising. It was tested on backyard rearing system to investigate how it can be incorporated as an alternative feeds in a farm with backyard rearing system with a tropical climate in Lamacan, Argao in Cebu Province, Region VII, Philippines. The rations were based on an organic farm that campaigned cutting down commercial feeds by at least 50% and looking at almost 100% non-inclusion of commercial feeds in their feeding (PinoyBisnis.com 2010). 27-day old Dominant CZ chicks were used in this study for 90 days. They were randomly grouped to Treatment 1 (T1): control group with 100% commercial feeds, Treatment 2 (T2): the ration with 50% commercial feeds and 50% Azolla; and Treatment 3 (T3): ration with 25% commercial feeds and 75% Azolla. The standard management practices, rearing and environmental conditions were the same for all treatments. Feeding was provided in ad libitum during the booster stage. While during the starter and grower stages, feeding followed the feed intake guide used in the farm. The results showed that T2 and T3 have satisfactory performance versus T1 since

they were able to achieve the marketable weight of 1000 grams in 90 days. The average weight gain of T2 was 1,094.6 grams, which is just 8.2% lower than the T1 at 1,194.4, while T3 was 1,081.2 or 9.4% versus T1. The details in growth performance evaluation can be found in Table 1. A separate meat chemical composition analysis (Siacor 2019) on total carbohydrate, polyphenol, soluble protein, and proteolytic enzyme activity was done with results in Fig. 1. The results on growth performance and meat quality of the chickens fed with 50% AZM were favourable.



Source: (Siacor,2019)

Fig. 1: Meat Chemical Composition with various Azolla Loading.

a) Total carbohydrate b) polyphenol c) soluble protein d) proteolytic enzyme activity of the chicken carcass at varying levels of Azolla loading in the chicken feed (values represent an average for three replicates; error bars represent standard deviation)

Table 1: Growth Performance of CZ Chickens at different feeding stages

Attributes	T0 control	T1 50% *Azolla*	T2 75% *Azolla*	Mean	SEM
CUMMULATIVE WEIGHT (g)					
Initial	49.62	50.51	50.59	50.23	1.12
Booster	231.21	182.89	181.05	199.05	7.83
Starter	826.57	754.09	686.06	758.25	17.84
Grower	1,244.03	1,145.09	1,131.74	1,175.23	20.75
WEIGHT GAIN (g)					
Booster	181.59[a]	132.38[a]	130.46[a]	148.82	7.72
Starter	595.36[a]	571.20[b]	505.01[ab]	559.20	12.86
Grower	417.47	391.00	445.68	416.98	11.76
Total	1,194.41[a]	1,094.58[a]	1,081.15[a]	1,125.00	20.85
Ave. Daily	13.27	12.16	12.01	12.50	0.23
FEED INTAKE (g)					
Booster	320.25	310.07	295.19	308.50	8.18
Starter	1,433.01	1,471.15	1,463.23	1,455.80	7.68
Grower	2,190.02	2,185.31	2,170.43	2,181.92	7.80
Total	3,943.28	3,966.53	3,928.85	3,946.22	18.86
Ave daily	43.81	44.07	43.65	43.85	0.21
CONVERSION RATIO					
Booster	1.76	2.34	2.26	2.27	0.11
Starter	2.41[a]	2.58[b]	2.90[ab]	2.64	0.06
Grower	5.25	5.59	4.87	5.34	0.16
Total	3.30[a]	3.62[a]	3.63[a]	3.54	0.06

Mean values within a row with superscripts differ significantly ($p < 0.05$); Source: (Authors)

Environmental Impacts

One of the environmental concerns in any producing industry is global warming. It is the average increase in the temperature of the atmosphere near the Earth's surface and in the troposphere, which can contribute to changes in global climate patterns. Global warming can have many different causes, but it is most commonly associated with human interference, specifically the release of excessive amounts of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), water vapour, and fluorinated gases earth (EPA 2017). Based on World Resources Institute Climate Analysis Indicators Tool, the Philippines' total GHG emissions are at 57.6 million metric tons of carbon dioxide equivalent (Mt CO_2e), 0.33% of global GHG emissions. This was dominated by the energy sector (54%), followed by agriculture (33%), industrial processes (IP) (8%), and waste (7%). Between 1990-2012, total GHG increased by 54 $MtCO_2e$ or an equivalent of 53% over the period and an annual change of 2.1%. These were driven by energy (3.4%), agriculture (1.5%), IP (7.1%), waste (2.1%), and land use change and forestry (-12.4%) (USAID 2016). In response to the increasing GHG, the Philippine Climate Change Commission (CCC) (Climate Change Commission 2011) formulated the 2010-2028 National Framework Strategy on Climate Change which identified a long-term mitigation objective of facilitating the transition towards low GHG emissions for sustainable development. CCC outlined the national climate change action plan in 2011. For agriculture, it articulated the following activities: enhance site-specific knowledge on the vulnerability of agriculture and fisheries to the impacts of climate change; conduct researches and disseminate knowledge and technologies on CC adaptation to reduce vulnerability of the sector to climate change; and establish knowledge management on climate change information for agriculture. Moreover, the country committed to reducing its GHG emissions by 70% by 2030 compared to a business-as-usual scenario through mitigation measures in the energy, transport, waste, forestry, and industry sectors.

Aligned to climate change initiatives, this research aims to answer the following questions: What is the GWP impact of the studied backyard system? and How much GWP impact is reduced with the inclusion of Azolla Pinnata in the ration?

With the formulated questions, this study aimed to evaluate the potential GWP reduction with the inclusion of AZM.

MATERIALS AND METHODS

Life Cycle Assessment (LCA)

This study utilizes the Life Cycle Assessment (LCA), an environmental management tool to evaluate a certain product or process in view of assessing and optimizing the quality of a system, in terms of environmental impacts over its entire life cycle. The concept was introduced by SETAC (Society of Environmental Toxicology and Chemistry) and has become a credible technique in many sustainability and environmental assessment endeavours among policymakers, manufacturers and consumers in planning and decision-making activities (Jensen 2008).

Impact Categories

The study focuses on Climate Change, the Global Warming Potential based on TRACI 2.1 (the Tool for the Reduction and Assessment of Chemical and other Environmental Impacts). TRACI 2.1 utilizes global warming potentials (GWPs) for the calculation of the potency of greenhouse gases relative to CO_2, expressed as $kgCO_2$/equivalent (Bare 2012). The relevant gases in the analysis are carbon dioxide, methane and nitrous oxide, which are discussed by (EPA 2017) to be as follows.

Carbon dioxide (CO_2) enters the atmosphere through burning fossil fuels (coal, natural gas, and oil), solid waste, trees and wood products, and also as a result of certain chemical reactions. Carbon dioxide is removed from the atmosphere (or "sequestered") when it is absorbed by plants as part of the biological carbon cycle.

Methane (CH_4) is emitted during the production and transport of coal, natural gas, and oil. CH_4 emissions can also be generated from livestock and other agricultural practices as well as the decay of organic waste in municipal solid waste landfills. One kilogram of CH_4 has 25 GWP versus a kilo of carbon dioxide.

Nitrous oxide (N_2O) is emitted during agricultural and industrial activities, as well as during combustion of fossil fuels and solid waste. One kilogram of N_2O has 298 GWP versus a kilo of carbon dioxide

Data Collection

The data on the material flow included the various inputs and outputs within the system boundaries of the scope of the study farm. The relevant data were collected from interviews of the farm owner, caretaker and observations during the conduct of the feeding trial. The collected data were then classified and established based on their equivalence per gas category. Presented in Table 2 is the summary of GHG factors.

The databases on GHG emissions on transportation, agricultural wastes and biomasses were based from (Engineering Tool Box 2009, EPA 2017). For utilities, from the equivalent carbon footprint of water (Griffiths-Sattenspiel & Wilson 2009) while electricity from Philippine's national grid emission factor. (DOE, 2017) Lastly, for feed and manure, GLEAM-interactive (GLEAM-i) (FAO 2017) was used. The parameters on the production system, feed formulation, manure management and operational parameters on mortality rates, slaughter weight and manure management were inputted in GLEAM-i to generate the corresponding GHGs of the studied farm. The same system and process were done in establishing the corresponding GHG of feeds and manure for AZM inclusions.

RESULTS AND DISCUSSION

Material Flow Analysis of the Backyard Chicken Farm

The boundary and material analyses are shown in Fig. 2. The farm raises Dominant CZ breed on backyard free-range rearing system. One production cycle is composed of 200 birds in 90 days with 5% average mortality. The DOCs, weighing an average of 50 grams each, are transported to the farm using a multi-cab from a hatchery in a nearby town,

Table 2: GHG Emission Factors of different inputs used in the Backyard Chicken Farm.

Inputs	Unit	CO_2	CH_4	N_2O
		kg/Unit	g/Unit	g/Unit
Transpo-Delivery	L	2.3480	0.0026	0.0011
Transpo-Motorcycle	L	2.3190	0.0011	0.0001
Electricity	kWh	0.6030		
Water	m^3	0.8700		
Bedding	kg	0.9750	0.2640	0.0350
Feed	kg CW	1.6853	-	4.4760
Manure	kg CW	-	11.3649	11.1614

37.1 km in distance. To make sure their health and survival, DOCs stay in an area with brooding lights that facilitate the required temperature and humidity. The corresponding required electricity to maintain this artificial brooding is 1 watt per bird. Aside from catching the litters of DOCS, the rice hull bedding also provides the heat necessary for brooding. Approximately 125 kg of rice hulls is used for both brooding and rearing stages.

After 28 days, the birds are allowed to range. The typical area estimated for free-ranging is 1 sqm. per bird. One caretaker is in charge of maintaining the cleanliness of the barn and farm, feeding the birds and pasturing them. The caretaker went to the ranging area twice a day, morning and afternoon for feeding and pasturing. He rode a motorcycle along a distance of approximately 1.5 km from the owner's place to the farm.

In a 90 day-cycle, one bird can consume 3.9 kilos of commercial feeds, which comprises 8.4% starter feeds given over 28 days; 37.8% starter over 35 days; and 53.8% grower 53.8% over 27 days. The conventional feeds are commercially available in the market, which is approximately 3 km from the owner's place. The required feeds are procured every two weeks and are stored at the owner's place. Only the required amount per feeding is brought to the farm. To ensure health and safety, basic recommended vaccines are also provided to the growing chicks.

In terms of water consumption, one bird drinks 0.5 litres per day or a total of 45 litres over the cycle. Minimal water is used in cleaning and up-keeping the barn and farm. Both water and electricity are locally supplied by respective service providers. The average utilities in a cycle are 9.5 m3 for water and 145.2 kWh for electricity.

At the end of the cycle, the birds have an average live weight of 1.5 kg. They are usually sold live but sometimes are slaughtered at the farm and sold as roasted chicken. The average dressing percentage is 71%, which makes the dressed chicken equivalent to approximately 1 kg.

Environmental Impact

Life cycle inventory and GHG emissions of current set-up: The life cycle inventory and GHG emission, as given in Table 3, were quantified based on 1,000 birds or an equivalent of 5 cycles for easier reference and analysis in this study. The current set-up is an equivalent global warming potential of

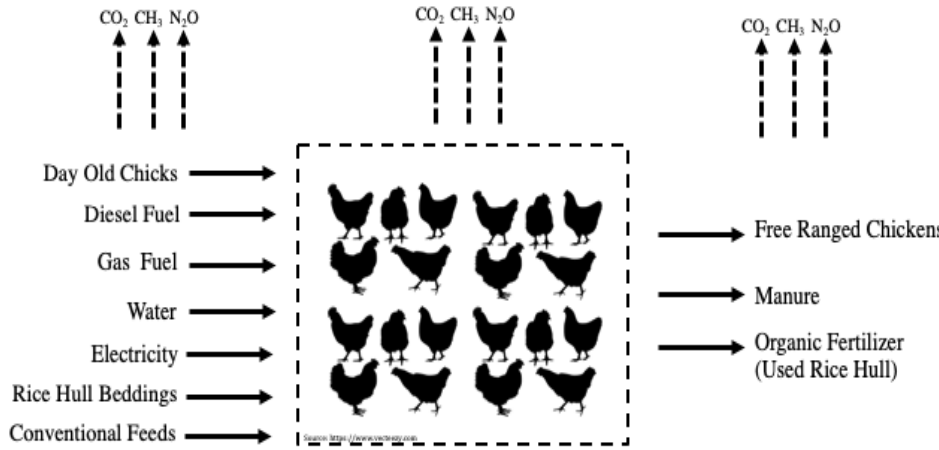


Fig. 2: Gate to gate system boundary of backyard chicken farm.

Table 3: Life Cycle Inventory and GHG Emissions per 1,000 birds of current set-up.

Inputs	Unit	Quantity	GWP in GWP in kg CO_2-eq/unit			
			CO_2	CH_4	N_2O	Total
Tranpo-Diesel	L	55.10	129.375	0.004	0.018	129.396
Transpo-Gasoline	L	67.50	156.533	0.002	0.002	156.536
Electricity	kWh	726.00	437.778	-	-	437.778
Water	m^3	47.50	41.325	-	-	41.325
Bedding	kg	625.00	609.375	4.125	6.519	620.019
Feed	kg CW	1,011.75	3,433.408	-	1,349.522	4,782.929
Manure	kg CW	1,011.75	-	287.462	3,365.168	3,652.630
Total			4,807.793	291.592	4,721.229	9,820.614

9,820.614 kg CO_2 eq. Among GHG gasses, carbon dioxide contributed the highest at 48.96% followed by nitrogen oxide at 48.07%. Carbon dioxide is generated much from the use of conventional feeds, followed by use of electricity and fuels. As discussed in the inventory analysis, there is limited transportation activities, with most of it coming from the mobility of the caretaker for feeding and pasturing. Also, the use of rice hull beddings is limited to the brooding area since the birds are allowed to range after 28 days. For nitrogen dioxide, it is basically driven by the use of conventional feeds and manure. The methane gas has minimal impact given that it has limited transportation activities, thereby, its major contributor is just manure.

The Gleam-I data does not include emissions from direct and indirect uses for backyard chickens. Thereby, comparison can be done with feed and manure related components only. The farm's 8,345.56 kg CO_2 eq/1,000 birds is much higher than Philippine's baseline average of 5,675.92 and East Asia and Southeast Asia regional average of 6,546.02 kg CO_2 eq/ 1,000 birds. Versus neighbouring countries, it is still much higher with Malaysia's 6,019.91, Thailand's 5,433.10 and Indonesia's 5,089.10.

The high GWP per 1,000 birds in this system can be de directly associated with the use of commercial feeds and the yield per cycle. Hence, the area for improvements in this system is to use alternative feeds with lower environmental impact as well as improve the average yield to distribute the impacts of the fixed inputs.

Global warming potential impact of AZM inclusion: The inclusion of Azolla Pinnata has no significant potential adverse effect in global warming potential on various forms as demonstrated on the studies (Kimani et al. 2018) on a rice paddy in Japan; (Xu et al. 2017) on double rice cropping system in southern China; (Jumadi et al. 2014) on the growth of upland Kangkong in silt loam soil in Indonesia. In providing a regular source of the floating fern, the farm uses a 2 sqm improvised water tub, wherein the Azolla Pinnata is propagated and harvested daily when needed. With the propagation method and application, there is no quantifiable impact from Azolla Pinnata to be considered.

The recommended 50% replacement of commercial feeds cut down the volume of feeds by half. This new feed mix ratio was inputted to the Gleam-I system, which changed the GHG profiles of feed and manure. As given in Table 4, the total GWP decreased to 7,024.83 kg CO_2 eq/1,000 bird. The 50% AZM resulted in a reduction of CO_2 by 35%; N_2O by 22.32%, CH_4 by 4.74%, and total GWP by 28.47%, as shown in Fig. 3.

In terms of relative contribution in Fig. 4, feeds decreased its share by 14.95% while manure increased by 9.3%, beddings by 2.51%, electricity by 1.77% and less than 1% for diesel and gasoline respectively.

Aside from the decrease in GWP, it is worth noting that the reduction in commercial feeds and replacement with AZM will also have a significant effect on production costs. Hence, the 50% AZM inclusion provides both environmental and economic impacts of this studied farm.

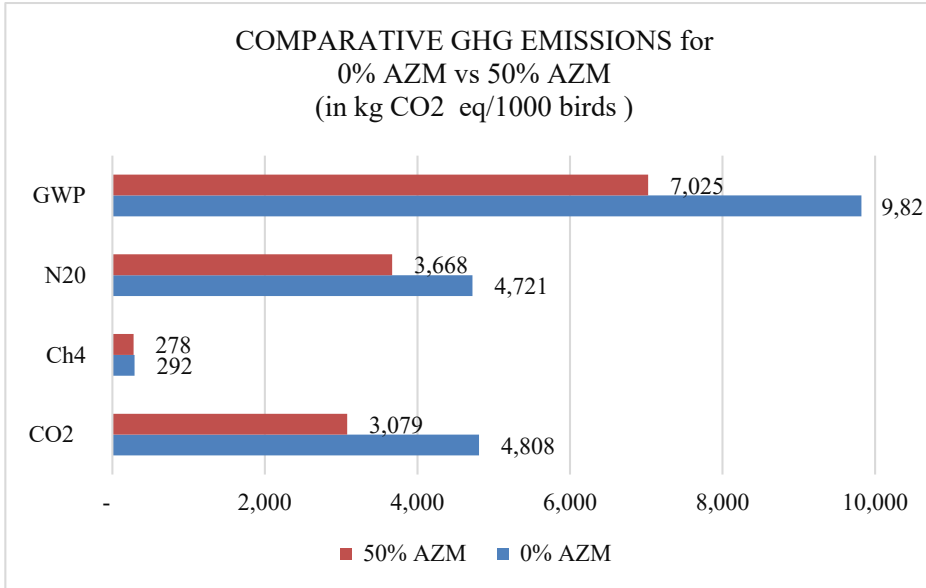


Fig. 3: Comparative GHG emissions for 0% AZM versus 50% AZM.

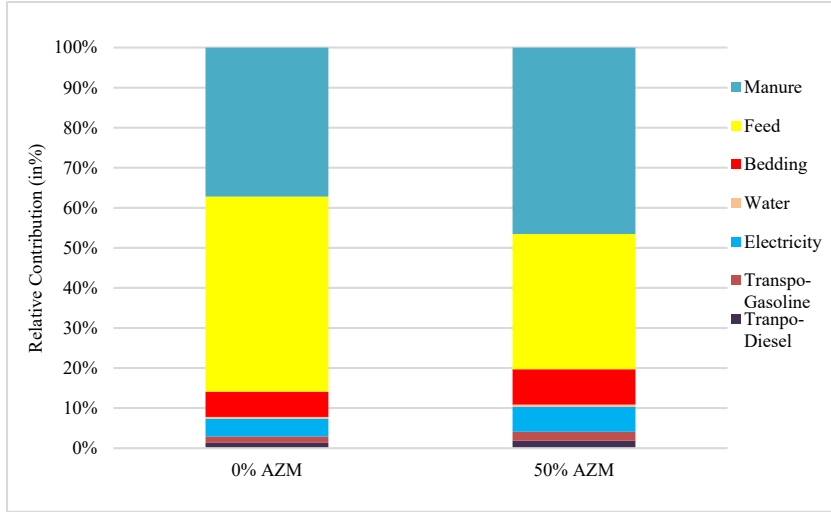


Fig. 4: Relative Contribution of Inputs to GWP.

Table 4: Life Cycle Inventory and GHG Emissions per 1,000 birds with 50% AZM inclusion.

Inputs	Unit	Quantity	GWP in GWP in kg CO_2-eq/unit			
			CO_2	CH_4	N_2O	Total
Tranpo-Diesel	L	55.10	2.3480	0.0001	0.0003	129.3964
Transpo-Gasoline	L	67.50	2.3190	0.0000	0.0000	156.5364
Electricity	kWh	726.00	0.6030	-	-	437.7780
Water	m^3	47.50	0.8700	-	-	41.3250
Bedding	kg	625.00	0.9750	0.0066	0.0104	620.0188
Feed	kg CW	1,011.75	1.6853		0.6585	2,371.3025
Manure	kg CW	1,011.75		0.2704	2.9601	3,268.4761
Total			3,079.491	277.757	3,667.586	7,024.8331

CONCLUSION AND PERSPECTIVES

The studied farm has a GWP impact of 9,820.614 kg CO_2-eq./1,000 birds with its current set-up using the commercial feed to Dominant CZ breed in backyard free-range rearing system. With the recommended inclusion of 50% AZM, GWP reduced to 7,024.83. This showed favourable results since there were reductions of CO_2 by 35%; N_2O by 22.32%, CH_4 by 4.74%. The gross effect of this reduction of conventional feeds is a climate change mitigation equivalent 28.47% of GWP kg CO_2-eq./1,000 birds. The environmental evaluation of AZM showed that the floating fern has favourable benefits, especially in the countryside. Moreover, potential savings can be generated from the 50% decrease in commercial feeds. The impacts indicate that Azolla Pinnata can be a cost-effective and sustainable feedstuff in backyard chicken rearing system especially that it requires simple propagation method. The environmental impact and savings can encourage more livelihood activities in rural.

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REFERENCES

Alalade, O. A., Iyayi, E. A. and Alalade, T. O. 2007. The nutritive value of Azolla (Azolla pinnata) meal in diets for growing pullets and subsequent effect on laying performance. The Journal of Poultry Science, 44: 273-277. https://doi.org/10.2141/jpsa.44.273

Bare, J. 2012. Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts. Retrieved July 10, 2019, from https://nepis.epa.gov/Adobe/PDF/P100HN53.pdf

Chang, H. C. 2007. Analysis of the Philippine chicken industry : Commercial versus backyard sectors. Asian Journal of Agricultural Development, 3(1): 1-16.

Climate Change Commission. 2011. National Climate Change Action Plan 2011-2018. Retrieved July 2, 2019, from http://climate.emb.gov.ph/wp-content/uploads/2016/06/NCCAP-1.pdf

DOE. 2017. 2015-2017 National Grid Emission Factor (NGEF). Retrieved August 1, 2019, from https://www.doe.gov.ph/national-grid-emission-factor-ngef

Dozier, W. A., Kidd, M. T. and Corzo, A. 2008. Dietary amino acid responses of broiler chickens. Journal of Applied Poultry Research, 17(1): 157-167. https://doi.org/10.3382/japr.2007-00071

Engineering Tool Box. 2009. Combustion from Fuels - Carbon Dioxide Emission. Retrieved March 5, 2019, from https://www.engineeringtoolbox.com/co2- emission-fuels-d_1085.html [

EPA. 2017. Understanding Global Warming Potentials. Retrieved March 5, 2019, from https://www.epa.gov/ghgemissions/understanding-global-warming-potentials

FAO, 2017. Global Livestock Environmental Assessment Model (GLEAM-i). Retrieved July 5, 2019, from www.fao.org/gleam/en/

Griffiths-Sattenspiel, B. and Wilson, W. 2009. The Carbon Footprint of Water. Retrieved July 10, 2019, from https://www.rivernetwork.org/resource/the-carbon-footprint-of-water/

Jaturasitha, S., Vearasilp, T., Leotaragul, A., Phongphaew, A., Apicharts-rungkoon, N., Simasathitkul, N. and ter Meulen, U. 2002. A comparative study of thai native chicken and broiler on productive performance, carcass and meat Quality. In: Conference on International Agricultural Research for Development. Witzenhausen. Retrieved from http://www.tropentag.de/2002/abstracts/full/213.pdf

Jensen, A. A. 2008. SETAC Europe LCA steering committee-the early years. International Journal of Life Cycle Assessment, 1: 1–6. https://doi.org/10.1065/lca2007.12.371

Jumadi, O., Hiola, S. F., Hala, Y. and Norton, J. 2014. Influence of Azolla (Azolla microphylla Kaulf .) compost on biogenic gas production , inorganic nitrogen and growth of upland kangkong (Ipomoea aquatica Forsk .) in a silt loam soil Influence of Azolla (Azolla microphylla Kaulf) compost on biogenic. Soil Science and Plant Nutrition, 60(5): 722-730. https://doi.org/10.1080/00380768.2014.942879

Kimani, S. M., Cheng, W., Kanno, T., Nguyen-sy, T., Abe, R., Oo, A. Z. and Sudo, S. 2018. Azolla cover significantly decreased CH_4 but not N_2O emissions from flooding rice paddy to atmosphere. Soil Science and Plant Nutrition, 64(1): 68-76. https://doi.org/10.1080/00380768.2017.1399775

MacLeod, M., Gerber, P., Mottet, A., Tempio, G., Falcucci, A., Opio, C. and Steinfeld, H. 2013. Greenhouse gas emissions from pig and chicken supply chains-A global life cycle assessment. Rome. Retrieved August 1,2019 from http://www.fao.org/3/i3460e/i3460e.pdf

Mananghaya, K. M. V. 2017. Enhancing your pasture for sustainable native chicken production ". Retrieved August 1, 2019, from http://www.pcaarrd.dost.gov.ph/home/portal/index.php/quick-information-dispatch/2866-enhancing-your-pasture-for-sustainable-native-chicken-production

Namra, M. M. M., Hataba, N. A. and Wahed, H. M. A. 2010. The productive performance of growing fayoumi chicks fed restricted diets supplemented with free fresh Azolla. Egypt Poultry Science, 30(III): 747-762.

Padhi, M. K. 2016. Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. Scientifica, 2016, 1-9. https://doi.org/10.1155/2016/2604685

Pillai, P., Premalatha, S. and Rajamony, S. 2005. Azolla: a sustainable feed for livestock. Leisa Magazine, 4: 26-27. Retrieved from http://www.eriksjodin.net/projects/ theazollacookingandcultivationproject/research/Azolla, a sustainable feed for livestock.pdf

PinoyBisnis.com. 2010. Raising Solraya's sunshine chickens. Retrieved March 5, 2019, from https://www.pinoybisnes.com/livestock-business/raising-solraya-sunshine-chickens/

PSA. 2018. Chicken Industry Performance Reports 2011-2017. Retrieved July 7, 2019, from https://psa.gov.ph/content/chicken-situation-report

Rascio, N. and La Rocca, N. 2008. Biological Nitrogen Fixation. Encyclopedia of Ecology, Five-Volume Set, 412-419. https://doi.org/10.1016/B978-008045405-4.00273-1

Roxas, N. P. 2000. Growth performance of Philippine native and broiler. Philippine J. Vet Animal Science, 26: 185-196.

Siacor, F. D. C. 2019. Meat Chemical Composition Analysis of Azolla Pinnata.Unpublished Data.

Sison, J. A. 2014. Feed use estimation-data, methodology and gaps the case of the Philippines. Metro Manila. Retrieved from http://www.amis-outlook.org/fileadmin/user_upload/amis/docs/resources/3c Feed use estimation – Data, methodologies and gaps (Philippines) September 2014.pdf

Thieme, O., Sonaiya, E. B., Rota, A., Alders, R., Saleque, M. A., De' Besi, G. 2014. Family poultry development – issues, opportunities and constraints. Animal Production and Health Working Paper. No. 12. Rome.

USAID. 2016. Greenhouse Gas Emissions in the Philippines. Retrieved July 2, 2019, from https://www.climatelinks.org/resources/greenhouse-gas-emissions-factsheet-philippines

Wong, J., de Bruyn, J., Bagnol, B., Grieve, H., Li, M., Pym, R. and Alders, R. G. 2017. Small-scale poultry and food security in resource-poor settings: A review. Global Food Security, 15: 43-52.

Xu, H., Zhu, B., Liu, J., Li, D., Yang, Y. and Zhang, K. 2017. Azolla planting reduces methane emission and nitrogen fertilizer application in double rice cropping system in southern China. Agron. Sustain. Dev., 37(29): 1-9. https://doi.org/10.1007/s13593-017-0440-z

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Temporal Variations of $PM_{2.5}$ and PM_{10} Concentration Over Hyderabad

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Relative humidity

ABSTRACT

The association between urbanization and health at the global level, as well as the role of air pollution, has increased the interest in studies, aimed to improve the air quality of urban areas. Addressing the challenges of pollution caused by urbanization plays a crucial role in developing sustainable urbanization. Understanding the temporal characteristics of particulate matter mass concentrations with an aerodynamic diameter of less than 2.5 μm and 10 μm ($PM_{2.5}$ and PM_{10}) is very important to counter the effect of air pollution. We have analysed and interpreted the diurnal, monthly and seasonal variations of one-hour average PM concentrations taken from Central Pollution Control Board (CPCB) for six stations over Hyderabad, India during March 2018 to February 2020. Average concentrations of $PM_{2.5}$ (41.5 μg/m^3) and PM_{10} (91.52 μg/m^3) for two consecutive years (2018 and 2019) are found to exceed the standard values of World Health Organization (WHO) standards ($PM_{2.5}$ = 10 μg/m^3 and PM_{10} = 20 μg/m^3) and National Ambient Air Quality Standards (NAAQS) ($PM_{2.5}$ = 40 μg/m^3 and PM_{10} = 60 μg/m^3). A clear diurnal and seasonal variations are observed for all the stations. In diurnal cycle, a large PM concentration was observed between 8 AM to 10 AM and again between 6 PM to 9 PM with a minimum at 3 PM in all seasons and also for all stations which clearly shows semidiurnal variations. Data analysis shows a high concentration of particulate matter in winter compared to other seasons. The $PM_{2.5}$ (PM_{10}) concentrations in winter were found to be increased by three (two times) when compared to monsoon. The ratio of $PM_{2.5}$ to PM_{10} is very close to 0.5 during post-monsoon and winter, and 0.4 in summer and monsoon seasons, which clearly shows that $PM_{2.5}$ comprises a major portion of PM_{10}. The $PM_{2.5}$ and PM_{10} are highly correlated with correlation coefficient 0.9. Out of 6 stations, Zoo Park is contributing more particulate matter pollutant concentrations.

INTRODUCTION

Today, with rapid urbanization and industrialization, there is every possibility of increasing pollution concentrations. According to the World Health Organization (WHO), 92% of the world's population is currently living in areas where the air quality level exceeds the WHO standards (WHO 2016). In a natural environment, atmospheric air is an important element which does not have any natural protective barrier that can be isolated, and therefore the control and analysis of the impact of pollutants are essential not only on a global but also important at continental, national and local scale (Cichowicz & Wielgosiński 2015a, 2015b, Ménard et al. 2016, Vallero 2014). Both industrial smog and photochemical smog are forms of air pollution. According to the National Institutes of Health, both can create major health risks, including asthma, lung tissue damage, bronchial infections and heart problems. The main sources of air pollutants are combustion processes, various technological processes as well as vehicular emissions (Cichowicz & Wielgosiński 2015a, 2015b, Gurney et al. 2012, Lelieveld et al. 2015, Nemitz et al. 2002). It should be taken to count, that low-emission sources emit pollutants primarily during the heating season, whereas remote systems do it with varying intensity throughout the entire calendar year (Cichowicz & Wielgosiński 2015a, 2015b, Lin et al. 2011).

Hyderabad, the capital city of Telangana, is one of the fastest-growing metropolitan areas in India, located on the banks of the Musi River at an average altitude of 542 m above sea level. It covers an area of 650 sq. km and has a current population of around 10 million with an increase of **2.7%** from 2019, which makes it the 6th most populous urban agglomeration in India. It is a tropical urban city with wet and dry climatic conditions. The annual and monthly mean temperatures are 26.6°C and 21-33°C respectively. The summer months (March-May) are hot and humid with maximum temperatures often exceed 40°C and winter occur in the months (December – February) with the lowest temperature occasionally dipping below 10°C. In the last few years, a stunning growth of vehicle population was observed leading to ~60 lakhs vehicles by the end of 2020. The major sources contributing to particulate matter air pollution are from vehicular emission, road dust and industrial emission.

The air quality in Hyderabad often exceeds the NAAQ standards especially for particulate matter (PM).

In this paper, an attempt has been made to analyze and interpret the diurnal, weekly, monthly and seasonal cycles of $PM_{2.5}$ and PM_{10} concentrations measured for 6 stations over Hyderabad having high traffic density zones, industrial areas and mixed conditions. The impact of meteorological parameters such as temperature, relative humidity and wind speed on $PM_{2.5,}$ PM_{10} concentrations are also discussed.

DATA DETAILS AND ANALYSIS

The Telangana State Pollution Control Board (TSPCB) is operating six Continuous Ambient Air Quality Monitoring Stations (CAAQMS) [IDA Bollaram (BLM); Hyderabad Central University (HCU); ICRISAT Patancheru (PTC); IDA Pashamylaram (PSM); Zoo Park (ZOO); Sanathnagar (SNN)] over Hyderabad. An hourly particulate matter ($PM_{2.5}$ and PM_{10}) data, downloaded from CAAQMS website (https://cpcb.nic.in/automatic-monitoring-data/) for these six stations were used in our study. It provides instant data generation, online data dissemination, meteorological parameters, etc., using sophisticated analysers for various parameters which include the particulate matter of size less than 2.5 µm and 10 µm ($PM_{2.5}$, PM_{10}). The meteorological parameters (temperature, relative humidity and wind speed) are also used to study the effect of these parameters on the PM concentrations. The details of the stations and their significance are mentioned in Table 1.

To access the air quality status about PM concentrations in Hyderabad, we analysed the recent two years of CAAQMS data for $PM_{2.5}$ and PM_{10} from all six stations. The PM_{10} concentration is not available for Sanathnagar station during the study period. Fig.1 shows the complete number of usable days of data in the form of a histogram. The total number of observational days for the period 1st March 2018 to 29th February 2020 was varying with minimum 698 and maximum 727 days across all stations, which

Table 1: Continuous ambient air quality monitoring stations.

S. No	Name of the station	Significance of station	Latitude (ºN)	Longitude (ºE)
1	Bollaram Industrial Area, Hyderabad – TSPCB	Industrial Residential Rural and Other Area	17.54	78.34
2	Central University, Hyderabad – TSPCB	Downstream of industrial area and sensitive zone	17.45	78.34
3	ICRISAT Patancheru, Hyderabad – TSPCB	Industrial Residential Rural and Other Area	17.51	78.27
4	IDA Pashamylaram, Hyderabad – TSPCB	Industrial Residential Rural and Other Area	17.53	78.18
5	Zoo Park, Hyderabad – TSPCB	Industrial Residential Rural and Other Area	17.34	78.45
6	Sanathnagar, Hyderabad – TSPCB	Centre of the city and Balanagar IDA	17.45	78.44

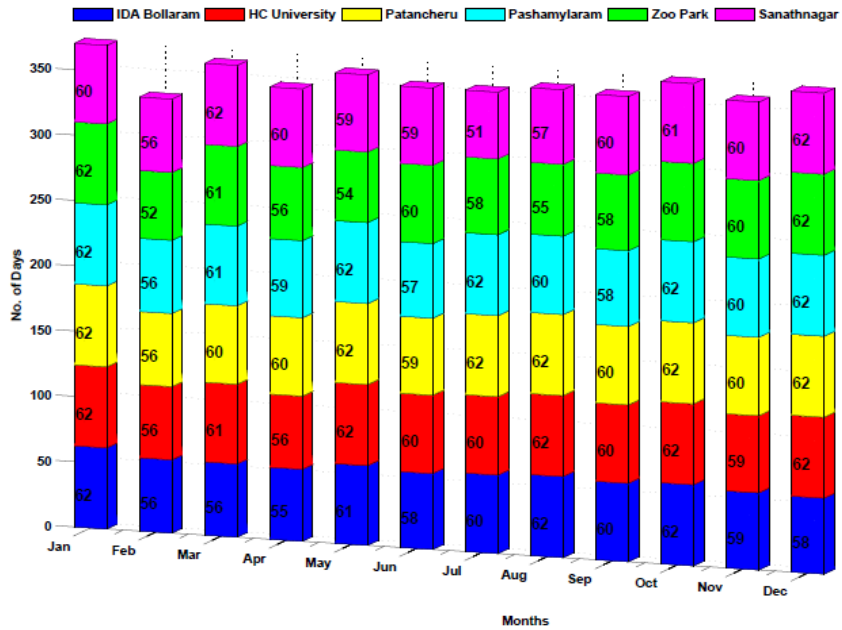


Fig. 1: Histogram of the monthly usable days for two years 2018 and 2019.

meets the valid standards for averaging, station wise (Xiao et al. 2018). Proper averaging was done to study diurnal, monthly and seasonal variations of PM concentration and effect of meteorological parameters.

RESULTS AND DISCUSSION

Temporal Variations of $PM_{2.5}$ and PM_{10} Concentrations

To evaluate the air quality status regarding the particulate matter, we attempted to analyze two years of ambient monitoring data for $PM_{2.5}$ and PM_{10} over Hyderabad. The hourly values of PM concentrations are averaged initially day-wise and subsequently averaged over a year for all six stations. The annual means are tabulated in Table 2 along with their averages. Rate of change (ROC) was used to compare the variance in $PM_{2.5}$ and PM_{10} concentrations for different stations using:

$$ROC\ (\%) = [(X - Y)/Y] \times 100 \quad \ldots(1)$$

Where, Y and X represent the average PM concentrations in 2018 and 2019 respectively, and they are tabulated in Table 2.

Coefficient of variation (CV) shown in Table 3 describes the degree of spatial variation of PM concentration in a given area and it can be expressed as

$$CV = STD/\overline{X} \quad \ldots(2)$$

We observed a significant difference in annual concentrations for six stations over Hyderabad (Table 2). The particulate matter concentrations in 2019 are found to be less than 2018 for all stations. The concentrations are much lower in Central University when compared to other stations. The percentage of days per year of particulate matter concentrations in all stations during the study period are depicted in Fig. 2.

Table 2: PM concentrations and rate of change.

Station	Parameter	2018	2019	Mean	ROC (%)
BLM	PM 2.5	45.6	40.08	43.25	-11
	PM10	100.27	95.14	97.7	-5.4
HCU	PM 2.5	38.2	29.80	34.01	-28
	PM10	90.78	78.44	84.6	-15.7
PTC	PM 2.5	40.22	35.04	37.63	-14
	PM10	93.05	81.78	87.4	-13.8
PSM	PM 2.5	42.43	37.31	39.88	-13
	PM10	93.74	89.11	91.4	-5.2
ZOO	PM 2.5	49.28	47.21	48.25	-4.4
	PM10	99.68	92.99	96.3	-7.2
SNN	PM 2.5	47.36	47.20	47.28	-0.35
	PM10	-	-	-	-

Daily concentrations of $PM_{2.5}$ (PM_{10}) ranges from 10 $\mu g/m^3$ - 75 $\mu g/m^3$ (35 $\mu g/m^3$ - 100 $\mu g/m^3$) respectively. For $PM_{2.5}$ (PM_{10}) the proportion of days with values greater than 35 $\mu g/m^3$ (70 $\mu g/m^3$) is 60%. The percentage of days with $PM_{2.5}$ concentration values of 35 - 50 $\mu g/m^3$ decrease by 10 % from 2018 to 2019 except in Zoo Park. A similar decrease in percentage was observed for PM_{10} concentration values of 75 - 100 $\mu g/m^3$.

The levels of PM concentration for different stations during the study period are shown in Fig.3. The central box comprises values of 25 and 75 percentiles, and whiskers show the range of values falling within 1.5 times the interquartile range beyond the box. The solid lines within the box represent the median values. The outliers, defined as data points beyond the inner fence are represented with '+' symbols. PM_{10} concentration data at Sanathnagar is not available and it was not shown in the figure. The average value of PM (both $PM_{2.5}$ and PM_{10}) concentration is found to be minimum at Central University. But the maximum value of $PM_{2.5}$ and PM_{10} occur at two different stations, Zoo Park and IDA Bollaram, respectively. The PM concentrations averaged for two years over Hyderabad are found to be 41.5 $\mu g/m^3$ ($PM_{2.5}$) and 91.52 $\mu g/m^3$ (PM_{10}) exceeding the values of WHO standard ($PM_{2.5}$ = 10 $\mu g/m^3$ and PM_{10} = 20 $\mu g/m^3$) and NAAQ standards ($PM_{2.5}$ = 40 $\mu g/m^3$ and PM_{10} = 60 $\mu g/m^3$) (WHO air guideline 2005, NAAQ 2009). Sarath et al. (2019) also reported similar observations from the same site.

ROC for 2019 following 2018 indicate a sharp decrease in PM concentrations at Central University when compared to other stations. The possible reasons for high negative ROC and low PM concentrations at Central University may be attributed to the improvement of roads, construction of flyovers and bypass the traffic through outer ring road. It may also due to green cover around Central University which prevent dust particles from building up in the environment. The CV is observed to be in the same order for all the stations, which indicates there is not much spatial

Table 3: Coefficient of variation (CV) of PM 2.5 and PM10 for different stations.

Station	PM 2.5			PM 10		
	2018	2019	Average	2018	2019	Average
BLM	0.39	0.42	0.41	0.31	0.37	0.34
HCU	0.51	0.62	0.57	0.44	0.5	0.47
PTC	0.54	0.55	0.55	0.45	0.52	0.49
PSM	0.51	0.53	0.52	0.45	0.48	0.47
ZOO	0.44	0.48	0.46	0.44	0.48	0.46
SNN	0.43	0.55	0.49	-	-	-

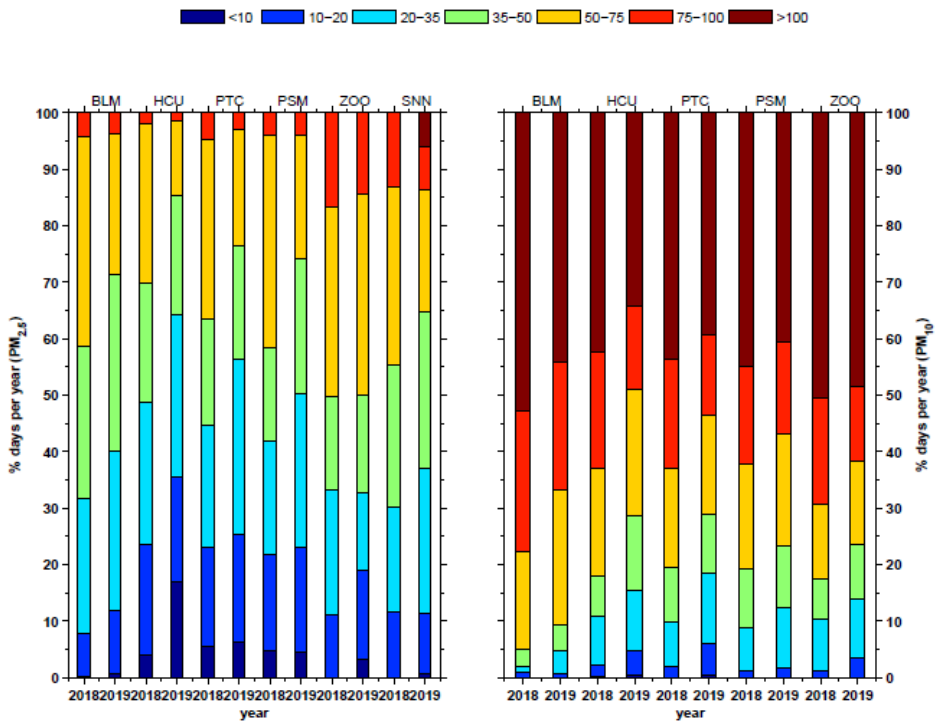


Fig. 2: The annual range of daily particulate concentrations for different stations. Left panel: $PM_{2.5}$; Right panel: PM_{10}.

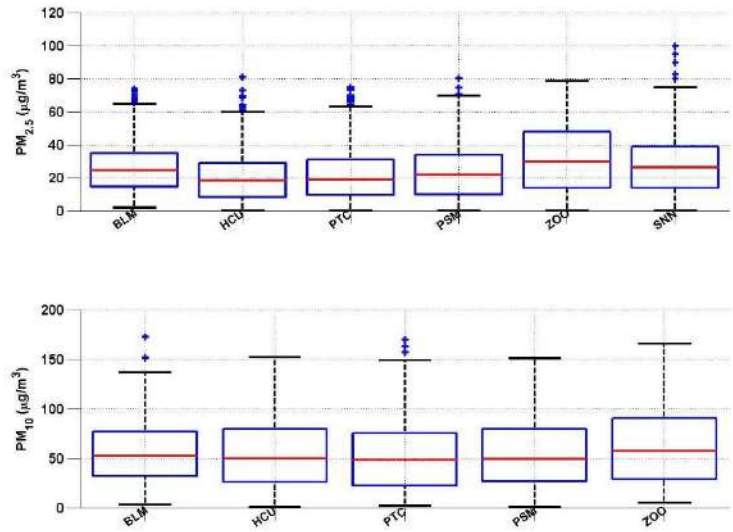


Fig. 3: The levels of PM concentration for different stations during the study period with median values (solid line within box), 25 and 75 percentiles and whiskers. The outliers are represented with '+' symbols.

variation (Yang et al. 2018). A strong correlation between $PM_{2.5}$ and PM_{10} was observed over Hyderabad with a correlation coefficient of 0.9, which could be explained by the similar sources like emissions from the combustion process, dust (due to movement of the vehicles on the road) (Tecer et al. 2008). The observed PM concentrations over six stations clearly indicate the influence of local combustion processes which includes usage of fuel in domestic heating, industrial activities, construction, transportation, and traffic.

A. Diurnal and Seasonal Variation of PM Concentrations

To study diurnal variation of particulate matter concentration, hourly values of PM for two years were averaged accordingly to get a set of 24 hour data points. Results, depicted in Fig.4, show a diurnal variation in PM concentrations which varies bimodally having two peaks between 8 AM – 10 AM and 6 PM – 9 PM, with minimum concentration observed at 3 PM. During night-time, after 9 PM the concentration was significantly decreased because of low traffic flow resulting in low emission rate (Luis et al. 2014). This behaviour mostly depends on traffic conditions in urban areas. Contributions to the annual means of PM concentrations are mainly from combustion processes and vehicle emissions. Low values of concentration are observed between 10 AM to 6 PM. One of the reasons may be the restriction of heavy-duty diesel vehicles into the city limits from 8 AM to 10 PM. The other reason is likely due to favourable dispersion conditions. Another noticeable observation is that a prominent year to year variation is observed in the case of Central University and ICRISAT Patancheru.

To study the effect of weekend and weekday on PM concentrations, mean diurnal variations of weekday (Monday - Friday), weekend (Saturday and Sunday) and their average for all stations during the study period are plotted in Fig. 5. It was observed that the difference in the trends and concentration values is marginal for weekdays and weekends. This indicates there is no noticeable weekday/weekend effect on PM concentration (Federico et al. 2015).

Further, we have analysed the averaged diurnal variations of PM concentration seasonally in all the sites during the study period for four seasons: Summer (March-May), Monsoon (June-August), Post Monsoon (September-November) and Winter (December-February). These variations are shown in Fig. 6. The variations are significantly more pronounced in winter than the other seasons. In case of $PM_{2.5}$, the post-monsoon concentration is slightly more than in summer whereas in PM_{10} it is reversed. The semi-diurnal variation was seen in all seasons except in monsoon. The seasonal variability of the PM concentrations shows a similar trend in all stations. Averaged concentrations over Hyderabad and their ratio are depicted in Table 4 for all seasons. Positive or negative correlations reflect the physical response mechanisms. A low positive and reasonable negative correlation was seen between temperature and RH with PM concentration, respectively. We also observed a better correlation between wind speed and $PM_{2.5}$ but low correlation with PM_{10}. This suggests that the dependency of $PM_{2.5}$ on wind speed was more significant than that of PM_{10} during winter (Xiao et al. 2018).

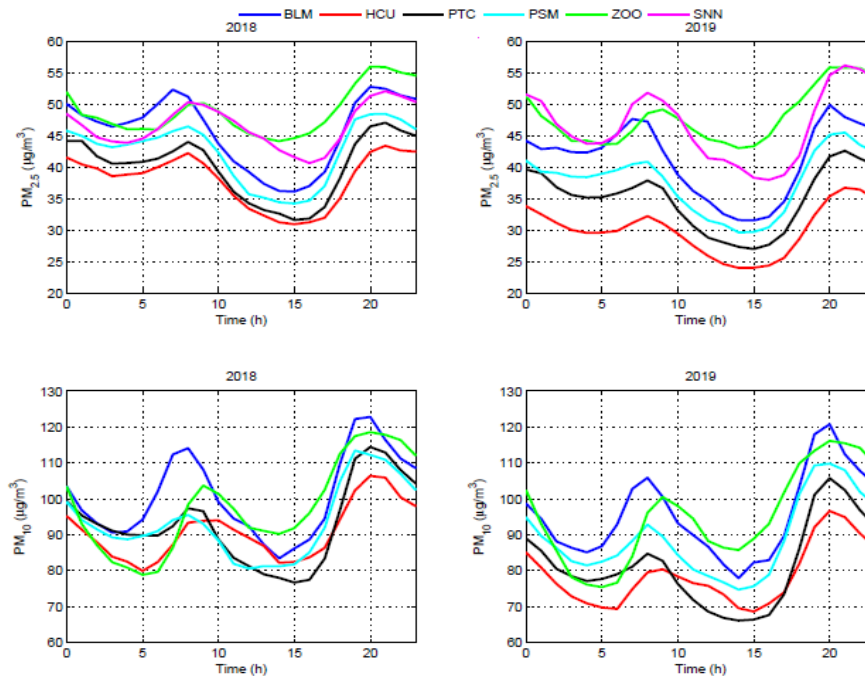


Fig. 4: Top Panel: Diurnal variations of $PM_{2.5}$ concentration of 2018 and 2019 for all stations. Bottom Panel: Diurnal variations of PM_{10} concentration.

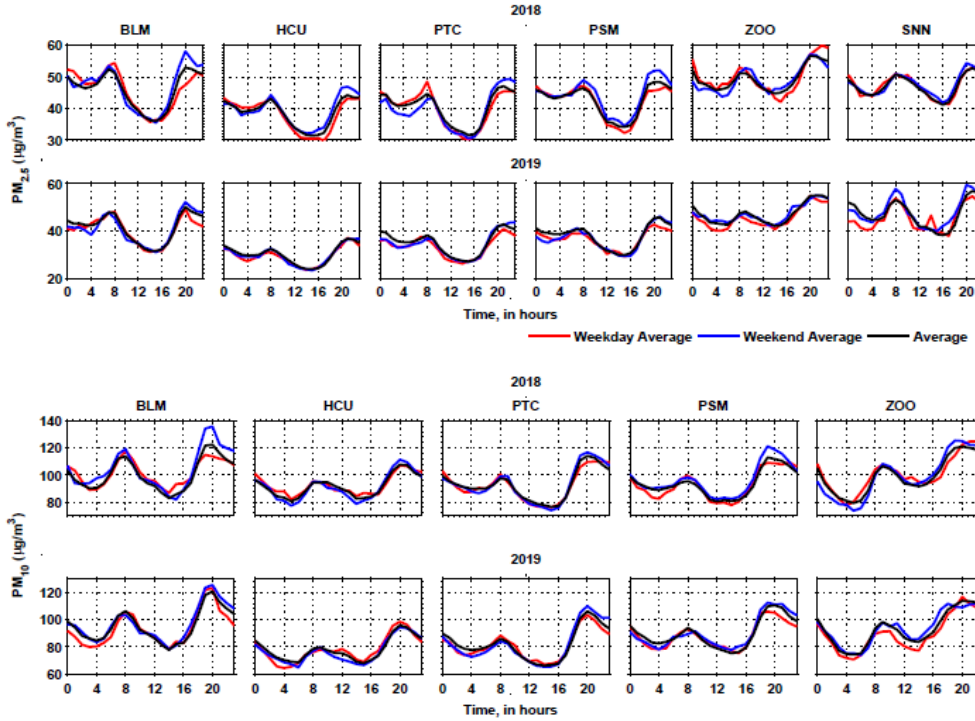


Fig. 5: Diurnal variation of PM concentrations during the weekday (Monday-Friday, Red), weekend (Saturday and Sunday, blue) and average (black) for different stations.

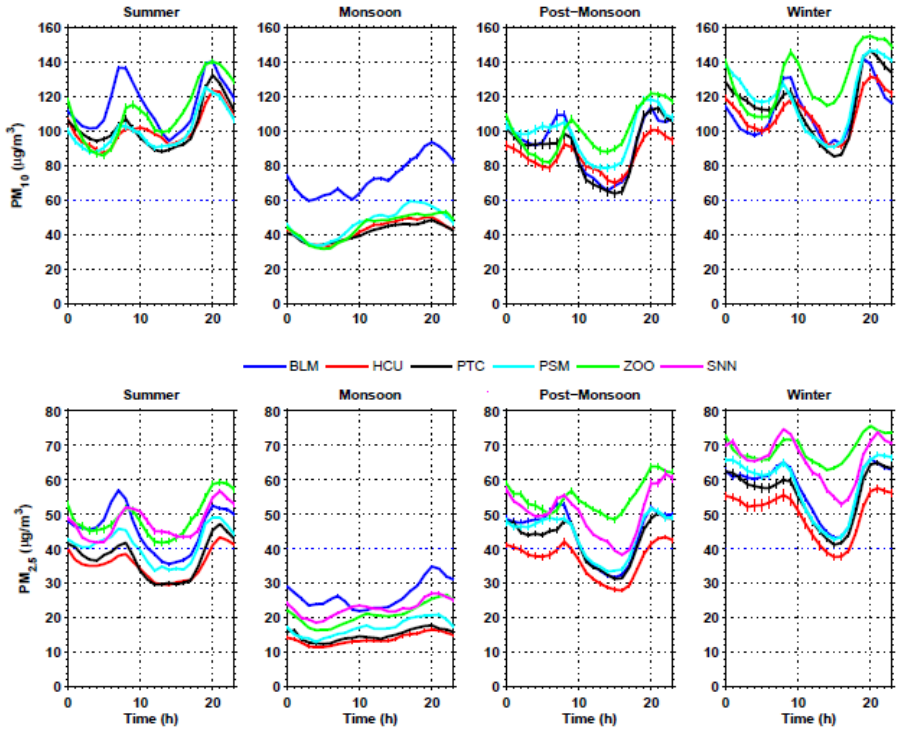


Fig. 6: Top Panel: Diurnal variations of PM_{10} concentration during summer, monsoon, post monsoon and winter seasons for different stations. Bottom Panel: Similarly, for $PM_{2.5}$ concentration.

Table 4: Seasonal variation of PM concentrations and their ratio.

Season	Mean $PM_{2.5}$	Mean PM_{10}	$PM_{2.5}$ / PM_{10}
Summer	42.7	106.1	0.4
Monsoon	19.4	49.74	0.39
Post Monsoon	45.53	93.12	0.49
Winter	59.41	117.62	0.51

High values of concentration observed in winter can be related to specific thermal inversions and domestic heating emissions. Similar observations have been examined by others (Tecer et al. 2008, Cichowicz et al. 2015). The percentage contribution of $PM_{2.5}$ in PM_{10} shows a gradual increase from summer to winter (Srimuruganandam et al. 2010). This indicates the inclusion of anthropogenic fine particles over Hyderabad. The ratio of concentrations for post-monsoon and winter is ~ 0.5 i.e., PM_{10} concentration is nearly double the $PM_{2.5}$, suggesting a large dust source in the city. A $PM_{2.5}/PM_{10}$ ratio of 0.5 is typical for developing country urban areas and is at the bottom of the range found in developed country urban areas (0.5–0.8) (WHO 2005).

B. Influence of Meteorological Parameters on Particulate Matter

To study the effect of meteorological parameters on particulate matter, daily means of $PM_{2.5}$ and PM_{10} are subsequently averaged monthly, represented by bars, and plotted in Fig. 7 along with corresponding Temperature, Relative Humidity (RH) and Wind Speed. Mean values of daily maxima of concentration in a particular month are also plotted in Fig. 7. It shows high concentrations in winter months (DJF) and low in monsoon months (JJA). Usually pollution levels in monsoon fall below the National Air Pollution standards due to precipitation, however unable to attain these standards at the other times of the year.

CONCLUSIONS

In the present study, the measurements of hourly particulate matter concentrations during March 2018 to February 2020 from six stations over Hyderabad are used to understand the temporal variations of PM concentrations and the effect of meteorological parameters. Our main conclusions are as follows: The annual mean concentrations of $PM_{2.5}$ and PM_{10} exceeded the standards of NAAQ and WHO at all stations. The concentrations are found to decrease from 2018 to 2019 and Rate of change (ROC) is declined for all station. This suggests that the measures taken by Greater Hyderabad Municipal Corporation (GHMC), as a part of the action plan (2017-2024), gave good results in controlling the particulate matter concentrations over Hyderabad. The spatial variations

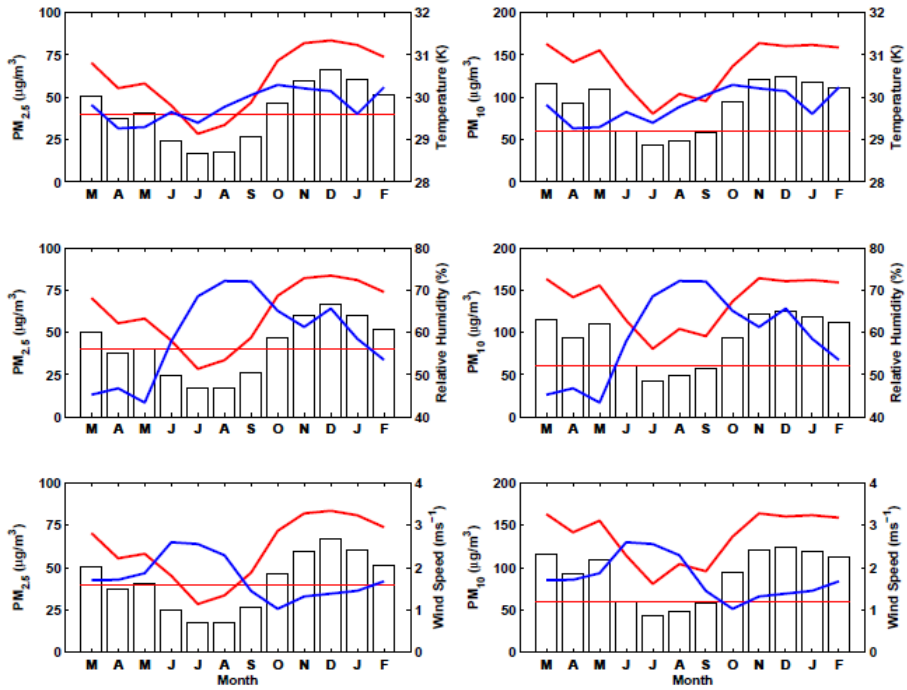


Fig. 7: Left Panel: Comparison of $PM_{2.5}$ concentration with Temperature, RH and Wind Speed. Right Panel: Similar comparison for PM_{10}. Bar graphs indicate daily means of monthly averages of PM concentration and corresponding meteorological parameters (Temperature, RH and Wind Speed, Blue); Mean values of daily maxima concentration in a particular month (Red).

in the annual average PM concentrations were observed to be in the same order for all stations. The $PM_{2.5}$ and PM_{10} concentrations are highly correlated (0.9) which indicates the sources of pollutants are mainly due to emissions from combustion sources and dust. Semidiurnal variations were seen in PM concentrations with two peaks during high traffic hours which can be attributed to vehicular emissions. A strong seasonal trend was observed in all stations, with the highest value in winter and lowest in Monsoon. The analysis showed that the meteorological parameters do not have an appreciable effect on PM concentrations. The results suggest that there is a need for a quantitative approach in evaluating the air pollutants related to the air quality over Hyderabad. To reduce further particulate matter pollutant concentrations, the developing Hyderabad city should establish and implement more joint regional air pollution control programs.

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REFERENCES

Cichowicz, R. and Wielgosiński, G. 2015a. Effect of meteorological conditions and building location on CO_2 concentration in the university campus. Ecological Chemistry and Engineering S, 22(4): 513-525.

Cichowicz, R. and Wielgosiński, G. 2015b. Effect of urban traffic on the emission of carbon dioxide in the university campus. Ecological Chemistry and Engineering S, 22(2): 189-200.

Cichowicz, R., Wielgosiński, G. and Fetter, W. 2017. Dispersion of atmospheric air pollution in summer and winter season. Environ. Monit. Assess., 189(605), doi:10.1007/s10661-017-6319-2.

Federico, M., San Martini, Christa, A. Hasenkopf and David, C. Roberts, 2015. Statistical analysis of $PM_{2.5}$ observations from diplomatic facilities in China. Atmospheric Environment, 110: 174 -185.

Gurney, K. R., Razlivanov, I., Song, Y., Zhou, Y., Benes, B. and Massih, M. A. 2012. Quantification of fossil fuel CO_2 emissions on the building/street scale for a large U.S. City. Environmental Science & Technology, 46(21): 12194-12202.

Lelieveld, J., Evans, J. S., Fnais, M., Giannadaki, D. and Pozzer, A. 2015. The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature, 525: 367-371.

Lin, W., Xu, X., Ge, B. and Liu, X. 2011. Gaseous pollutants in Beijing urban area during the heating period 2007–2008: variability, sources, meteorological, and chemical impacts. Atmospheric Chemistry and Physics, 11: 8157-8170. https://doi.org/10.5194/acp-11-8157.

Luis, F., Pineda-Martínez, Noel Carbajal, Arturo Campos-Ramos, Antonio Aragón-Piña and Agustín R. García, 2014. Dispersion of atmospheric coarse particulate matter in the San Luis Potosí, Mexico, urban area. Atmósfera, 27(1): 5-19.

Ménard, R., Deshaies-Jacques, M. and Gasset, N. 2016. A comparison of correlation-length estimation methods for the objective analysis of surface pollutants at Environment and Climate Change Canada. Journal of the Air & Waste Management Association, 66: 9,874-9,895.

NAAQ 2009. https://scclmines.com/env/DOCS/NAAQS-2009.pdf

Nemitz, E., Hargreaves, K.J., McDonald, A. G., Dorsey, J. R. and Fowler, D. 2002. Micrometeorological measurements of the urban heat budget and CO2 emissions on a city scale. Environmental Science & Technology, 36(14): 3139-3146.

Sarath, Guttikunda K. and Nishadh, PujaJawahar, K. A. 2019. Air pollution knowledge assessments (APnA) for 20 Indian cities. Urban Climate, 27: 124-141.

Srimuruganandam, B. and Shiva Nagendra Saragur Madanayak, 2010. Analysis and interpretation of particulate matter PM_{10}, $PM_{2.5}$ and PM_1 emissions from the heterogeneous traffic near an urban roadway. Atmospheric Pollution Research, 1: 184-194.

Tecer, L.H., Pinar Süren, Omar Alagha, Ferhat Karaca and Gürdal Tuncel, 2008. Effect of meteorological parameters on fine and coarse particulate matter mass concentration in a coal-mining area in Zonguldak, Turkey. Journal of the Air & Waste Management Association, 58(4): 543-552.

Vallero, D. (5th ed.) 2014. Fundamentals of Air Pollution, London: Elsevier Inc. Academic Press.

WHO, 2005. Air Quality Guidelines, https://www.who.int/phe/health_topics/outdoorair/outdoorair_aqg/en/

WHO, 2016. Ambient Air Pollution, A Global Assessment of Exposure and Burden of Disease; Working Papers; World Health Organization: Geneva, Switzerland.

Xiao, K., Wang, Y., Wu, G., Fu, B. and Zhu, Y. 2018. Spatiotemporal characteristics of air pollutants (PM_{10}, $PM_{2.5}$, SO_2, NO_2, O_3, and CO) in the Inland Basin City of Chengdu, Southwest China. *Atmosphere, 9*: 74.

Yang, X., Lei Jiang, Wenji Zhao, Qiulin Xiong, Wenhui Zhao and Xing Yan, 2018. Comparison of ground-based $PM_{2.5}$ and PM_{10} concentrations in China, India, and the U.S. Int. J. Environ. Res. Public Health, 15(7): 1382.

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Assessment of Heavy Metal, Arsenic in Chhilpura Pond Water and its Effect on Haematological and Biochemical Parameters of Catfish, *Clarias batrachus*

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ABSTRACT

Heavy metal poisoning means an excess of the required concentration that is found naturally on earth. In the present experiment, it has been observed that the natural water source surrounded by mountains is also polluted with arsenic because these heavy metals like arsenic are naturally contained in rocks that accumulate in the water source with soil erosion and rain. In this experiment arsenic was found in the water of Chhilpura pond, in which the haematology and biochemistry of catfish (*Clarias batrachus*) were studied, it was found that the RBC, haemoglobin, PCV, MCV, MCH, platelets, glucose and protein contents of catfish were found to be relatively low as compared to the control, whereas the WBC was observed to be higher to control and differential leucocyte count fluctuations were observed. It can be inferred from this experiment that heavy metals such as arsenic alter the haematology and biochemistry of catfish (*Clarias batrachus*).

INTRODUCTION

The aquatic environment is heavily degraded due to many geographical and anthropogenic activities that cause industrial and domestic effluents. Particularly, river, lakes, ponds and the reservoirs (aquatic ecosystem) situated near industrial and urban areas are the potential targets for the disposal of the environmental adverse elements like organic and inorganic contaminants (Kamaludeen et al. 2003; DeForest et al. 2007). Heavy metals are largely occurring in dispersed form in stone and rock formations. Industrialization, urbanization and anthropogenic contribution have an increasing amount of heavy metals in the biosphere and it has the largest availability in aquatic ecosystems and to a relatively smaller proportion in the atmosphere as particulates (Prakash et al. 2014; Pichhode & Gaherwal 2019a). Heavy metals are of particular burden due to their non-biodegradable nature and their damaging effect to human health. These heavy metals have high efficiency to bioaccumulate in various organs (such as liver and kidney) and muscle tissue.

In drinking water, arsenic contamination has been reported from over 70 countries, indicating a serious health risk for an estimated 150 million people worldwide. Around 200 million people living in ten countries (including India) of South and South-East Asia are exposed to arsenic through drinking water as well as by the airborne metalloid in the areas with coal burning and industrial emissions. The level of arsenic contamination in Asian countries is more severe than the rest of the world (Agusa et al. 2008; Asati et al. 2016). The permissible limit of As-concentration in potable water was set at 50 µg/L in India, and the same level was followed in Bangladesh and many other countries. The World Health Organization (WHO) recommends lowering this limit to 10 µg/L, certified by the Bureau of Indian Standards (2003). Although well-intentioned, the infrastructure available to test and/or monitor the aggregate in India and many other countries arsenic concentration in water in the range of 10–50 µg/L is acceptable (Compounds 2001, Acharyya 2005). Arsenic is a natural ubiquitous element and it occurs in several environmental segments as a result of natural and anthropogenic activities, while anthropogenic sources include mining activity, use of pesticides (insecticides, herbicides and rodenticides) and many types of wood preservatives which result in contamination of soil and other related substances (Baldissarelli et al. 2012, Aruljothi et al. 2013).

Natural water resources are continuously contaminating by heavy metals and their harmful impacts on various economically important aquatic animals like fishes and others. Although the level of contamination in natural ecosystems is usually well below the limit that can cause mortality in exposed animals, it may be sufficient to impair the normal functioning of tissues (Saleh et al. 2011, Mandour et al. 2012,

Pichhode & Nikhil 2015a, b). These types of contaminants or pollutants cause disturbances in various physiological and biochemical mechanisms of fish. Freshwater fish are the best sentinels for monitoring and determining the health status of an aquatic ecosystem. The initial toxic effect of any contaminant is only apparent at the cellular or tissue levels and then becomes prominent in morphological or behavioural changes (Pichhode et al. 2020). Various heavy metals, agrochemicals and industrial processes continuously release various wastes into natural freshwater sources and adversely affect soil and aquatic biota monitoring. Identification and management of these pollutants are important to minimize their adverse effects on aquatic ecosystems (Witeska et al. 2014).

Arsenic contamination and their toxicity have global effects and the major arsenic repositories are freshwater. Arsenic toxicity depends on several intercellular factors that make it difficult to estimate the effect. The use of aquatic algae is often advocated for bioremediation of arsenic-contaminated water because they collect arsenate and transform it into arsenite and methylated chemical species. Fish are another major key component of aquatic ecosystems (Magellan et al. 2014). The blood shows the early effect of toxicity of arsenic in fish. It enters in the blood predominantly through extensive gill surface area where the barrier between the blood and the metal salt is very thin (Kumar & Banerjee 2012, Pichhode & Gaherwal 2019b) as well as through buccal cavity. Over the past several decades, fishes have been widely used as a model organism to evaluate the effects of contaminated water. Very few researchers like Roy & Bhattacharya (2006), Singh (2007) have worked on the toxicity of arsenic in fish. The hardy nature of catfish, *Clarias batrachus* makes it an excellent bioindicator animal model for toxicological investigations.

Arsenic contaminated fish consumption could result in arsenic exposure to humans and lead to adverse effects on health (Kar et al. 2011). Arsenic could bring up a series of molecular events involved in oxidative stress, lipid metabolism disorder, iron homeostasis and carcinogenesis (Xu et al. 2013, Pichhode & Gaherwal 2020). Due to the effect of arsenic, there are many changes in the biochemistry, haematology and histology of catfish, *Clarias batrachus*. A decrease in haemoglobin concentration, erythrocyte counts, packed cell volume, monocyte, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), serum total proteins and blood glucose while increased values of total leukocytes lymphocyte, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were observed in exposed fish due to arsenic. The lower values of haemoglobin concentration (Hb) and erythrocyte counts in the study could be due to the inability of fish to carry the able amount of oxygen to hematopoietic tissue (Hussain et al. 2014, Pichhode & Gaherwal 2019c). In this investigation, arsenic contamination in Chhilpura pond water was evaluated and study of haematology and biochemistry of *Clarias batrachus* was conducted.

MATERIALS AND METHODS

Materials

The material for the present study comprised of blood was taken from catfish (*Clarias batrachus*) that were kept in the study area water sample and laboratory condition water sample (control) for a specific exposure time.

Study area: The present investigation site was a pond of Chhilpura village, Tehsil Baihar, District Balaghat, Madhya Pradesh, India. Balaghat district is located in the southern part of Jabalpur Division (Fig. 1). It occupies the southeastern portion of the Satpura Range and the upper valley of the Wainganga river. Chhilpura is located at Madhya Pradesh and Chhatisgarh state border in latitude 22º18'55'' and longitude 80º95'50''in the 121 kilometres north-east part away from the district headquarter according to Geological Survey of India, Toposheet No. 64 B/12 (Paul 1971) that comes in Supkhar forest reserve of Kanha National Park buffer zone and the village is surrounded by mountains and dense forests.



Fig. 1: Balaghat district position in India map and location of Chhilpura village, District Balaghat, Madhya Pradesh, India.

Experimental animal: The healthy catfishes, *Clarias batrachus* were used as an experimental animal and it was collected from the local fish market of Indore and acclimatized to the laboratory for one week during which they were regularly fed with prawn powder and soya meal.

Collection of blood sample: The blood collected by disposable syringe and needles from cardiac puncture of *Clarias batrachus* and kept in sterilized appropriate vials then processed for various haematological and biochemical analyses (Dacie & Lewis 1975).

Experimental design: In the present investigation experimental fishes were divided into two groups. Ten (10) fish were kept in the control group and exposed to normal water and forty (40) fishes were exposed to Chhilpura pond water sample.

Experimental duration: In both the control and experimental group fishes were exposed to a maximum of 96 hrs.

Experimental condition: The properties of Chhilpura pond water was tested before its use. The physicochemical conditions of sample water are given in Table 1.

Methods

Determination of arsenic in Chhilpura pond water sample: The water was collected from study site for analysis of contamination of arsenic by adopting the method of Feldmann (2009) and using arsenic test kit (WT025) CAS No. 387443, was taken from Himedia Laboratories Pvt. Ltd., Mumbai, India.

Table 1: Showing physicochemical conditions of Chhilpura pond sample water sample.

S. No.	Physicochemical Parameters	Range
01	Temperature (ºC)	30.8±0.5
02	pH	7.6±0.6
03	Dissolved oxygen (mg/L)	7.2±0.6
04	Chemical oxygen demand (mg/L)	14.7±0.5
05	Suspended solids (mg/L)	39.3±1.5
06	Chlorides (mg/L)	17.0±1.5
07	Hardness as $CaCO_3$ (mg/L)	173.0±0.8

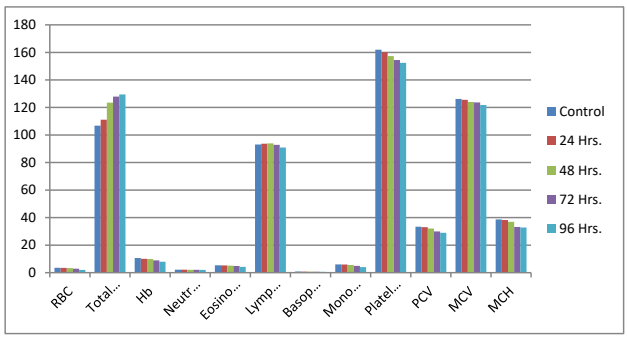


Fig. 2: Haematological changes in *Clarias batrachus* of Chhilpura pond water.

Haematological analysis: RBC, WBC, DLC, platelets, PCV, MCV and MCH were counted by Haemocytometer method (Sharma & Singh 2000). Haemoglobin concentrations were estimated by Sahil's method (Ratan 1978), differential leucocyte count by Leishman method (Rümke 1960).

Biochemical analysis: The activity of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were determined by adopting the method of Boyer (2000), total protein by biuret method (Harris 2003) and blood glucose level by Follin-wu method (Folin & Malmros 1929).

RESULTS

In the present investigation arsenic concentration in Chhilpura pond water was found to be 0.3 mg/L.

Haematological Estimation

In control group haematological values were, RBC (3.53 million/cmm), total WBC (106.80 × 10^3/cmm), Hb (10.64 g/dL), Neutrophils (2.18%), Eosinophils (5.32%), Lymphocytes (93.12%), Basophils (0.82%), Monocytes (6.00%), Platelets (162 cells/cmm), PCV (33.36%), MCV (126.20 fl) and MCH (38.64 pg) (Fig. 2).

In Chhilpura pond experimental group haematological values were at the 24 h RBC (3.44 million/cmm), total WBC (111.10 × 10^3/cmm), Hb (10.03 g/dL), Neutrophils (2.12%), Eosinophils (5.24%), Lymphocytes (93.71%), Basophils (0.80%), Monocytes (5.90%), Platletes (160.20 cells/cmm), PCV (33.08%), MCV (125.50 fl) and MCH (38.22 pg).

Moreover, in the present investigation at the 48 h haematological values were RBC (3.31 million/cmm), total WBC (123.50 × 10^3/cmm), Hb (9.90 g/dL), Neutrophils (2.08%), Eosinophils (5.10%), Lymphocytes (93.90%), Basophils (0.78%), Monocytes (5.55%), Platletes (157.32 cells/cmm), PCV (32.04%) , MCV (124.00 fl) and MCH (36.94 pg).

In the present experiment at the 72 h haematological values were RBC (2.90 million/cmm), total WBC (127.90 × 10^3/cmm), Hb (8.95 g/dL), Neutrophils (2.04%), Eosinophils (4.84%), Lymphocytes (92.84%), Basophils (0.73%), Monocytes (4.91%), Platelets (154.50 cells/cmm), PCV (29.98%), MCV (123.70 fl) and MCH (33.18 pg).

In the present investigation at the 96 h haematological values were RBC (2.00 million/cmm), total WBC (129.44 × 10^3/cmm), Hb (7.92 g/dL), Neutrophils (1.98%), Eosino-

phils (4.16%), Lymphocytes (90.90%), Basophils (0.61%), Monocytes (4.02%), Platelets (152.44 cells/cmm), PCV (28.93%), MCV (121.5 fl) and MCH (32.78 pg).

In the present investigation due to arsenic-contaminated water of Chhilpura pond (0.3 mg/L) RBC, Hb, Neutrophils, Eosinophils, Basophils, Monocytes, Platelets, PCV, MCV and MCH values were decreased as compared to control value at 24, 48, 72 and 96 h. Total WBC values were increased at 24, 48, 72 and 96 h interval. Lymphocytes were also increased at 24 and 48 h and then decreased at 72 and 96 h as compared to the control value.

Biochemical Estimation

The total protein concentration, SGOT activity, SGPT activity and blood glucose level of control fishes was 5.20 g/dL, 137.00 µmole/L, 42.42 µmole/L and 117.40 mg/dL respectively.

Total protein in blood (TP): The quantity of total protein in blood serum of experimental fishes was found decreased in Chhilpura pond water sample. The decreased total protein concentration in blood of experimental fishes after 96 h was 22.30 per cent. The decreased in protein value of blood after 24, 48 and 72 hrs. were 3.84, 4.23 and 11.53 per cent respectively (Fig. 3).

Serum glutamate oxaloacetate transaminase activity (SGOT): The SGOT activity was found increased in Chhilpura pond water sample. The increased SGOT activity after 24, 48, 72 and 96 h were 0.87, 3.13, 3.43 and 4.52 per cent respectively (Fig. 4).

Serum glutamate pyruvate transaminase activity (SGPT): The SGPT activity was found increased in Chhilpura pond water sample. The increased SGPT activity after 24, 48, 72 and 96 h was 1.13, 3.37, 3.91 and 5.65 per cent respectively (Fig. 5).

Total blood glucose: The blood glucose level was found to be decreased in Chhilpura pond water samples. The decreased levels of blood glucose after 24, 48, 72 and 96 h were 0.68, 3.74, 4.77 and 11.24 per cent respectively (Fig. 6).

In the present investigation due to arsenic-contaminated water of Chhilpura pond (0.3 mg/L) total protein and blood glucose level was decreased as compared to control at 24, 48, 72 and 96 h and serum glutamate oxaloacetate transaminase activity (SGOT) and serum glutamate pyruvate transaminase activity (SGPT) was increased as compared to control value at 24, 48, 72 and 96 h.

DISCUSSION

Heavy metals, such as arsenic, are one of the ten chemicals of WHO's major public health concerns. The work of the World Health Organization (WHO) to reduce and eliminate arsenic risk involves establishing guideline importance, reviewing evidence, and providing risk management recommendations. The WHO publishes a guideline value for arsenic in its guidelines for drinking water quality. The Guidelines are intended to be used as the basis for worldwide regulation and standard setting. The arsenic contents in groundwater of different countries are varied. The maximum permissible concentration of arsenic in drinking water is 50 µg/L and the recommended value is 10 µg/L (UNEPA 1975, WHO 2001).

Haematological profiles of fish are widely applied and studied to monitor environmental pollution and contaminants in aquatic ecosystems because haematological profiles are indicative of the stress and physiological status of animals (Adhikari et al. 2004). In fish, haematological parameters are commonly and frequently used to measure the toxicological effect as well as functional status of aquatic organisms by using blood (Thrall 2004). The haematological parameters include RBC, WBC, PCV, haemoglobin and other

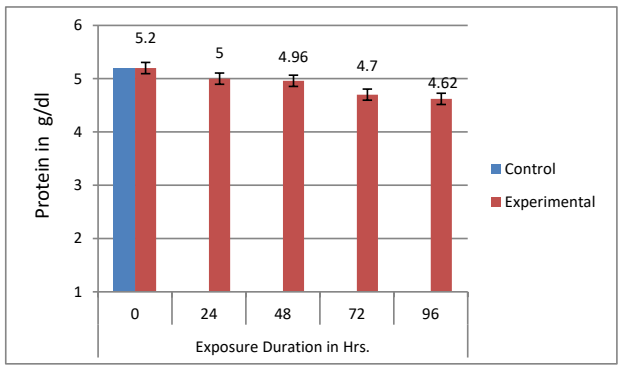


Fig. 3: Total protein in the blood of *C. batrachus* exposed to Chhilpura pond water sample (0.3 mg/L) for different durations.

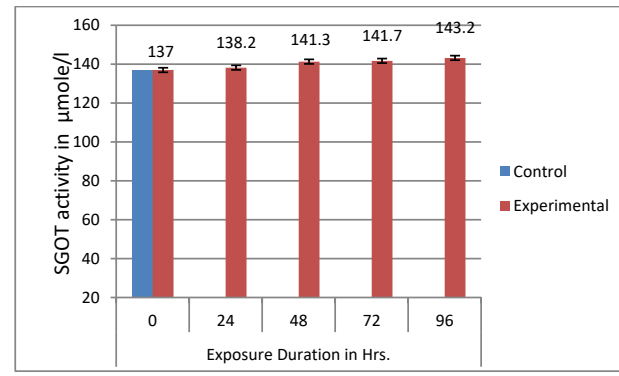


Fig. 4: SGOT activity of *C. batrachus* exposed to Chhilpura pond water sample (0.3 mg/L) for different durations.

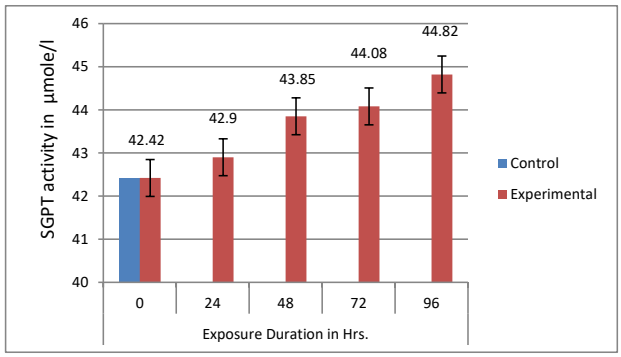


Fig. 5: SGPT activity of *C. batrachus* exposed to Chhilpura pond water sample (0.3 mg/L) for different durations.

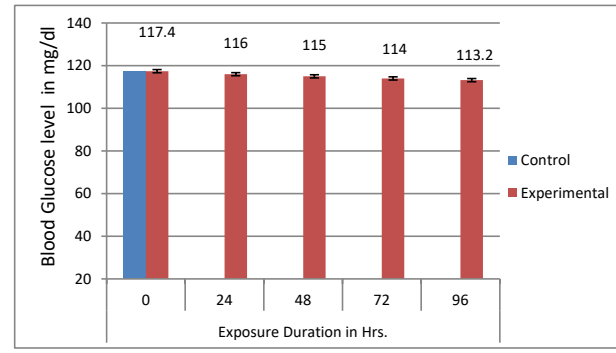


Fig. 6: Blood glucose level in *C. batrachus* exposed to Chhilpura pond water sample (0.3 mg/L) for different durations.

haematological indices like MCV, MCH and platelets are generally used to evaluate the health status of fish (Nussey et al. 1995). The quality of water can be affected by the presence of toxins in aquatic media, which affects the value of the haematological parameters of fish due to their proximity to the external environment. Reduction of RBC, haemoglobin and PCV content in fish at arsenic risk may be due to disorder in hematopoietic processes, accelerated dissolution of RBC (Svobodova et al. 1997, Pichhode & Gaherwal 2019d). The accumulation of arsenic in the gill region leads to haemolysis which can also be exerted at low levels of RBC. Cockel et al. (1991) found that fish treated with sub-chronic doses of arsenic represented low haemoglobin level resulting in anaemic behaviour. Arjun et al. (2002) reported that lack in the number of RBC may be caused by the interruption in erythropoiesis or by the destruction of red cells. They also reported that these changes may be due to anaemic condition and haemolysis caused by heavy metals. Another reason for the decreased amount of haemoglobin and RBC might be due to hypochromic microcytic anaemia which is contributed to the lack of iron or its decreased utilization for erythrogenesis (Natarajan 1981). A significant decrease in erythrocyte RBC counts, Haemoglobin and an increase in WBC in *C. punctatus* can be related to pollution due to the effluents (Rao & Hymavathi 2000).

Goel & Sharma (1987) reported some haemato-chemical characters of *Clarias batrachus* under arsenic metallic stress. It decreased total RBC, Hb% and PCV in *Clarias batrachus* from a sub-acute injection of 15 doses of 1000 µg of disodium ortho arsenate indicating an anaemic condition. However, total WBC increased.

The fish were observed for the study of behavioural and/or physical changes. Blood sample (1.50 mL) was taken from the caudal vein of each fish at 3rd, 6th and 9th days of the experiment respectively by using with and without anticoagulant (Ethylene diamine tetra acetic acid, 1 mg/mL). Haematological parameters such as leucocyte counting, haemoglobin concentration, erythrocyte counting, pack cell volume, erythrocyte indices like mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, lymphocyte and monocyte were determined (Ghaffar et al. 2014, Pichhode & Gaherwal 2019c).

Ghaffar et al. (2016) brief the report of fish to different concentrations of arsenic showed the mixed type of impacts on different evaluated points. The combined exposure with higher concentrations significantly decreased haemoglobin concentration, erythrocyte count, corpuscular haemoglobin contents, lymphocyte, monocyte count and packed cell volume. Whereas, the leucocyte count and mean corpuscular volume were increased in respective days in fish as compared to those in the control group.

The blood parameters and haematopoietic system are known to be the best biomarkers to monitor the pathological and physiological observation of organisms exposed to various toxicants (Kousar & Javed 2015). Furthermore, an increased degree of mean corpuscular volume can be related to an increased number of immature erythrocytes suggestive of anaemia. Decreased degree of mean corpuscular haemoglobin concentration reported as an inability of the hematopoietic system to produce haemoglobin, with inflammation in the erythrocytes.

Kumar et al. (2015) explained the impacts of the various doses of arsenic salt sodium meta-arsenite (SMA) on the blood parameters of catfish, *Clarias batrachus*. Mortality rate increased with increasing concentrations of arsenic salt. All the blood parameters (leucocytes count, erythrocytes, haematocrit and haemoglobin concentration) decreased with increasing concentration of toxicant and become lower at higher concentration when compared with the control. The available haematological indices of mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC)

were equally lowered. In conclusion, the changes observed indicate that haematological parameters can be referred to as an indicator of arsenic-related stress in fish on discovering the arsenic levels. Jain & Ali (2000) reported that the impact of arsenic induces significant alteration in haematological parameters. RBC count decreased in the exposure level, whereas, WBC count was increased at all concentration of dose-dependent arsenic treatment. The concentration of arsenic in natural water bodies mainly depends on the degree of pollution and geochemical composition. In the present study, RBC, haemoglobin, PCV, MCV, MCH and platelets contents were decreased and increased in the number of WBC and lymphocytes due to effect of arsenic in the Chhilpura pond water (0.3 mg/L).

The decrease in the protein content of brain, gill, intestine, kidney, liver, blood and muscle has been observed in the fish exposed to nickel (Thatheyus et al. 1992). The reduction in the protein level of liver, kidney and blood has also been observed in the fish *Labeo rohita* exposed to pesticides due to intensive proteolysis during stress conditions (Rajamannar & Manohar 1998). Jana & Bondyopadhyay (1987) have reported such a reduction in protein content when the fish *Channa punctatus* was exposed to heavy metals such as mercury, arsenic and lead. A decrease in protein content also affects the other biochemical parameter. Depletion in tissue protein in *H. fossilis* due to copper and arsenic toxicity may be attributed to either rapid utilization of body protein or poor intake of dietary protein by fish, which support to the view of Syverson (1981) who opined that the heavy metal, in general, interferes with protein synthesis. Humtsoe et al. (2007) explained serum ALT and AST are good indicators of the overall health status of an individual especially hepatocyte injury and related stress. The increased levels of aspartate amino transferase (AST) and alanine amino transferase (ALT) enzymes also suggestive of adverse effects of arsenic and urea on the hepatocytes and abnormal cellular metabolism leading to cell death (Sharma et al. 2007, Haque & Roy 2012). Previous studies demonstrated that basal levels of glucose varied in ecologically-distinct species, in part influenced by environmental and non-environmental factors such as feeding habits and life mode of the fish. It is reported that glucose in blood serum is the best indicator of stress in fish (Percin & Konayalioglu 2008, Kumari et al. 2017).

The carbohydrate stored as reserve fuel in the liver and muscle tissue of fish and provides energy during acute and chronic stress (Soni et al. 2018). Glucose is referred to as an instant and immediate source of energy in stressful condition. In blood plasma reduction in carbohydrate content of the exposed fish in present investigation seems to be due to rapid utilization of blood glucose and depletion of stored glycogen to provide energy for catfish under toxicity of arsenic. Glycogen is the main reserve source of energy for animals during metabolism and their content in the tissue of fish exposed to chemical substance may indicate the health condition of the fish (Ananth et al. 2014). Glycogen plays an important role in carbohydrate metabolism. The unbalancing in glycogen profile was referred to as one of the most outstanding lesions to the biological system due to the mode of heavy metals (DeBruin 1976). The lower and higher concentrations of arsenic trioxide exposed the decreasing glycogen level of the liver and kidney tissues in fish *Labeo rohita* exhibits during the 7, 14, 21 and 28 days of exposure. The glycogen level in different tissues was less in control fish and treated fish. The significant reduction in plasma glucose levels might be due to a hypoxic situation caused by arsenic, due to an excess uses of stored carbohydrates during acute treatment (Kumari et al. 2015).

In the present investigation total protein and blood glucose level was decreased as compared to control value at 24 hours interval and Serum glutamate oxaloacetate transaminase activity (SGOT) and Serum glutamate pyruvate transaminase activity (SGPT) was increased as compared to control value at 24 hours interval due to effect of arsenic which present in Chhilpura pond water (0.3 mg/L).

CONCLUSION

Finally, we conclude that apart from geographical changes, industrialization and urbanization increase the heavy metal toxicity which causes harm to all types of organisms, especially those that live aquatic life. Similarly, arsenic has also become a worldwide problem, the standard amount of which has been set by WHO. In the present experiment, it was also seen that in such water source which is surrounded by mountains, the water flowing through the mountains in the rainy season also increases the amount of heavy metals. In future, the amount of such source can increase even more. In the present experiment, it was seen that the use of this water led to many changes in the haematology and biochemistry of *Clarias batrachus*, the number of RBC, haemoglobin, PCV, MCV, MCH, platelets, glucose and protein contents started decreasing as well as the number of WBC, lymphocyte cell, SGOT and SGPT were increased. From this, it can be concluded that arsenic changes the haematology and biochemistry of catfish (*Clarias batrachus*).

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REFERENCES

Acharyya, S. K. 2005. Arsenic levels in groundwater from Quaternary alluvium in the Ganga Plain and the Bengal Basin, Indian subcontinent: insights into influence of stratigraphy. Gondwana Research, 8 (1): 55-66.

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C. T. and Ayyappan, S. 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicology and Environmental Safety, 58(2): 220-226.

Agusa, T., Takagi, K., Kubota, R., Anan, Y., Iwata, H. and Tanabe, S. 2008. Specific accumulation of arsenic compounds in green turtles (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) from Ishigaki Island, Japan. Environmental Pollution, 153 (1): 127-136.

Ananth, S., Mathivanan, V., Aravinth, S. and Sangeetha, V. 2014. Impact of arsenic metal toxicant on biochemical changes in the grass carp, *Ctenopharyngodon idella*. Int. J. Mod. Res. Rev, 2: 74-78.

Arjun, J., Das, M., Dkhar, P. S., Dey, S. and Das, M. K. 2002. Role of vitamin C pre-treatment in reducing the blood lead level and lead-induced toxic effect in erythrocyte cell membrane. Proceedings-National Academy of Sciences India Section B, 72 (3/4): 257-266.

Aruljothi, B. 2013. Effect of arsenic on bioenzymological studies in fresh water fish, *Labeo rohita*. Int. J. Modern Res. and Reviews, 1(1): 13-19.

Asati, A., Pichhode, M. and Nikhil, K. 2016. Effect of heavy metals on plants: an overview. Int. J. Appl. Innov. Eng. Manage, 5: 2319-4847.

Baldissarelli, L. A., Capiotti, K. M., Bogo, M. R., Ghisleni, G. and Bonan, C. D. 2012. Arsenic alters behavioral parameters and brain ectonucleotidases activities in zebrafish (*Danio rerio*). Comparative Biochemistry and Physiology Part C: Toxicology Pharmacology, 155(4): 566-572.

Boyer, R. 2000. Modern Experimental Biochemistry, 3rd ed.; Addison Wesley Longman, Inc.: California.

Bureau of Indian Standards. (22nd ed.) 2003. Indian Standard: Drinking Water Specifications. New Delhi.

Cockell, K.A., Hilton, J.W. and Bettger, W.J. 1991. Chronic toxicity of dietary disodium arsenate heptahydrate to juvenile rainbow trout (*Oncorhynchus mykiss*). Archives of Environmental Contamination And Toxicology, 21(4): 518-527.

Compounds, W.A. 2001. Environmental Health Criteria 224. World Health Organisation, Geneva.

Dacie, V. and Lewis, S. M. 1975. In: Practical Haematology, ELBS, Longman, Singapore, publishers.

DeBruin, A. 1976. Blood coagulation disorders. In: Biochemical Toxicology of Environmental Agents, Elsevier/North-Holland Biomedical Press Amsterdam, 1493-1481.

DeForest, D. K., Brix, K. V. and Adams, W. J. 2007. Assessing metal bioaccumulation in aquatic environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. Aquatic Toxicology, 84 (2): 236-246.

Feldmann, J. 2009. Onsite testing for arsenic: Field test kits. In: Reviews of Environmental Contamination, 197: 61-75. Springer, New York, NY.

Folin, O. and Malmros, H. 1929. Blood glucose determination method. J. Biol. Chem., 89: 121.

Ghaffar, A., Ashraf, S., Hussain, R., Hussain, T., Shafique, M., Noreen, S. and Aslam, S. 2014. Clinicohematological disparities induced by triazophos (organophosphate) in Japanese Quail. Pak. Vet. J., 34: 257-259.

Ghaffar, A., Hussain, R., Aslam, M., Abbas, G. and Khan, A. 2016. Arsenic and urea in combination alters the hematology, biochemistry and protoplasm in exposed Rahu Fish (*Labeo rohita*) (Hamilton, 1822). Turkish Journal of Fisheries and Aquatic Sciences, 16 (2): 289-296.

Goel, K. A. and Sharma, S. D. 1987. Some haematochemical characteristics of *Clarias batrachus* under metallic stress of arsenic. Comparative Physiology and Ecology, 12 (2): 63-66.

Haque, S. and Roy, S. K. 2012. Acute effects of arsenic on the regulation of metabolic activities in liver of fresh water fishes (*Taki*) during cold acclimation. Jordan Journal of Biological Sciences, 147 (618): 1-7.

Harris, D. 2003. Quantitative Chemical Analyses, 6th ed.; W. H. Freeman and Company: USA.

Humtsoe, N., Davoodi, R., Kulkarni, B. G. and Chavan, B. 2007. Effect of arsenic on the enzymes of the rohu carp *Labeo rohita* (Hamilton 1822). Raffles Bull. Zool., 14: 17-19.

Hussain, R., Khan, A., Mahmood, F., Rehan, S. and Ali, F. 2014. Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (*Coturnix japonica*). Pesticide Biochemistry and Physiology, 109: 58-63.

Jain, C.K. and Ali, I. 2000. Arsenic: occurrence, toxicity and speciation techniques. Water Research, 34 (17): 4304-4312.

Jana, S. R. and Bandyopadhyaya, N. 1987. Effect of heavy metals on some biochemical parameters in the freshwater fish *Channa punctatus*. Environment and Ecology, 5 (3): 488-493.

Kamaludeen, S.P.B.K., Arunkumar, K.R., Avudainayagam, S. and Ramasamy, K. 2003. Bioremediation of chromium contaminated environments. Indian Journal of Experimental Biology, 41: 972-985.

Kar, S., Maity, J. P., Jean, J. S., Liu, C. C., Liu, C. W., Bundschuh, J. and Lu, H. Y. 2011. Health risks for human intake of aquacultural fish: Arsenic bioaccumulation and contamination. Journal of Environmental Science and Health, Part A, 46 (11): 1266-1273.

Kousar, S. and Javed, M. 2015. Studies on induction of nuclear abnormalities in peripheral blood erythrocytes of fish exposed to copper. Turkish Journal of Fisheries and Aquatic Sciences, 15 (4): 879-886.

Kumar, R. and Banerjee, T. K. 2012. Impact of sodium arsenite on certain biomolecules of nutritional importance of the edible components of the economically important catfish *C. batrachus* (Linn.). Ecology of Food and Nutrition, 51 (2): 114-127.

Kumar, R., Mishra, S. and Banerjee, T. K. 2015. Studies of dose dependent effects of sodium meta-arsenite on the blood parameters of nutritionally important catfish *C. batrachus* (Linn.). International Journal of Environment and Natural Sciences, 7: 7-12.

Kumari, B., Kumar, V. and Ahsan, J. 2015. Effect of arsenic on glucose and glycogen content in a walking catfish (*Clarias batrachus*). In: Conference Proceeding: World Aquaculture.

Kumari, B., Kumar, V., Sinha, A. K., Ahsan, J., Ghosh, A. K., Wang, H. and DeBoeck, G. 2017. Toxicology of arsenic in fish and aquatic systems. Environmental Chemistry Letters, 15 (1): 43-64.

Magellan, K., Barral-Fraga, L., Rovira, M., Srean, P., Urrea, G., García-Berthou, E., and Guasch, H. 2014. Behavioural and physical effects of arsenic exposure in fish are aggravated by aquatic algae. Aquatic Toxicology, 156: 116-124.

Mandour, M. A., Al-Shami, S. A. and Hssein, Y. A. 2012. Effect of feeding graded levels of urea on growing New Zealand White rabbit performance. Global Vet., 9: 761-8.

Natarajan, G. M. 1981. Changes in the biomodal gas exchange and some blood parameters in the air-breathing fish, *Channa striatus* (Bleeker) following lethal (Lc 50/48 hrs.) exposure to metasystoc (Demeton). Current Science, 40-41.

Nussey, G., Van Vuren, J. H. J. and Du Preez, H. H. 1995. Effect of copper on blood coagulation of *Oreochromis mossambicus* (Cichlidae). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 111 (3): 359-367.

Paul, D.K., 1971. Government of India, Geological Survey of India.

Percin, F. and Konyalioglu, S. 2008. Serum biochemical profiles of captive and wild northern bluefin tuna (*Thunnus thynnus* L. 1758) in the Eastern Mediterranean. Aquaculture Research, 39 (9): 945-953.

Pichhode, M. and Gaherwal, S. 2019a. Biochemical response of heavy metal, sodium arsenate exposure in catfish, *Clarias batrachus*. International Journal of Current Advanced Research, 8 (08), 19676-19678. DOI: http://dx.doi.org/10.24327/ijcar.2019.19678.3809

Pichhode, M. and Gaherwal, S. 2019b. Toxic effect of arsenic trioxide on biochemical response in catfish, *Clarias batrachus*. Int. J. Recent Sci. Res., 10 (08): 34033-34036. DOI: http://dx.doi.org/10.24327/ijrsr.2019.1008.3804

Pichhode, M. and Gaherwal, S. 2019c. Toxicological effects of arsenic trioxide exposure on haematolical profile in catfish, *Clarias batrachus*. International Journal of Current Research and Review, 11(17): 09-12. DOI: http://dx.doi.org/10.31782/IJCRR.2019.11163

Pichhode, M. and Gaherwal, S. 2019d. Study of sodium arsenate induced haematological changes in catfish, *Clarias batrachus*. Pranama Res. J., 9 (8): 163-169.

Pichhode, M. and Gaherwal, S. 2020. Effect of heavy metal toxicity, arsenic trioxide on the biochemical parameter of fresh water fish, *Clarias batrachus*. Pollution Research, 39: 123-125.

Pichhode, M. and Nikhil, K. 2015b. Effect of copper mining dust on the soil and vegetation in India: a critical review. International Journal of Modern Sciences and Engineering Technology (IJMSET), 2 (2): 73-76.

Pichhode, M., Gaur, P., Khan, H. R., Dudwe, J. and Gaherwal, S. 2020. Histological alteration caused by arsenic trioxide in catfish, *Clarias batrachus*. Journal of Xidian University, 14 (3): 124-137.

Pichhode, M. and Nikhil, K. 2015a. Effect of copper dust on photosynthesis pigments concentration in plants species. International Journal of Engineering Research and Management (IJERM), 2 (2): 63-66.

Prakash, M. M., Gaherwal, S., Soni, R. and Nageshwar, W. 2014. Cypermethrin induced biochemical changes in kidney of *Clarias batrachus*. World Applied Sciences Journal, 32 (1): 18-21.

Rajamannar, K. and Manohar, L. 1998. Sublethal toxicity of certain pesticides on carbohydrates, proteins and amino acids in *Labeo rohita*. Journal of Ecobiology, 10 (3): 185-191.

Rao, L. M. and Hymavathi, V. 2000. Effect of slaughter house pollution on the haematological characters of *Channa punctata*. Pollution Research, 19 (2): 195-198.

Ratan, V. 1978. Hb% Estimation by Sahil's Method in Handbook of Human Physiology, 3rd edn.

Roy, S. and Bhattacharya, S. 2006. Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of *Channa punctatus*. Ecotoxicology and Environmental Safety, 65 (2): 218-229.

Rümke, C. L. 1960. Laboratory aids. Variability of results in differential cell counts on blood smears. Triangle; The Sandoz Journal of Medical Science, 4: 154.

Saleh, S. Y., Abdl-Rahman, M. A., Amal, A. Z. and Safaa, S. A. E. H. 2011. Growth performance, cecal fermentation and blood biochemistry of rabbits fed diet supplemented with urea-bentonite combination. Journal of Agricultural Science, 3 (1): 14.

Sharma, A., Sharma, M. K. and Kumar, M. 2007. Protective effect of Mentha piperita against arsenic induced toxicity in liver of Swiss Albino mice. Basic Clinical Pharmacology Toxicology, 100 (4): 249-257.

Sharma, I. J. and Singh, H. S. 2000. Students Laboratory Manuals of Veterinary Physiology, 1st edn. Kalyani Publisher, New Delhi, India.

Singh, A. K. 2007. Toxicopathological effect of the arsenic salt, sodium arsenate on the respiratory organs of the walking catfish *Clarias batrachus* (L.). Unpublished Ph. D. Dissertation, Banaras Hindu University, Varanasi, India.

Soni, R., Gaherwal, S. and Shiv, G. 2018. Effect of herbicide 2, 4-D on hematological parameters of *Clarias batrachus*. International Journal of Current Research in Life Sciences, 7 (07): 2441-2444.

Svobodova, Z., Groch, L., Flajšhans, M., Vykusova, B. and Machova, J. 1997. Effect of long-term therapeutic bath in malachite green on common carp (*Cyprinus carpio* L.). Acta Veterinaria Brno., 66 (2): 111-116.

Syverson, T. L. M. 1981. Effects of methyl mercury on protein synthesis in vitro. Acta. Pharmacol. et. Toxicol., 49: 422-426.

Thatheyus, A. J., Selvanayagam, M. and Raja, S. S. 1992. Toxicity of nickel on protein content in tissues of *Cyprinus carpio communis* (Linn). Indian Journal of Environmental Health, 34 (3): 236-238.

Thrall, M. A. 2004. Veterinary Haematology and Clinical Chemistry, Vol. 19. Williams and Wilkins cap, Philadelphia, PA, USA, 277-289.

United Nation Environmental Protection Agency, 1975. Interim Primary Drinking Water Standards, Fed. Reg. 40, (11): 990.

WHO, 2001. Arsenic Compounds, Environmental Health Criteria 224, 2nd ed., World Health Organisation, Geneva.

Witeska, M., Sarnowski, P., Ługowska, K. and Kowal, E. 2014. The effects of cadmium and copper on embryonic and larval development of ide *Leuciscus idus* L. Fish Physiology and Biochemistry, 40 (1): 151-163.

Xu, H., Lam, S. H., Shen, Y. and Gong, Z. 2013. Genome-wide identification of molecular pathways and biomarkers in response to arsenic exposure in zebra fish liver. PloS One, 8(7): e68737.

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A New Index Contributing to an Early Warning System for Cyanobacterial Bloom Occurrence in Atlantic Canada Lakes

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ABSTRACT

Cyanobacterial harmful algal blooms (cyanoHAB) have become more frequent and prominent in Atlantic Canada freshwater bodies over the last several years, especially in Nova Scotia (NS). Inspired by the trophic index of Vollenweider, a new index was developed with modification and adaptation for freshwater systems. Our model TRINDEX shows the effectiveness of estimation for the variation of cyanobacterial dominance in phytoplankton communities. TRINDEX can assist in determining the threshold for cyanobacterial bloom onset. Combinations of nutrients and pigments under TRINDEX were tested by a binary discrimination test to find the optimal range of threshold for cyanoHAB formation in freshwater lakes.

INTRODUCTION

Many drinking waters and recreational reservoirs around the globe suffer from cyanobacterial HABs (cyanoHABs) and the used restrictions have caused negative social and economic impacts on local communities and businesses. The desire to protect valuable freshwater and marine resources have motivated extensive research on methods for predicting and mitigating algal blooms. Mitigation strategies for HABs have been divided into two categories, namely precautionary prevention (or early warning protocols) and bloom controls (Kim 2006). Precautionary prevention refers to monitoring and predicting events while bloom control involves both direct controls applied after an HAB has begun and indirect controls deal with strategies, such as management of land-derived nutrient inputs (Kim 2006).

The wish to predict the algal bloom occurrence and proliferation under a complex environmental situation has led to developing many indices for the estimation of eutrophication. There is a real need for improving the knowledge of the eco-physiological mechanisms leading to cyanoHABs; but this cannot be achieved only through reliance on the bulk indices (chlorophyll-a (chl-a), temperature, nutrients).

Most research on eutrophication are based on chemical components, i.e. nutrient characteristic of water bodies such as total phosphorus (TP) and its mineral part (PO_4-P, the dissolved and bioavailable form of phosphate easily consumed by algae) and dissolved inorganic nitrogen (DIN), which contribute significantly to algal growth. All single indices or combined parameters (Novotny & Olem 1994, Bartram et al. 1999, Brient et al. 2008, Brylinsky 2009, Chorus 2012, Ndong et al. 2014, Ahn et al. 2017), despite their usefulness, can show only the real-time conditions and do not predict adequately the cyanobacterial growth in mass and the situation of bloom occurrence as well as the thresholds of bloom onset. They were primarily developed for the determination of trophic status only. There were a few studies which focused on the bloom forecasting using simulations (Anderson et al. 2016). However, almost no work has been done to determine the thresholds that can predict exactly the HAB occurrence in freshwater ecosystems, except for the model from Downing et al. (2001) which provided statistical analysis for predicting the risk of cyanobacterial dominance.

A warning system is an essential tool, from our perspective, which should be able to adequately foresee the irregular patterns such as massive blooms and contribute to water management and decision making. Nowadays, some modern approaches use sophisticated tools and devices including remote sensing, imaging process, etc. to observe and predict blooms. However, two main issues have persisted: (1) They are costly (especially for computational cost) and are used primarily for long-term forecasting (Anderson et

al. 2016); (2) The biophysical coupling effects involving bloom occurrences and the distinction between cyanoHAB and other algal blooms were not satisfactorily considered (Kudela et al. 2015, Anderson et al. 2016).

In this paper, a new index is developed to forecast the potential bloom occurrence in freshwater bodies and to assess the freshwater quality relating to the cyanoHAB presence. Our goal is to determine the bloom threshold based on the nutrient level combined with algal pigments and then estimate the appearance of bloom patterns. Specifically, the three following objectives were addressed: (1) To develop a new index, the Threshold Index (or colloquial TRINDEX) for cyanoHAB onset prediction; (2) To validate the TRINDEX using field data from two Nova Scotian lakes, Mattatall (Colchester and Cumberland counties) and Torment (Kings County). Determining the threshold TRINDEX for bloom prediction is assessed by binary discrimination test (Receiver Operating Characteristic (ROC) analyses); (3) To suggest a practical scheme for bloom prediction based on TRINDEX definition with the expectation that our approach can be applied at a larger scale for different trophic waterbodies where blooms could happen.

MATERIALS AND METHODS

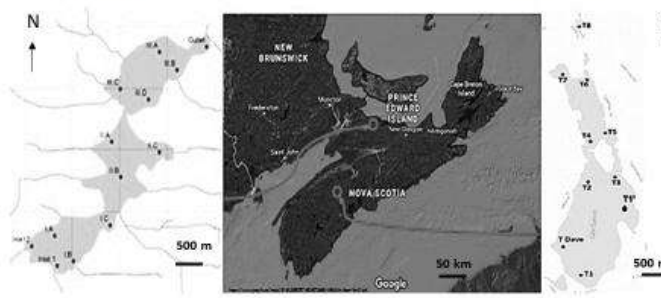
Study Sites

Two sites in the province of Nova Scotia (NS) Canada, were our targets. Mattatall lake (ML), between Colchester and Cumberland counties, was served as the main site; and lake Torment LT (Kings County) was used for independent verification. The datasets collected from both lakes are independent as LT is located over 200 km from ML in a different geographic area. Both locations are shown in Fig. 1 with their information in Table 1.

ML is mainly spring-fed with some brooks. In terms of human activities, there are blueberry fields and forestry on the west side of the lake. There are approximately 60 residences (both seasonal and year-round) with varying lot sizes and ages. With the data from three years (2015-2017), ML showed a moderately eutrophic level based on chlorophyll-a and TP measurements and contained potentially toxic cyanobacterial species (Hushchyna & Nguyen-Quang 2017). There was a bloom of green algae (*Mougeotia* sp.) in the middle of summer (2015, 2016) following by a cyanobacterial bloom of *Dolichospermum planctonicum* in late summer-autumn.

LT is in East Dalhousie, Kings County. The lake is used for residential and recreational purposes. It covers 261 hectares. There are 250 cottages and homes around the lake. It is surrounded by a forest with no significant agricultural activity on the watershed. The lake is dystrophic with brown water (colour changes 70-145 mg.L^{-1} Pt), low pH (5-6), and high organic content (DOC was 6.5-8.5 mg.L^{-1}) (Marty & Reardon 2016, Nguyen-Quang et al. 2017). The frequency of HAB has increased every year, i.e. since summer 2016. The cyanobacterial blooms were dominated by other cyano-

Location	N-Coordinate	W-Coordinate
IA	45.683914	-63.483759
IB	45.681845	-63.480922
IC	45.684767	-63.475384
IIA	45.691568	-63.475148
IIB	45.688689	-63.47378
IIC	45.690711	-63.467932
IIIA	45.698875	-63.467907
IIIB	45.697408	-63.46526
IIIC	45.695859	-63.473843
IIID	45.695011	-63.469584
Inlet 1	45.681467	-63.483369
Inlet 2	45.683072	-63.487229
Outlet	45.699303	-63.460799



Location	N-Coordinate	W-Coordinate
T1	44.71798	-064.74467
T1'	44.72619	-064.73376
T2	44.72710	-064.74387
T3	44.72715	-064.73530
T4	44.73933	-064.74036
T5	44.74369	-064.73926
T6	44.74960	-064.74142
T7	44.75008	-064.74615
T8	44.75116	-064.74110
T Dave	44.72186	-064.74819

Fig. 1: The location of Mattatall lake (left) and Torment lake (right).

Table 1: Background information about the studied lakes.

Lake	Elevation, m	Surface area, ha	Length, km	Max depth, m
Mattatall	49	120	5	9.3
Torment	172	261	4	6.5

bacteria species (*Dolichospermum flos-aquae*) in LT and appeared randomly from June to November (Nguyen-Quang et al. 2017).

Field Sampling Process and Lab Analysis

Samples were taken bi-weekly or every month, depending on the weather conditions, starting in May through to November, at the surface and bottom levels. Sampling locations are presented in Fig. 1. DO was measured by YSI probe (Professional Plus, Hoskin Scientific LTD, USA). The data on phosphate (PO_4), nitrate (NO_3), chlorophyll-a (chl-a) and phycocyanin (PC) were analysed by our Laboratory.

To determine the concentration of pigments, water samples were filtered through the GF/A Whatman filters. The filters were extracted after that in 90% acetone for chlorophyll-a or in phosphate buffer saline for phycocyanin, then sonicated (50% amplitude for 30 seconds) and centrifuged twice (first centrifugation at room temperature with 3500 *g* for 10 mins; second centrifugation at 4ºC, 13000 *g* for 1.5 hours). The pigment concentrations (chl-a and PC) were measured in $\mu g.L^{-1}$ unit by using the Turner 10AU Fluorometer (Turner Designs, USA) based on the calibration standard curve for both pigments. A dissolved fraction of phosphate and nitrate were measured after filtration through GF-A filters by a photometer using a tablet reagent system (YSI 2010).

Mathematical Formulations

We believe the TRIX concept suggested by Vollenweider et al. (1998) for coastal marine zones is reasonable to be employed for freshwater resources, as it uses the combination of key biological and hydrochemical parameters in a logarithmic relationship without specific characteristics of the marine environment. However, this conception was not well known in freshwater literature. To deal with the non-normal distribution of most of the environmental data, the logarithmic transformation is an appropriate way to 'transform' random data into the normal distribution form. Inspired by the logarithmic transformation of Vollenweider et al. (1998), we suggest herein our Threshold Index (hereafter named TRINDEX) formula as follows.

$$\text{TRINDEX} = \frac{k}{n}\sum_{1}^{i=n}\left[\frac{(logM_i - logL_i)}{(logU_i - logL_{i)}}\right], \quad \ldots(1)$$

Where,

TRINDEX – Threshold Index to be considered

M_i – measured parameter *i*

L_i – lower limit (concentration) of the considered parameter *i*

U_i – upper limit (concentration) of the considered parameter *i*

k – factor standing for the maximum value of considered range (0,10), so k=10 by default

n – total of parameters M_i we expect to consider

It is our view that chl-a is not a perfect parameter to represent the cyanobacterial bloom detection, because chl-a can be produced by all algal species including microalgae. We propose that the pigment PC, therefore, needs to be introduced into the index as an alternative parameter to reflect the cyanobacterial presence in all phytoplankton communities. There will be hence two scenarios of TRINDEX to be considered by our study.

Scenario 1: Four parameters M_i: PC, D%O, NO_3 and PO_4 will be used (*n = 4)* …(2)

Scenario 2: Five parameters M_i: Chl-a, PC, D%O, NO_3 and PO_4 will be used (*n = 5)* …(3)

The absolute deviation of oxygen from 100% (D%O) shows the main processes of phytoplankton growth which can be used for the detection of bloom onset; nitrogen and phosphorus were chosen in the form of nitrate (NO_3) and phosphate (PO_4) as the main sources of nutrients for cyanobacteria growth. These components can be easily measured daily. The DO fluctuation could be high depending on each period of the day in eutrophic waters. Our observations on various Nova Scotian mesotrophic lakes showed that the period between 8 AM to 2 PM was the optimal time for the development and accumulation of phytoplankton. This period was, therefore, suggested to be used in our monitoring purposes.

The quantity ($logU_i$- $logL_i$) is defined by the difference between upper and lower limits. When these limits are determined, all values being out of this range should be excluded. Therefore, to have an appropriate range to cover different trophic conditions, we used limits of detection (LOD) as the lower limit and maximum value obtained in measurements of the considered variable for the upper limit.

Data Analysis and Discrimination Test for the Thresholds

Definition of onset of blooms: The onset of a bloom can be defined as the start or beginning of any visible signs of blooms, i.e. the first visible appearance of signs or symptoms of some surface scums of a waterbody. However, our definition of bloom onset herein is not only associated with visible signs of algal appearance, but also with scenarios where there are no visible algal signs (but certain amounts of phycocyanin present). Therefore, we suggest that when PC concentration is over 0.03 $mg.L^{-1} \pm 0.002$, these cases can be considered as onset of bloom (PC criteria based on Brient et al. 2008), equivalent to the cell count 20,000 cells per mL of cyanobacteria.

The onset could be a visible bloom or scum situation, but this may not be stable. The surface bloom at onset status can be observed appearing and disappearing unstably in a short period (critical phase) while the supercritical phase of blooms can show a stable situation where blooms or scums can last visibly for long periods (many hours or many days). The onset status can lead to the 'stable blooms' if ambient conditions allow them to develop, or completely vanish, also due to the ambient conditions.

Discrimination Test for Threshold: Receiver Operating Characteristic (ROC) Curve

As in the clinical practice (Carter et al. 2016), a 'yes or no' decision is usually required for 'diseased or non-diseased' situation, herein two states for the bloom: 'yes - bloom occurrence and no - no bloom' are also defined. The bloom threshold T is based on the variable TRINDEX that will drive the outcomes of the decision, as positive (yes - bloom) or negative (no - no bloom) as follows:

$$Decision(TRINDEX) = \begin{cases} + & positive, or\ bloom\ occurence\ if\ TRINDEX > T \\ - & negative, or\ no\ bloom \qquad if\ TRINDEX < T' \end{cases} \quad \ldots(4)$$

If TRINDEX does have some ability to adequately discriminate between positive and negative situations, there are an infinite number of possible decision thresholds. These include three following possibilities (Fig. 2a): (1) threshold T_1, calling all patterns positive with TRINDEX $\geq T_1$, would correctly identify nearly every positive pattern, although a large proportion of negative patterns would inappropriately be called positive; (2) threshold T_2 more of a balance is struck, as both positive and negative events are missed, and finally, (3) T_3 most negative events are correctly identified, but a large proportion of the positive patterns are incorrectly deemed negative.

Four possible outcomes can result for each trial: correctly positive, correctly negative, incorrectly positive and incorrectly negative. At this point, the cut-off area will be introduced as the area which measures the discrimination, i.e. the ability of the TRINDEX test to correctly classify those with, or without the 'disease', as a binary variable. That is equivalent to bloom occurrence (yes) or no bloom (no) respectively.

The ROC analysis is a binary discriminator test which assesses the predictive power of a binary classification system to evaluate a model in a decision-making process and it helps to identify the threshold T. This test is recognized as a useful tool for interpreting medical test results and in many other fields as a method for evaluating the accuracy of analyses (***Lerman et al. 2010***). For more details of ROC curves and related metrics, refer to Brown & Davis (2006).

A curve illustrating the model performance can then be determined by plotting CPF (correct positive fraction or sensitivity) on the vertical axis and (1 - CNF) (CNF is correct negative fraction or specificity) on the horizontal axis (Fig. 2a). The sensitivity is the probability that case X was classified correctly as above the threshold while specificity is the inverse, namely probability that X classified correctly as below the threshold.

The perfect model (Fig. 2b) corresponds to a point in the top left-hand corner of the Y-axis (i.e. CNF = CPF = 1), the top right (CPF = 1, CNF = 0) and bottom left (CPF = 0 and CNF = 1) of the diagram correspond to the extremes of the decision process where every trial is always deemed either positive or negative. A random predictor (CP = IP and CN = IN) gives a straight line CPF = 1 – CNF (X = Y, line of equality or random change). This can be explained

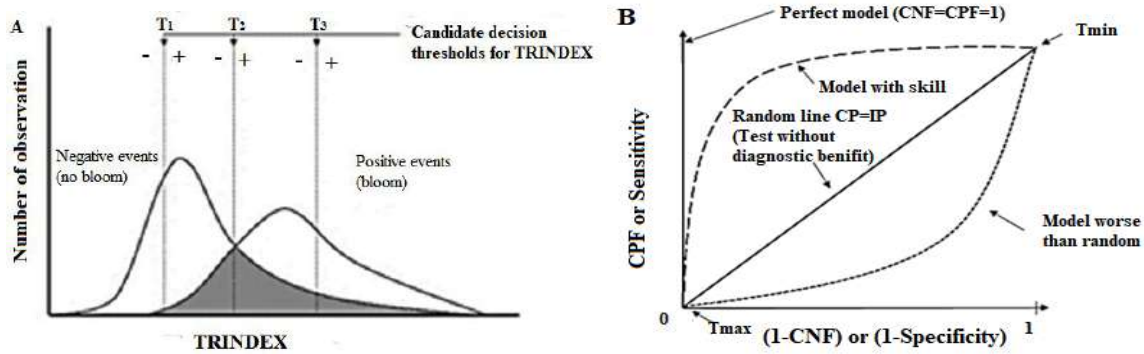


Fig. 2: (a) Illustration of the occurrence of positive and negative observations *versus* TRINDEX on the left (adapted from Brown & David 2006); (b) Binary discrimination skill assessment curves on the right (Adapted from Stow et al. 2009).

by a value of index reaching equalling numbers of true and false positives occur. This value is considered as critical or threshold. The definition of the area under the ROC curve (AUC) was introduced as a criterion to evaluate the overall performance of the discrimination test. This is the percentage of randomly drawn pairs for which this is true. AUC may take values ranging from 0.5 (no discrimination) to 1 (perfect discrimination). A rough practical guide for evaluating the accuracy of a discrimination test with the AUC criteria described as in Table 2 (Carter et al. 2016).

Another factor to estimate the effectiveness of our test is the Youden index *J*. The Youden index *J* (Youden 1950) is defined as:

$$J = \max \{ \text{sensitivity}_c + \text{specificity}_c - 1 \}, \quad \ldots(5)$$

Where *c* ranges over all possible criterion values.

The Youden index *J,* ranging between 0 and 1, is commonly used to measure overall diagnostic effectiveness (Schisterman et al. 2005). When *J* values are close to 1, it indicates that the effectiveness is relatively good, while values close to 0 indicate limited effectiveness.

We use the dataset from ML (2015-2017) for TRINDEX development and data from LT (2015-2018) to validate our approach. In the following calculations, our parameters *sensitivity* (correct positive fraction) and *specificity* (correct negative fraction) are displayed in the percentage (%) instead of the fraction (see Fig.2b).

In our model, the real sample size of two lakes is different (170 samples of LT compared to 266 ones of ML, greater than the required minimum number 132), hence it is statistically significant.

Our experimental data related to HAB for both lakes (Mattatall and Torment) are not normally distributed. Using log transformation as above mentioned is to convert them into the *'normal distribution'* and TRINDEX can be then processed. The statistical software R combined with Excel and MedCalc is used to carry out all steps.

RESULTS AND DISCUSSION

TRINDEX Calculations and the ROC Curve for Performance of Bloom Prediction

Data used for determining lower and upper limits are given in Table 3.

Based on Table 3, formulas (2) and (3) for TRINDEX will become:

Table 2: The AUC criteria to evaluate the accuracy of the diagnostic test.

AUC value	0.9-1.0	0.8-0.9	0.7-0.8	0.6-0.7	0.5-0.6
Evaluation	excellent (A)	good (B)	fair (C)	poor (D)	fail (F)

Table 3: Limits and ranges - Mattatall lake data.

Limits and ranges	L_i	U_i	$LogL_i$	$LogU_i$	$LogU_i$-$LogL_i$
Phycocyanin, mg.L^{-1}	4×10^{-5}	1.855	-4.4	0.3	4.7
Chlorophyll-a, mg.L^{-1}	5×10^{-5}	1.692	-4.3	0.2	4.5
Oxygen |100–%O|	0.01	1000	-2	2.0	4.0
Phosphate, mg.L^{-1}	0.01	1.52	-2	0.2	2.2
Nitrate, mg.L^{-1}	0.01	1.72	-2	0.2	2.2

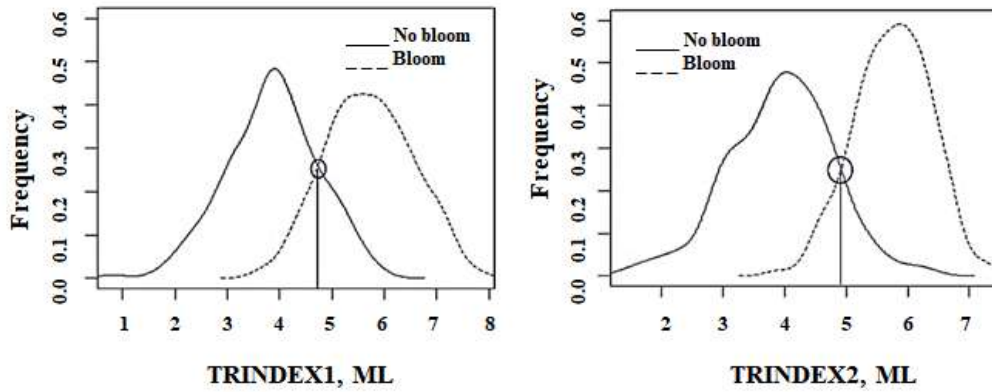


Fig. 3: Distribution of no bloom and bloom cases for TRINDEX1 and TRINDEX2, Mattatall lake.

$$TRINDEX1 = 2.5 \times [\frac{logPC+4.4}{4.7} + \frac{logD\%O+2}{4.0} + \frac{logPO4+2}{2.2} + \frac{logNO3+2}{2.2}], \quad \ldots(6)$$

$$TRINDEX2 = 2 \times [\frac{logPC+4.4}{4.7} + \frac{logChl-a+4.3}{4.5} + \frac{logD\%O+2}{4.0} + \frac{logPO4+2}{2.2} + \frac{logNO3+2}{2.2}], \quad \ldots(7)$$

Data were divided into two groups: (i) bloom occurrence and (ii) no bloom. The distinct scenario for both bloom and no bloom conditions for TRINDEX1 and TRINDEX2 using *rnorm* in R software is graphically represented in Fig. 3.

The cut-off point 5.0 was estimated from Fig. 3. However, this cut-off point should be validated by field observations via ROC curve analysis to precisely determine the threshold value for cyanobacterial bloom. This discrimination test was processed with field observation data (Fig. 4 and Table 5).

Sensitivity (true positive cases) was calculated by assuming that every TRINDEX value can lead to bloom. Inversely, specificity of false positive was done by assuming that every TRINDEX cannot lead to blooms. All calculations of TRINDEX were rounded at 0.2 unit. Formulas for false positive and false negative are as follows.

True positive = Sensitivity = ∑Number of TRINDEX with bloom/ Total of bloom case, …(8a)

False positive = (100 - Specificity) = ∑Number of no bloom TRINDEX /Total of no bloom cases, …(8b)

The dataset of 266 values of TRINDEX1 during 2015-2017 was used for TRINDEX in ML, among them 74 cases with bloom and 192 cases without bloom. Single cases of bloom were detected when TRINDEX1 started from value 4 (Table 5). The higher TRINDEX1 (greater than 5.0), more frequent bloom cases were recorded than no bloom cases; and maximum bloom cases (14 cases) happened when TRINDEX1 = 6.2. Therefore, it can be said that the TRINDEX1 range from 4.0 to 5.0 is the marginal situation, where there is likely to be no sign of a visible bloom but just small disturbances of the environmental conditions (leading to a higher TRINDEX1) could trigger the cyanobacterial bloom.

There were 249 calculated values of TRINDEX2 (Table 5) with 75 bloom cases and 174 no bloom cases. The lowest TRINDEX2 showing bloom was 4.4, but when TRINDEX2 = 5.2 the number of bloom cases was more prevalent than no bloom cases. The maximum number of no bloom cases was noticed when TRINDEX2 = 4.0 and the maximum bloom cases were when TRINDEX2 = 6.2. From the above analyses, the proposition of a transition range for TRINDEX2 was from 4.4 to 5.2 and the suggested threshold value for bloom occurrence suggested was 5.2.

From ROC curves (Fig. 4), the appropriate threshold for bloom onset can be chosen. It should have the maximum sensitivity and at the same time the minimum false positive cases. As two axes of our ROC curve are determined by the *sensitivity* 100% (the probability of true positive results) and (*100% - specificity*) (the probability of false positive results). As such, the false positive cases show TRINDEX are high but no blooms are occurring, while the false negative ones show the opposite scenario: TRINDEX are low but blooms are observed.

ROC curve for TRINDEX1 has the best combination of high sensitivity (81%) and low false positive (12%) (Fig. 4, left side), equivalent to the point 5.0 in Table 5. So, all results of TRINDEX1 equal or greater than 5.0 must be resulting in cyanobacterial blooms. TRINDEX2 (Fig.4 right) has the best combination of high sensitivity (83%) and low false positive (6%), equivalent to 5.2 in Table 5.

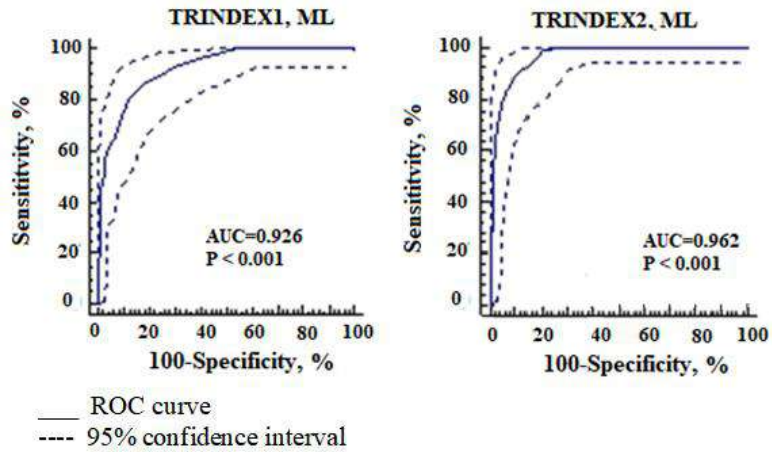


Fig. 4: ROC curves for TRINDEX1 and TRINDEX2 in Mattatall lake.

The significant level represented by p-value stands for the probability that the observed sample AUC (area under the curve) is found when the true (population) AUC is 0.5. When p is small ($p < 0.05$) then it can be concluded that AUC differs significantly from 0.5. Carter et al. (2016) have mentioned that a ROC curve test has (at least) some discriminatory power if the 95% confidence interval of AUC does not include 0.50. In our case of ML, the AUC is 0.926 (95% CI: 0.887 to 0.954; $p < 0.0001$) for TRINDEX1 of ML and AUC is 0.961 (95% CI: 0.929 to 0.981; $p < 0.0001$) for TRINDEX2. This confirms the good fit of our threshold 5.0 for ML as the AUC = 0.926 and 0.961, the discrimination test was then excellent (Table 2).

An AUC over 0.9 (0.926 and 0.961 for TRINDEX1 and TRINDEX2, respectively) implies that in a hypothetical experiment in which we randomly select pairs of positive cases (no bloom) a false negative result is deemed comparable to that of a false positive result. With the environmental factors that can affect a lake system, the random excitation can cause a change of stability around the equilibrium point and beyond this equilibrium point, blooms occur, i.e. instability will cause the HAB.

The range of values of TRINDEX1 from 4.0 to 5.0 can be classified as the transition phase, i.e. potential for a bloom occurrence in the near future. Considering TRINDEX as 'predictor' for bloom, the Youden index J is significant in our tests: 0.69 for TRINDEX1 and 0.78 for TRINDEX 2 (Table 4c). Hence, it is concluded that for ML, two following cut-off points are considered as thresholds: 5.0 for TRINDEX1 while 5.2 for TRINDEX2 with a goodness of fit of discrimination test.

Independent Verification by Lake Torment Data

The same procedure was followed by using data from lake Torment (LT) and cut-off point was found approximately 4.6 for TRINDEX1 and TRINDEX2 (Fig.5). Fig. 6 shows the ROC curve analyses for LT.

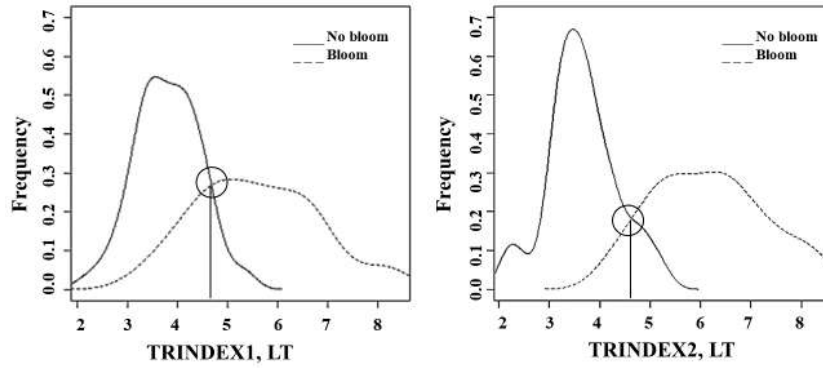


Fig. 5: Distribution of no bloom and bloom cases for TRINDEX1 (Left) and TRINDEX2 (Right), lake Torment.

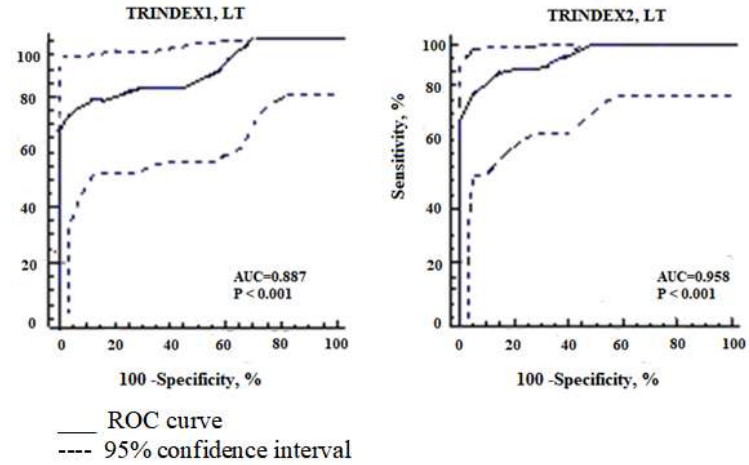


Fig. 6: ROC curves for TRINDEX1 and TRINDEX2 in lake Torment.

TRINDEX1 (Fig. 6 left) had the best combination of high sensitivity (79%) and low false positive (6%) at value 4.8, while TRINDEX2 (Fig. 6 right) had the best combination of high sensitivity (79%) and false positive (2%), at value 5.2.

The transition phase of TRINDEX1 for LT data was 3.4-4.8 and the cut-off point was 4.8. The AUC = 0.887 confirmed the discrimination test was excellent (95%CI: 0.830 to 0.930; $p < 0.0001$). For TRINDEX2, the transition range is 4.0-5.2 and cut-off point at 5.2, and AUC = 0.956 also showing that the discrimination test is excellent (95%CI: 0.914 to 0.982; $p < 0.0001$). Youden index J is also significant: 0.73 for TRINDEX1 and 0.78 for TRINDEX2.

Tables 4 a,b,c show comparison between LT and ML data in term of threshold and ROC curve analyses.

As TRINDEX2 combines both pigments PC and chl-a, it seems inaccurate for the prediction of cyanobacterial bloom thresholds due to the increase of chl-a by other phytoplankton rather than just cyanobacteria, hence increasing TRINDEX2 above the real threshold. Therefore, TRINDEX1 based only on PC seems the better indicator to estimate the threshold for cyanobacterial bloom than TRINDEX2. The lowest value of TRINDEX1 when blooms appear in the 2 lakes was chosen for the transition phase and the cut-off point is the threshold for bloom onset. TRINDEX1 thresholds for the prediction of cyanobacterial blooms can be defined:

- TRINDEX1 < 3.4: no bloom happens as the system is stable.
- TRINDEX1 is between 3.4 and 4.8: there will be a high risk of cyanobacterial bloom development; this range is called 'transition phase'. Other environmental components such as weather conditions should be triggering factors to predict bloom development. In this transitional phase, the situation tends to the onset tendency, that means blooms are happening but can be unstable (appearing and then disappearing in a short period) or becoming stable, depending on ambient conditions.
- TRINDEX1 > 4.8: cyanobacterial blooms could happen and become stable during a certain period (hours or even days).

The performance of our model was evaluated via Accuracy, Precision, Recall and F1 score metrics. Precisely, among these 40 observations, we have 4 false positive cases (10%); 22 true positive (TP) cases; 1 false negative (FN) case (5%); and 13 true negative (TN) cases. It is important to note when we have a large number of true negative cases, it can influence the accuracy of our predictions.

Table 4a: Statistical analyses of TRINDEX1 and 2 for both lakes (by using MedCalc).

	TRINDEX1		TRINDEX2	
	Mattatall	Torment	Mattatall	Torment
Sample size	266	170	249	170
Positive group[a]	74 (27.82%)	24 (14.12%)	74 (29.72%)	24 (14.12%)
Negative group[b]	192 (72.18%)	146 (85.88%)	175 (70.28%)	146 (85.88%)

[a]results = 1 (having bloom); [b]results = 0 (no bloom)

Table 4b: Area under the ROC curve (AUC) for two lake data.

	TRINDEX1		TRINDEX2	
	Mattatall	Torment	Mattatall	Torment
Area under the ROC curve	0.926	0.887	0.961	0.956
Standard Error[a]	0.0162	0.0465	0.0106	0.0226
95% confidence interval[b]	0.887 to 0.954	0.830 to 0.930	0.929 to 0.981	0.914 to 0.982
z statistic	26.237	8.324	43.505	20.172
Significance level p (Area=0.5)	< 0.0001	<0.0001	< 0.0001	<0.0001

[a]DeLong et al., 1988 (the method recommended by MedCalc to calculate standard error and CI95%); [b]Binomial exact

Table 4c: Youden index for data from two lakes.

	TRINDEX1		TRINDEX2	
	Mattatall	Torment	Mattatall	Torment
Youden index J	0.69	0.73	0.78	0.78

Table 5: Data for ROC curve of Mattatall lake.

TRINDEX	TRINDEX1					TRINDEX2				
	Bloom	No bloom	Total	Sensitivity	(100-Specificity)	Bloom	No bloom	Total	Sensitivity	(100-Specificity)
1.60		1	1	100	100					
1.80		1	1	100	99		1	1	100	100
2.00		5	5	100	99		1	1	100	99
2.20		3	3	100	96		3	3	100	99
2.40		3	3	100	95		4	4	100	97
2.60		4	4	100	93		4	4	100	95
2.80		12	12	100	91		4	4	100	93
3.00		12	12	100	85		18	18	100	90
3.20		5	5	100	79		6	6	100	80
3.40		6	6	100	76		10	10	100	76
3.60		17	17	100	73		18	18	100	71
3.80		21	21	100	64		17	17	100	60
4.00	2	21	23	100	53		26	26	100	51
4.20	3	24	27	97	42		13	13	100	36
4.40	3	13	16	93	30	1	13	14	100	28
4.60	2	10	12	89	23	5	11	16	99	21
4.80	4	11	15	87	18	2	7	9	92	14
5.00	5	6	11	81	12	5	7	12	89	10
5.20	7	4	11	75	9	4	3	7	83	6
5.40	5	7	12	65	7	7	3	10	77	5
5.60	8	2	10	59	3	10	2	12	68	3
5.80	3	3	6	48	2	8	1	9	55	2
6.00	4		4	44	1	5		5	44	1
6.20	14		14	39	1	15	1	16	37	1
6.40	5	1	6	20	1	7	1	8	17	1
6.60	6		6	13	0	2		2	8	0
6.80						1		1	5	0
7.00	2		2	5	0	1		1	4	0
7.20	1		1	3	0	1		1	3	0
7.40						1		1	1	0
Total	74	192	266			75	174	249		

In summary, the TRINDEX1 model which is applied to the real observation data from LT for 3 summers is 87.5% accurate, with a recall 0.95 (which is excellent as far above 0.5), a precision of 84.6%, and a F_1 score near 0.9 (which is also very good as F_1 defined in the range from 0 (bad test) to 1 (excellent test)).

Practical Scheme for Application

As indicated, TRINDEX1 is a more appropriate indicator to predict the cyanobacterial blooms. The transition phase can point out the need and significance of a frequent monitoring program for the waterbody. For the possibility of bloom occurrence: the closer TRINDEX1 to threshold values, the higher probability of cyanobacterial growth. The scheme in Fig. 7 is suggested as a practical tool for bloom onset prediction and management based on TRINDEX1.

From this scheme, three scenarios of risk could lead to the management decision for waterbody dealing with algal bloom issue: (1) When no bloom is observed and TRINDEX1<3.4, the monitoring plan for waterbody should follow its established routine; (2) When TRINDEX1 of the lake goes between 3.4-4.8, the risk for a cyanoHAB growth increases. In this case, there might not have any visible sign of bloom in a waterbody, but a more frequent sampling plan with all nutrient parameters, plus taxonomy and toxin analyses should be initiated. Also, the early warning signs could be placed in all accesses to the lake to inform residents about the algal growth concerns. (3) If TRINDEX1 is calculated greater than 4.8, the risk of blooming issues is high and stable blooms could be either observed (on the surface) or not (blooms dissipate in the water column). In this last scenario, any activities of people and pets must be restricted in the

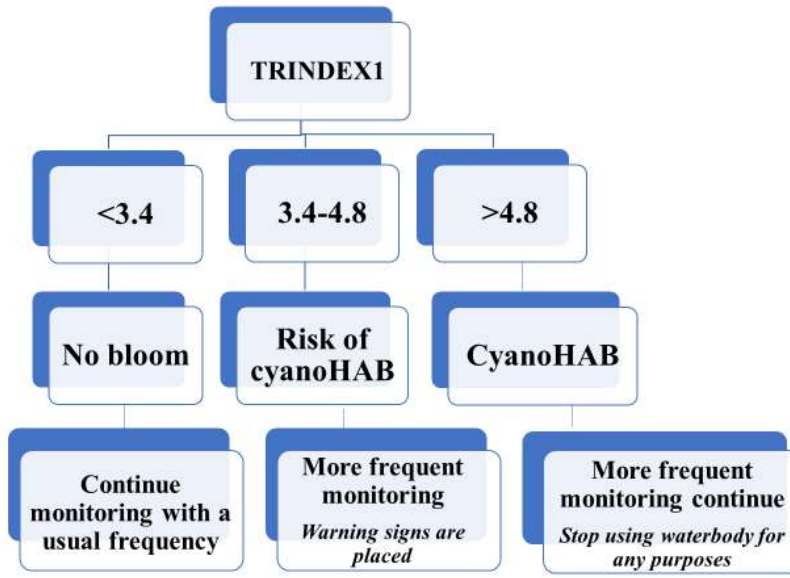


Fig. 7: Practical scheme of TRINDEX application for bloom onset prediction.

concerned waterbody. The monitoring plan should be more intensive during the bloom episodes.

TRINDEX1 is our recommendation for the bloom prediction indicator for monitoring purposes.

Advantages and Limitations of TRINDEX

A "one-size-fits-all" approach (a term used by Anderson et al. 2015) for HAB modelling is not practical and even utopic. Whether forecasting the potentially harmful bloom occurrence or tracking its path, models should always be linked to the local chemistry, physics, and biology of the waterbody and based on the in-situ data. Alert systems and mitigation strategies will be dictated by the history of human resource use in the region and will hinge on local to federal government mandates for protecting those resources (Anderson et al. 2015). From this perspective, we would underline here the advantages and limitations that could lead to conceive TRINDEX for monitoring purposes in each waterbody.

(1) TRINDEX, especially TRINDEX1, is suggested as an indicator for the cyanobacterial bloom onset. This can tell about cyanobacteria presence and bloom occurrence in the waterbody.

(2) The range called 'transition phase' should be understood as a potential risk, or 'a situation involving exposure to bloom' possibility, i.e. that can lead to (i) stable bloom situation (supercritical); or (ii) unstable blooming immediately (critical); or (iii) nothing happening (subcritical), depending on many other factors (such as light, wind, temperature etc.). The transition phase must be considered as an important step of the bloom onset and need to be carefully studied due to its high sensitivity for both false positive and false negative cases.

(3) Temperature was not yet considered in our TRINDEX model herein, because different potentially toxic cyanobacterial species grow with various temperature ranges. We suggest our TRINDEX1 could be used when the temperature is greater than 15°C (which is the lowest temperature range observed in Atlantic Canada for cyanobacterial blooms development).

(4) The inaccuracy of the model may be caused by community metabolism, which was not considered yet. For the potential users for other lakes (oligotrophic to medium eutrophic), our TRINDEX thresholds suggested herein can be appropriately applicable; while for high eutrophic ones, we should recommend that the users would define their own upper and lower limits and would go through all necessary steps to adjust correctly their own lake thresholds.

(5) Also, the dominant species generating blooms can be a significant factor that could intervene in the accuracy of TRINDEX. Both lakes in our consideration (Mattatall and Torment) have the same genus *Dolichospermum*. Further investigation needs to be undertaken with other waterbodies containing different species generating blooms and community metabolism.

CONCLUSIONS

The prediction of cyanobacterial bloom occurrence has always been a challenging subject in both marine and freshwater environments for many decades, and the emphasis on the determination of thresholds for bloom onset, especially in the freshwater ecosystem was not strong. An ideal alert system should quantitatively predict cyanoHAB likelihood, intensity, and potential blooming. The number of approaches for monitoring, detecting, predicting, and forecasting the onset, fate, and demise of algal blooms is arguably comparable to the diversity of species being studied.

Here we focus our work on the prediction of cyanoHABs using index capable to show the thresholds determining the transitional phase to blooming aspects, above which, cyanobacterial blooms in freshwater bodies could happen. All our efforts rely on a close relationship between observations and a simple model leading to developing a forecasting capability. TRINDEX could be practically developed and used in the potential application of smart systems for water management.

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REFERENCES

Ahn, C.Y., Joung, S.H., Yoon, S.K. and Oh, H.M. 2007. Alternative alert system for cyanobacterial bloom, using phycocyanin as a level determinant. Journal of Microbiology, 45(2): 98-104.

Anderson, C.R., Kudela, R.M., Kahru, M., Chao, Y., Rosenfeld, L.K., Bahr, F.L., Anderson, D.M.T. and Norris, A. 2016. Initial skill assessment of the California Harmful Algae Risk Mapping (C-HARM) system. Harmful Algae, 59: 1-18.

Anderson, C.R., Moore, K.S., Tomlinson, M.C., Silke, J. and Cusack, C.K. 2015. Living with harmful algal blooms in a changing world: strategies for modeling and mitigating their effects in coastal marine ecosystems. In: Shroder, J. F., Ellis, J.T. and Sherman, D. J. (eds.) Coastal and Marine Hazards, Risks, and Disasters. Chapter 17.

Bartram, J., Burch, M., Falconer, R., Jones, G. and Kuiper-Goodman, T. 1999. Situation assessment, planning and management. In: Chorus, I. and Bartram, J. (eds) Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management, WHO.

Brient, L., Lengronne, M., Bertrand, E., Rolland, D., Sipel, A., Steinmann, D., Baudin, I., Legeas, M., Le Rouzic, B. and Bormans, M. 2008. A phycocyanin probe as a tool for monitoring cyanobacteria in freshwater bodies. Journal of Environmental Monitoring, 10: 248-255.

Brown, C.D. and Davis, H.T. 2006. Receiving operating characteristics curves and related decision measures: A tutorial. Chemometrics and Intelligent Laboratory Systems, 80: 24-38.

Brylinsky, M. 2009. Lake Utopia Water Quality Assessment. Final report, Dept. Environment of New Brunswick, Canada, pp.92.

Carter, J.V., Pan, J., Rai, S.N. and Galandiuk, S. 2016. ROC-ing along: Evaluation and interpretation of receiver operating characteristic curves. Surgery, 59(6): 1638-1645.

Chorus, I. 2012. Current approaches to cyanotoxin risk assessment, risk management and regulations in different countries. Federal Environ Agency, Germany, pp.151.

Downing, J.A., Watson, S.B. and McCauley, E. 2001. Predicting cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences, 58(10): 1905-1908.

Hushchyna, K. and Nguyen-Quang, T. 2017. Using the modified Redfield ratio to estimate harmful algae blooms. Environmental Problems, 2(2): 101-108.

Kim, H.G. 2006. Mitigation and controls of HABs. In: Granéli, E. and Turner, J.T. (eds) Ecology of Harmful Algae, Springer.

Kudela, R.M., Palacios, S.L., Austerberry, D.C., Accorsi, E.K., Guild, L.S. and Torres-Perez, J. 2015. Application of hyperspectral remote sensing to cyanobacterial blooms in inland waters. Remote Sensing of Environment, 167: 196-205.

Lerman, D.C., Tetreault, A., Hovanetz, A., Bellaci, E., Miller, J., Karp, H., Mahmood, A., Strobel, M., Mullen, S., Keyl, A. et al. 2010. Applying signal-detection theory to the study of observer accuracy and bias in behavioral assessment. Journal of Applied Behavior Analysis, 43(2): 195-213.

Marty, J. and Reardon, M. 2016. King county lake monitoring program 2015 season. King County Lake Monitoring Program 2016 Season. Report. Municipality of the County of Kings.

Ndong, M., Bird, D., Nguyen-Quang, T., Boutray, M., Zamyadi, A.,Vincon-Leite, B., Lemaire, J.B., Prevost, M. and Dorner, S. 2014. Estimating the risk of cyanobacterial occurrence using an index integrating meteorological factors: Application to drinking water production. Water Research, 56: 98-108.

Nguyen-Quang, T., McLellan, K., Hushchyna, K. and Murdock, A. 2017. Harmful Algal Bloom (HAB) Monitoring for Lake Torment and Armstrong Lake. A systematic investigation in 2016-2017 versus 2014 Kings County water quality results. Technical report.

Novotny, V. and Olem, H. 1994. Water quality: prevention, identification, and management of diffuse pollution. Technology & Engineering, Wiley, pp. 1072.

Schisterman, E.F., Perkins, N.J., Liu, A. and Bondell, H. 2005. Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology, 16(1): 73-81.

Stow, C.A., Jolliff, J., Mc Gillicuddy, D.J., Doney, S.C., Allen, J.I., Friedrichs, M.A.M., Rose, K.A. and Wallhead, P. 2009. Skill assessment for coupled biological/physical models of marine systems. Journal of Marine Systems, 76: 4-15.

Vollenweider, R.A., Giovanardi, F., Montanari, G. and Rinaldi, A. 1998. Characterization of the trophic conditions of marine coastal waters, with special reference to the NW Adriatic Sea / Proposal for a trophic scale, turbidity and generalized water quality index. Environmetrics, 9: 329-357.

Youden, W.J. 1950. An index for rating diagnostic tests. Cancer, 3: 32-35.

YSI 9300 and 9500 direct-read photometer. 2010. User manual. YSI Incorporated.

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Cost Estimation of Electrokinetic Soil Remediation for Removal of Six Toxic Metals from Contaminated Soil

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ABSTRACT

The purpose of the study is to estimate the operating cost of the electrokinetic methods in the removal of toxic metals (TMs) from granite mine tailing soil with the help of the proposed cost estimation models. The conventional electrokinetic technologies have not considered the cost estimation in the removal of TMs from polluted soils. In this study, we incorporated the chelates such as citric acid and ethylenediaminetetraacetic acid (EDTA) enhanced electrokinetic soil remediation process followed by a cost estimation of the processes. Our study proposed the cost estimation models to determine the operating cost of the conventional and enhanced electrokinetic treatment processes, specifically for the removal of six TMs such as chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni), zinc (Zn) and manganese (Mn) from granite mine tailing soil. We investigated the chelating enhanced electrokinetic removal of TMs about six times more than the conventional process for 20 days of operation. Furthermore, we estimated that the operating cost of the conventional and enhanced electrokinetic processes was about US$ 110 to US$ 508 per cubic meter of treated soil. The total operating cost was about US$ 110 to US$ 1006 per cubic meter of treated soil including enhancers cost. We believe the chelating enhanced electrokinetic treatment of soil was more effective than conventional treatment for removal of TMs from contaminated soil.

INTRODUCTION

Soil environment has been polluted by incorporation of toxic heavy metals and organic pollutants. Improper management and human activities cause soil contamination (Shukla & Chandel 2005). Recent studies reported the toxic metals(TMs) such as chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn) and manganese (Mn) at elevated concentrations in granite mining waste (Reddy & Yarrakula 2019). During the mineral and milling process, a vast quantity of granite waste is produced and accumulated at dump yards, which may affect the environment (Santhosh et al. 2019). Recently, star-shaped microfluidic channel techniques have been used to detect the TMs (Satish et al. 2017). The concentrations of TMs assessed with advanced remote sensing technique and geography information technology system (GIS) particularly for groundwater (Asadi et al. 2017, Monica et al. 2018). The environmental impact assessment of TM polluted water and soil was determined to minimize environmental pollution (Koteswara & Kiran 2019). Conventional methods such as biosorption, phytochemical oxidation, coconut coir, saxaul tree ash and nickel oxide/carbon nanotube composites (NiO/CNT) investigated for the removal of TMs and organic pollutants from industrial waste disposal (Aravind et al. 2017, Pratapa et al. 2019). Most of the conventional remediation studies are ineffective and not feasible on a large scale at the field level. Electrochemical behaviour of different metals and metal alloys studied with sodium chloride solution for environmental applications (Suresh et al. 2018). The solubility of metals and TMs in the soil affected by the chemical composition of the soil as well as groundwater (Sposito 2008).

An electrokinetic soil remediation technology has emerged and successfully been applied for removal of TMs and organic contaminants from industrial wastewater and soil (FAO/WHO 2000). The removal of toxic TMs from clayey soil applied in several field applications by using electric current (Collins & Kinsela 2011). Electrokinetic remediation is an advanced technique used in-situ and ex-situ removal of toxic metals, organic pollutants, and radio-nuclide materials from contaminated soil. Recent studies investigated the reducing agents enhanced electrokinetic remediation for removal of TMs from polluted soil (Reddy et al. 2019). The metal or TM ions would be desorbed under an acidic or low pH environment at the anode during the electrokinetic treatment (Sivapullaiah et al. 2015). Recently, many studies have focussed on zero waste/mitigation of waste/prevention

of waste using reducing and chelating enhanced reagents in electrokinetic soil remediation technologies (Peng & Tian 2010, Liu et al. 2017).

Conventional electrokinetic methods focussed on the removal of TMs from contaminated water and soil, but not considered the cost estimation of the process (Rosestolato et al. 2015). Several electrokinetic soil remediation studies not considered the cost estimation of the process however, each remediation step involved a cost in a real process (Gao et al. 2013, Reddy et al. 2019). We cope with this problem and proposed the operating cost estimation models soil remediation processes particularly in the electrokinetic removal TMs from mine tailing soils. Generally, an electrokinetic soil remediation process depends upon the treatment time (Ma et al. 2010), by means that longer treatment may also increase the operating cost of the process in terms of cost of chemical reagents and cost of the electrical energy (Pedersen et al. 2015). The basic idea of this study is to determine the operating cost of the electrokinetic treatment of contaminated soils particularly in the remediation of toxic metals. Our previous study investigated the removal of these six TMs using reducing agents enhanced electrokinetic soil remediation process (Reddy & Yarrakula 2019). The TM ionic species would easily migrate from the soil surface to the electrolytic solutions in the presence of electric current (Giannis & Gidarakos 2005, Giannis et al. 2009). Our previous study investigated the removal of these six TMs using reducing agents enhanced electrokinetic soil remediation process (Reddy et al. 2019). In this study, we incorporated the chelating agents (Citric acid and EDTA) for enhanced electrokinetic soil remediation process followed by the estimation of operating cost of the process.

The purpose of the study is to estimate the operating cost of the electrokinetic experiments for removal of TMs from granite mining soil with the help of proposed cost estimation models. The study is classified into two sections, the first section dealt with the electrokinetic removal of TMs by using most efficient chelating agents such as Citric acid and EDTA and subsequent section dealt with the estimation of the operating cost with help of proposed cost estimation models in this study.

MATERIALS AND METHODS

Experimental Design

Recent studies used the electrokinetic reactor with a design of 30cm × 20cm × 15cm in laboratory scale and fabricated with Plexiglas along with two electrode chambers with dimensions of 5cm × 20cm × 15cm and a working volume of 1.5L (Fig. 1) (Reddy et al. 2019). We used the same design of the electrokinetic reactor, and two graphite electrodes act as anode and cathode with a length of 15cm and a diameter of 1.5cm. We conducted four different EKSR experiments with prior prepared anolyte and catholyte solutions as represented in Table 1.

Determination of TMs Concentration and Electricity Consumption

Atomic absorption spectroscopy (AAS) Varian AA110 spectrophotometer used to measure TMs concentration of the soil in pre-treatment and post-treatment of four electrokinetic experiments (Wang et al. 2014, Reddy et al. 2019). The removal efficiency or removal performance can be determined using the Equation (1) (Tang et al. 2014, Bahemmat et al. 2016).

$$\text{Removal efficiency (\%)} = \frac{C_0 - C_f}{C_0} \times 100 \qquad ...(1)$$

Where, C_0: Initial concentration, C_f: Final concentration (mg/kg) of the soil pre-treatment and post-treatment of electrokinetic removal of TMs.

The expenditure of electrical energy is directly related to the remediation time using the integral time for the passing of current during the electrokinetic process across the electrokinetic reactor. The consumption of electricity to be determined with the Equation (2) (Ma et al. 2010, Pedersen et al. 2015).

$$\text{Energy expenditure } (E_e) = P = \frac{1}{V_s}\int_0^t VIdt \qquad \ldots(2)$$

Where,

E_e: The energy expenditure (Wh/m^3),

P: The electrical power (W)

Table 1: Experimental pattern of four different EKSR experiments.

Exp. No.	Anolyte	Soil saturation	pH	Catholyte	Duration (Days)	Electric potential (V/cm)
Exp.1	Distilled water	Distilled water	9.7	Distilled water	5, 10, 15, 20	2
Exp.2	Distilled water	0.1M Citric acid	5.7	0.1M Citric acid	5, 10, 15, 20	2
Exp.3	Distilled water	0.1M Citric acid	5.9	0.1M EDTA*	5, 10, 15, 20	2
Exp.4	0.1M NaOH	Distilled water	9.7	Distilled water	5, 10, 15, 20	2

* EDTA: Ethylenediaminetetraacetic acid

V_S: The volume of the soil (m^3)

I: The current passage across the electrokinetic reactor (A)

V: The voltage (V)

t: The treatment time (h)

Proposed Cost Estimation Models

The operating cost of the electrokinetic experiments was estimated through the consumption of enhancing solutions and electrical energy during electrokinetic removal of TMs from mine tailing soil. The total cost of the process was estimated by adding the individual costs of enhancers and cost of electricity expenditure. Generally, the total cost of the electrokinetic process is directly related to the remediation time which includes the electricity supplied across the electrokinetic reactor. The cost of the enhancers was estimated by the quantity of enhancers consumed in the process multiplied by the cost of one unit. In the same way, the cost of electricity was estimated by the electricity consumption charges in India. Our study considered the operating cost of the electrokinetic experiments rather than the capital investment of the process.

Several studies used the equation (2) for estimating the amount of electricity consumed during electrokinetic removal of heavy metals from polluted soils (Ma et al. 2010, Pedersen et al. 2015, Reddy et al. 2019). We simplified the equation (2) into equations (4 and 5) for quick estimation of the cost of enhancers and the cost of the electricity for four different electrokinetic experiments. In this study, we proposed the following three cost estimation models to determine the enhancers cost, electricity cost and the total operating cost of the electrokinetic process. The models were well correlated with manual calculations in the estimation of enhancers cost and electricity cost for four different electrokinetic experiments.

Enhancers cost (E_C) can be estimated by the following Equation (3):

$$E_C = \frac{1}{V_S}\left[\sum_i^n \left(m_i \times c_i\right)\right] \quad \ldots(3)$$

Electricity cost (EE_C) can be estimated by the following Equation (4):

$$EE_C = \frac{1}{V_S}\left[\left(10^{-3}\int_0^t VIdt\right)C_c\right] \quad \ldots(4)$$

The Total cost (T_C) can be estimated by the following Equation (5):

$$T_C = \frac{1}{V_S}\left[\sum_i^n \left(m_i \times c_i\right) + \left(10^{-3}\int_0^t VIdt\right)C_c\right] \quad \ldots(5)$$

Here,

T_C: The total cost of the process including enhancers and energy cost

V_S: The volume of the soil (m^3)

m_i: The amount of enhancing reagent (kg)

C_i: The cost of enhancing reagent ($/kg)

I: The current passage across the electrokinetic reactor (A)

V: The voltage (V)

t: The treatment time (h)

RESULTS AND DISCUSSION

Conventional Electrokinetic Experiment

During the electrokinetic process, the toxic metal ionic species are dissociated from the soil surface and travelled between anode compartment and cathode compartment by electromigration, electrophoresis and electroosmosis. The metallic species was collected through anolyte and catholyte solutions and measured their concentration via acid digestion followed by atomic absorption spectrometry. A conventional electrokinetic experiment was performed for 5-20 days by purging distilled water for both the anode chamber and cathode chamber. Thereafter collected the anolyte and catholyte solution samples for detecting the TM species for every five days of treatment. The removal performance of TM species with respect to time was recorded and can be seen in the Experiment (1) (Fig. 1a). The removal percentage was about 6%, 9%, 16%, 24%, 11% and 32% respectively for Cr, Co, Ni, Cu, Zn and Mn after 20 days of treatment. The average percentage of removal of all metallic species found that around 13% by means that longer treatment time (>20days) is required to remove the TMs completely from mine tailing soil.

Enhanced Electrokinetic Experiments

During the enhanced electrokinetic process, the metallic species were collected through anolyte and catholyte solutions and measured their concentration via acid digestion followed by atomic absorption spectrometry. The enhanced electrokinetic experiments were performed for 5 to 20 days by purging citric acid and EDTA as chelates at the cathode chamber to suppress the increase in pH of catholyte. The acidic environment was most favoured for the dissociation

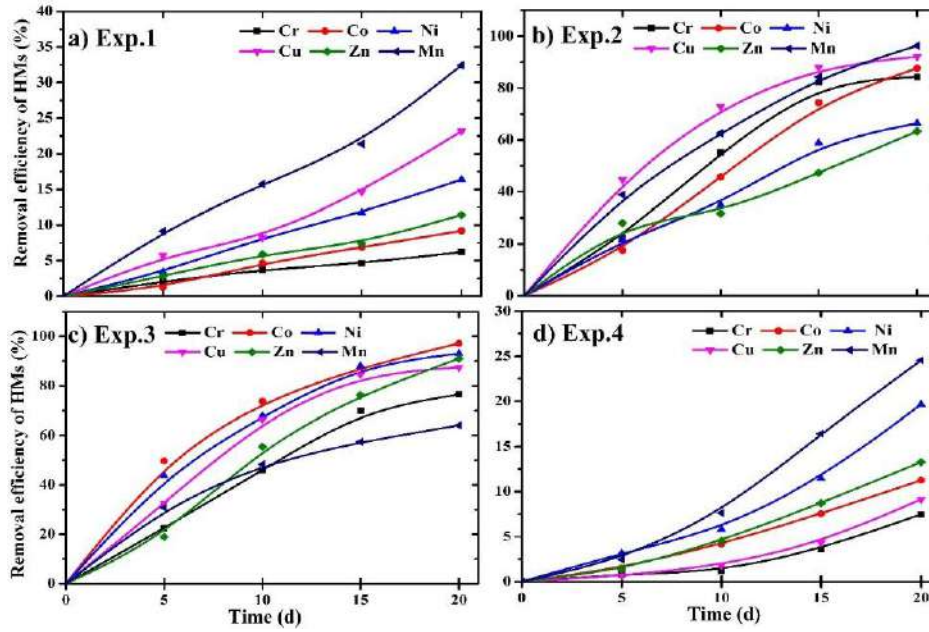


Fig. 1: Removal performance (%) of TMs in experiments (1, 2, 3 and 4) w.r.t. time for 20 days.

of metallic species from the soil surface to anolyte/catholyte electrolyte solutions. Thereafter collected the anolyte and catholyte solution samples for detecting the TM species for every five days of treatment. The removal performance of TM species with respect to time was recorded (Fig. 1b & 1c) and can be observed in Experiments (2 &3). The removal percentage was ranged about 76%-84%, 88%-97%, 66%-93%, 87%-92%, 63%-91% and 64%-96% respectively for Cr, Co, Ni, Cu, Zn and Mn after 20 days of treatment. The average removal performance (%) of all metallic species found that around 84% by means that 25-30 days treatment time is required to remove the TMs completely from mine tailing soil in the case of chelating enhanced electrokinetic experiments. However, the results demonstrated that the enhanced electrokinetic removal of TMs was about six times more than the conventional method.

Alkali Electrokinetic Experiment

The experiment (4) was performed for 5-20 days by purging sodium hydroxide (NaOH) at the anode chamber to increase the pH of the anolyte solution. Thereafter collected the anolyte and catholyte solution samples for detecting the TM species for every five days of treatment. The removal performance of TM species with respect to time was recorded (Fig. 1d). The removal percentage was about 7%, 11%, 19%, 9%, 13% and 24% respectively for Cr, Co, Ni, Cu, Zn and Mn after 20 days of treatment. The average percentage of removal of all metallic species found that around 14% by means that longer treatment time (>20days) is required to remove the TMs completely from mine tailing soil. The results indicated the removal of TMs in alkali electrokinetic experiment was close to conventional electrokinetic experiment.

Cost Estimation of Electrokinetic Experiments

In the case of the conventional electrokinetic experiment (1), the enhancers were not used, hence the total operating cost was directly related to the electricity consumption for 20 days of operation. The expenditure of electrical energy was about 1104 kWh/m^3 respectively. However, the total operating cost estimated by the proposed model was around 110.4 US$/$m^3$. The citric acid as enhancing electrokinetic experiment from Table 2 and Fig. 2, the total operating cost was directly related to the electricity consumption and the amount of citric acid utilized for 20 days of operation. The citric acid was consumed about 146 kg/m^3 with a cost of 293 US$/$m^3$. The consumption of electricity was about 2480 kWh/m^3 with a cost of 248 US$/$m^3$ respectively. However, the total operating cost was estimated at around 541 US$/$m^3$. In the case of EDTA, an enhancing electrokinetic experiment, the total cost was related to the electricity consumption and amount of citric acid consumed during removal of TMs for 20 days of operation. The consumption of EDTA was about 267 kg/m^3 with a cost of 800 US$/$m^3$ and citric acid cost was about 26 US$/$m^3$ for soil saturation. The consumption of electricity

was about 1800 kWh/m^3 with a cost of 180 US$/$m^3$ respectively. However, the total operating cost was estimated at around 1006 US$/$m^3$. In the case of the alkali electrokinetic experiment, the total operating cost was directly related to the electricity consumption and amount of NaOH utilized for 20 days of operation. The NaOH was consumed about 53.3 kg/m^3 with a cost of 112 US$/$m^3$. The consumption of electricity was about 5080 kWh/m^3 with a cost of 508US$/$m^3$ respectively. However, the total operating cost was estimated around 620 US$/$m^3$ (Fig. 2). The electricity cost of experiment 4 with NaOH was almost twice higher than other experiments 1-3, it might be the reason that the increase in pH of the soil matrix during the electrokinetic process.

The results demonstrated that conventional and alkali electrokinetic experiments were costly and not effective in the removal of TMs from contaminated soils. The citric acid enhanced electrokinetic experiment was more effective and economically feasible than the EDTA enhanced electrokinetic experiment in the removal of TMs from contaminated soils.

CONCLUSION

Our study proposed the cost estimation models to estimate the operating cost such as cost of chemical reagents, cost of electrical energy and total cost of the process in the electrokinetic removal of TMs from contaminated soil. We estimated the operating cost of conventional and enhanced electrokinetic treatment processes in specifically for the removal of six TMs such as chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni), zinc (Zn) and manganese (Mn) from granite mine tailing soil. We investigated that the chelating enhanced electrokinetic removal of TMs about six times more than the conventional process in 20 days of operation. Furthermore, we estimated the operating cost of the conventional and enhanced electrokinetic processes was about US$110 to US$508 per cubic meter of treated soil. The total operating cost becomes US$110 to US$1006 per cubic meter of treated soil including enhancers cost. We conclude that the chelating enhanced electrokinetic treatment of soil was more effective and economically feasible than conventional treatment for removal of TMs from contaminated soil.

Table 2: Cost estimation of four electrokinetic experiments.

Exp. No	Enhanced solution consumption						Energy consumption		[e]Total cost ($/$m^3$)
	Citric acid		EDTA		NaOH		Power		
	Consumption (kg/m^3)	[a]Cost ($)	Consumption (kg/m^3)	[b]Cost ($)	Consumption (kg/m^3)	[c]Cost ($)	Consumption (kwh/m^3)	[d]Cost ($)	
Exp.1	0	0	0	0	0	0	1104	110.4	110.4
Exp.2	146.6	293.2	0	0	0	0	2480	248	541.2
Exp.3	13.3	26.6	266.6	800	0	0	1800	180	1006.6
Exp.4	0	0	0	0	53.33	112	5080	508	620

[a] Cost of citric acid per kg: 2$; [b]Cost of EDTA per kg:3$; [c] Cost of NaOH pellets per kg: 2.1$; [d] Average cost of electricity consumption in India per kilo-Watt-hour(kWh): 0.1$.

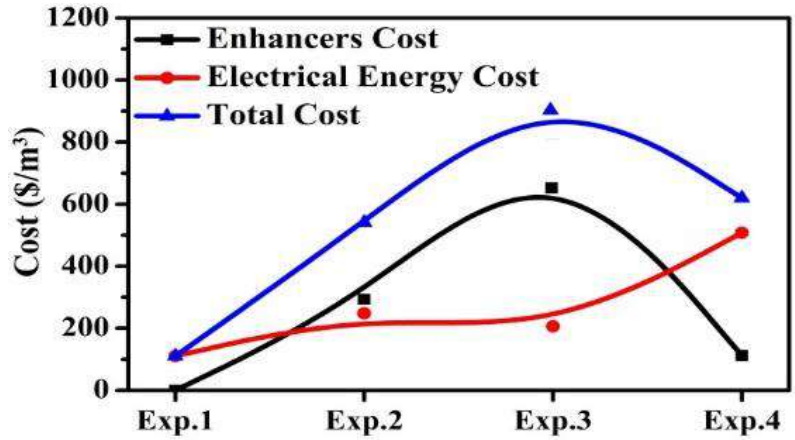


Fig. 2: Cost Estimation of four electrokinetic experiments.

REFERENCES

Aravind, C., Chanakya, K. and Mahindra, K. 2017. Removal of heavy metals from industrial waste water using coconut coir. Int. J. Civ. Eng. Technol., 8: 1869-1871.

Asadi, S.S., Lahari, K. and Sai Madhulika, K. 2017. Analysis of soil quality for environmental impact assessment -a model study. Int. J. Civ. Eng. Technol., 8: 798-805.

Bahemmat, M., Farahbakhsh, M. and Kianirad, M. 2016. Humic substances-enhanced electroremediation of heavy metals contaminated soil. J. Hazard. Mater., 312: 307-318.

Collins, R.N. and Kinsela, A.S. 2011. Pedogenic factors and measurements of the plant uptake of cobalt. Plant Soil., 339: 499-512.

FAO/WHO 2000. Health risk of heavy metals from long-range transboundary air pollution. Europe Guidelines.

Gao, J., Luo, Q. and Zhang, C. 2013. Enhanced electrokinetic removal of cadmium from sludge using a coupled catholyte circulation system with multilayer of anion exchange resin. Chem. Eng. J., 234: 1-8.

Giannis, A. and Gidarakos, E. 2005. Washing enhanced electrokinetic remediation for removal cadmium from real contaminated soil. J. Hazard. Mater., 123: 165-175.

Giannis, A., Nikolaou, A., Pentari, D. and Gidarakos, E. 2009. Chelating agent-assisted electrokinetic removal of cadmium, lead and copper from contaminated soils. Environ. Pollut., 157: 3379-3386.

Koteswara, Reddy. G. and Kiran, Y. 2019. A theoretical mechanism in the degradation of polyolefin plastic waste using phytochemical oxidation process. J. Solid. Waste Technol. Manag., 45: 468-477.

Liu, Y., Chen, J. and Cai, Z. 2017. Removal of copper and nickel from municipal sludge using an improved electrokinetic process. Chem. Eng. J., 307: 1008-1016.

Ma, J.W., Wang, F.Y., Huang, Z.H. and Wang, H. 2010. Simultaneous removal of 2,4-dichlorophenol and Cd from soils by electrokinetic remediation combined with activated bamboo charcoal. J. Hazard. Mater., 176: 715-720.

Monica, C.L., Raju, M.V. and Kumar, D.V. 2018. Assessment of heavy metal concentrations and suitability study of ground water (bore wells) quality for construction purpose: A model study. Int. J. Civ. Eng. Technol., 9: 1273-1282.

Pazos, M., Plaza, A., Martín, M. and Lobo, M.C. 2012. The impact of electrokinetic treatment on a loamy-sand soil properties. Chem. Eng. J., 183: 231-237.

Pedersen, K.B., Kirkelund, G.M. and Ottosen, L.M. 2015. Multivariate methods for evaluating the efficiency of electrodialytic removal of heavy metals from polluted harbour sediments. J. Hazard. Mater., 283: 712-720.

Peng, G. and Tian, G. 2010. Using electrode electrolytes to enhance electrokinetic removal of heavy metals from electroplating sludge. Chem. Eng. J., 165: 388-394.

Pratapa, Reddy. Y., Narayana, K.L. and Kedar, Mallik M. 2019. Electro-chemical behaviour of different metals in sodium chloride solution. Int. J. Emerg. Trends Eng. Res., 7: 715-719.

Reddy, G.K., Yarrakula, K. and Lakshmi, U.V. 2019. Reducing agents enhanced electrokinetic soil remediation (EKSR) for heavy metal contaminated soil. Iran. J. Chem. Chem. Eng., 38: 183-199.

Reddy, G.K. and Yarrakula, K. 2019. Geo-chemical exploration of granite mining waste using XRD, SEM/EDX and AAS analysis. Iran. J. Chem. Chem. Eng., 38: 215-228.

Reddy, K.R. 2013. Electrokinetic remediation of soils at complex contaminated sites. Coupled Phenom Environ. Geotech., 131-147.

Rosestolato, D., Bagatin, R. and Ferro, S. 2015. Electrokinetic remediation of soils polluted by heavy metals (mercury in particular). Chem. Eng. J., 264: 16-23.

Santhosh, C., Kishore, K.H. and Lakshmi, G.P. 2019. A detection of heavy metal ions using star-shaped microfluidic channel. Int. Emerg. Trends in Engg. Res.,7: 768-771.

Satish, Kumar.M., Asadi, S.S. and Vutukuru, S.S. 2017. Assessment of heavy metal concentration in ground water by using remote sensing and GIS. Int. J. Civ. Eng. Technol., 8: 1562-1573.

Shukla, R.S. and Chandel, P.S. 2005. A Textbook of Plant Ecology. S. Chand and Company LTD, 10th Edition.

Sivapullaiah, P.V., Nagendra Prakash, B.S. and Suma, B.N. 2015. Electrokinetic removal of heavy metals from soil. J. Electrochem. Sci. Eng., 5: 47-65.

Sposito, G. 2008. Chemistry of Soils. Oxford University Press, 2nd Edition, New-York.

Suresh, G., Narayana, K.L. and Mallik, M.K. 2018. Electrochemical behaviour of lens TM deposited Co-Cr-W alloy for bio-medical applications. Int. J. Mech. Prod. Eng. Res. Dev., 2018: 41-52.

Tang, W.W., Zeng, G.M. and Gong, J.L. 2014. Impact of humic/fulvic acid on the removal of heavy metals from aqueous solutions using nanomaterials: A review. Sci. Total Environ., 468-469: 1014-1027.

Traina, G., Morselli, L. and Adorno, G.P. 2007. Electrokinetic remediation of bottom ash from municipal solid waste incinerator. Electrochim. Acta., 52: 3380-3385.

Wang, Y., Fang, Z., Liang, B. and Tsang, E.P. 2014. Remediation of hexavalent chromium contaminated soil by stabilized nanoscale zero-valent iron prepared from steel pickling waste liquor. Chem. Eng. J., 247: 283-290.

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A Study on Chemical Disintegration of POP Ganesh Idols in Bhopal, Madhya Pradesh, India

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ABSTRACT

In India, festivals like Ganesh Chaturthi, Durga Puja form an integral part of its rich and diverse cultural heritage. The ritual of idol worshipping and immersion into water bodies signifies the cycle of creation and dissolution, acknowledging the rhythm of nature that all things come from nature and go seamlessly back to it for a cycle to begin. But, in recent times the practice of immersing Plaster of Paris (POP) made idols along with other decorative material into natural water bodies has immensely raised the severe issue of water pollution and creating an uncongenial aquatic ecosystem. In this purview, a pilot project for chemical disintegration of POP idols during Ganesh festival was run in Bhopal, Madhya Pradesh, India to study the effective application of a chemical method developed by CSIR-NCL, Pune by using ammonium bicarbonate. Water samples were collected before and after idol immersion activity from the tank. The detailed chemical analysis of the aqueous phase was carried out to study the effect on various parameters and application of residues. The analytical results reveal that the method has its practical utility as both the products of the reaction can further be used in chalk making and as fertiliser with dilution. The concentration of heavy metals was found minimal and in the limits in the treated aqueous phase. This paper also supports the sustainable management of such functions rather than the use of artificial materials.

INTRODUCTION

In India, idol worship has been in practice since ancient times. Ganesh festival is celebrated traditionally in all states of India with full enthusiasm as a social and community activity. The objective of the festival is mainly to bring people together and promote harmony. On festive occasions such as Vinayaka Chaturthi, Durga Puja, Sarswati Puja, etc., it has been a tradition to immerse idols in water bodies like rivers, lakes, ponds, estuaries, open coastal beaches, wells etc. Traditionally idols were made of natural clay decorated with natural things like flowers and natural colours (Reddy & Kumar 2001). However, in recent times due to many short comes like increasing demands of idols, non-availability of clay, high cost, delicacy of clay idols, beauty with artificial accessories etc. Plaster of Paris (POP) idols are in use in large numbers and have strongly abducted the markets. Plaster of Paris (POP) idols are very easy to make in moulds, comparatively cost less and have good strength and stability. But, POP idols when immersed in water, they don't dissolve in water and remains as such for a long period. This contributes excessively to water pollution in rivers, ponds, lakes and sea (Vyas et al. 2008). The idols are also found stuck in the water and block the natural flow of water bodies leading to stagnation. Decoration of POP idols with chemical paints and artificial accessories also supplements the issue by altering the physico-chemical attributes of water bodies like water hardness, acidic content, drop in oxygen level etc. Chemical paints along with varnish and polish used, contain many heavy metals like chromium, mercury, lead, cadmium etc. which harm the aquatic system and disturb the life cycle of aquatic animals (Bhattacharya et al. 2014). The polluted water bodies also raise a serious health issue when used for various purposes like drinking, bathing, in agriculture etc. Immersion of a large number of POP idols in natural water bodies not only deteriorates the quality of water but also poses adverse effects on the aquatic ecosystem and environment (Upadhyaya & Bajpai 2010).

Taking it into concern and in pursuance to the Hon'ble Bombay High Court directions issued in its order dated 22/07/2008, CPCB evolved the Guidelines for immersion of idols and other puja materials reaching in the water bodies during the festival. In the guidelines it is suggested "Use of traditional clay for idol making rather than backed clay, Plaster of Paris etc. may be encouraged, allowed and promoted. The painting for idols should be discouraged and the use of chemical paints must be strictly prohibited".

But, even after the release of guidelines for idol immersion, construction and trade of POP idols still have been observed in the market. The disintegration of POP idols by conventional crushing is not acceptable by people due to sentimental attachment to the ritual. Hence, there is a need to develop some scientific method to disintegrate these POP idols without posing any harm to the environment and sentiments of devotees. Keeping this at priority an eco-friendly scientific method for chemical disintegration of POP idols was first developed by CSIR- National Chemical Laboratory (NCL), Pune (Maharashtra) (Navale et al. 2018).

The objective of this study is to apply the chemical method developed by CSIR-NCL, Pune for the disintegration of POP Ganesh idols used in the Ganesh festival during the year 2019 as a pilot project in Bhopal city and verify the method by studying various parameters of water and its practical utility.

Plaster of Paris (POP) is chemically calcium sulphate hemihydrates having formula $CaSO_4.1/2\ H_2O$ and is prepared by heating Gypsum at about 150°C. When mixed with water POP hardens fast and so, have several applications like the protective or decorative coating of walls and ceilings and for moulding and casting decorative elements, in building material, in hospital for plastering injured bones, in dental casts, idol preparation etc (Vekinis et al. 1993, Ram & Sen 1959). The impact of idol immersion on aquatic bodies are given in Table 1.

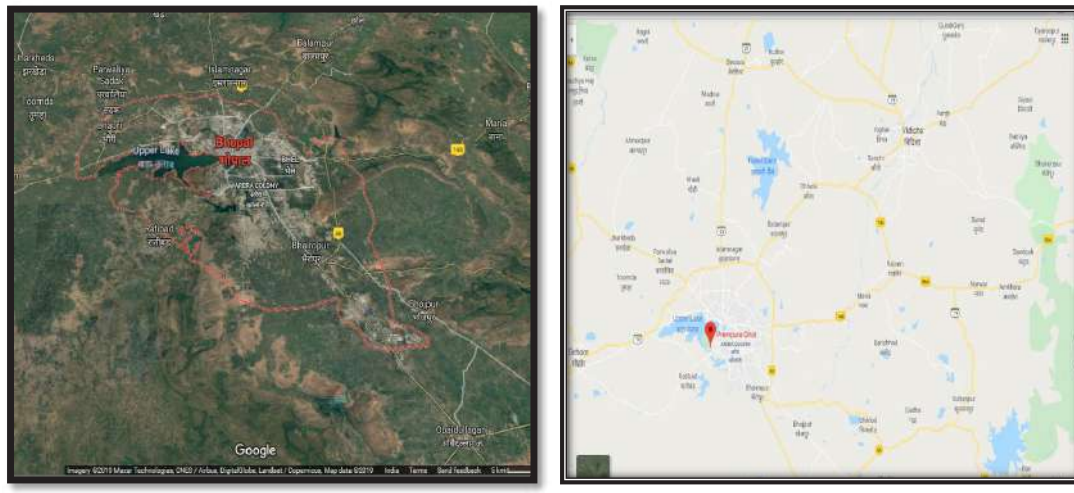
In the present study, a pilot project would have been taken into the city Bhopal, Madhya Pradesh, India for chemical disintegration of POP Ganesh idols at Prempura ghat in the year 2019. Ammonium bicarbonate (NH_4HCO_3) was used for the dissolution of POP idols at the ghat where artificial tanks were used for the process. Detailed chemical analysis of the aqueous phase was carried out to evaluate the change in physico-chemical parameters of the aqueous phase and possible applications of the residue.

STUDY AREA

Bhopal is a city in central India, and the capital of the state of Madhya Pradesh within the co-ordinates 23°15'0" N, 77°25'0" E and at an elevation of 1729 feet (527 M). Bhopal is the city of serene beauty having several natural and artificial lakes dominating the city giving it the title of "City of Lakes". Also, rich in culture, art and public work having numerous heritage structures of its period. Bhopal is a cosmopolitan city where the majority of the population is dominated by the Hindus (occupying around 55% of the total) and every year during Ganesh Festival around 6000 Kg POP idols are immersed in lakes playing a crucial role in water pollution. This anthropogenic activity leads to deterioration of water quality and reduces the self-purifying ability of water bodies which adversely affects flora and fauna around it. Prempura ghat of the city Bhopal was selected for the study where POP Ganesh idols from the entire city were collected and transported by Bhopal Municipal Corporation (BMC). The Google map of the study area is shown in Fig. 1.

MATERIALS AND METHODS

To achieve the objective of the study to disintegrate POP idols of Ganesh in Bhopal in the year 2019 by a chemical method



(a) (b)

Fig 1: (a) Google map of city Bhopal, Madhya Pradesh, India (Co-ordinates 23°15'0" N, 77°25'0" E).
(b) Prempura Ghat- Study area for the project.

Table 1: Impact of idol immersion on aquatic life.

S. No.	Materials contributed during immersion	Impact on the aquatic body
1.	Plaster of Paris	Increased dissolved solids, increased hardness, sludge, reduce life carrying capacity
2.	Decoration materials viz. cloths, polish, paints, ornaments, cosmetic items etc.	Contributes suspended matters, trace metals (zinc, lead, chromium, arsenic, mercury etc.), metalloids and various organic and inorganic matter, oil & grease etc.
3.	Flowers, garlands, oily substances	Increase floating suspended matter organic contamination, oil & grease and various organic and inorganic matter
4.	Polythene bags, plastic items	Contribute suspended, settleable matter and hazardous material to water and chokes the aquatic life
5.	Bamboo sticks, beauty articles	Big pieces got collected and recycled while small pieces remain floating in the water or settled at the river bottom inhabiting river flow
6.	Eatables, food items	Contributes oil & grease, organics to water bodies

developed by CSIR-National Chemical Laboratory (NCL), Pune (Maharashtra), India all the important immersion Ghats of Bhopal city were recognised. Major immersion ghats of Bhopal city are shown in Fig. 2. The main Ghats where immersion of idols has been done in the city include Prempura ghat, Jwahar bal udhyan, Shahpura visarjan ghat, Jhoolelal visarjan ghat and Khatlapura visarjan kund etc. The experimental set up for the study was done at Prempura ghat where all the POP Ganesh idols collected from different Ghats and localities were gathered and weighed with the help of a weighing machine.

For the method, 20 % w/v solution of ammonium bicarbonate (Merck India, ABC) was prepared and stored in five number large-sized plastic tanks arranged at Prempura ghat each of capacity around 5000 litres. Each tank containing ammonium bicarbonate solution is fitted with a motor pump and overhead shower to ensure proper mixing and recirculation of water. This would also help to fasten the reaction by removing the topmost layer of calcium carbonate formed at the surface of idols after the disintegration of POP with ammonium bicarbonate. The ratio of POP to ammonium bicarbonate was kept 1:1. During the study, a total of around 3000 kg of POP idols were disintegrated by the method where each tank containing around 4000 litres of the solution having 600 kg of ammonium bicarbonate dissolved in it.

POP is calcium sulphate hemihydrate ($CaSO_4.½H_2O$) when reacts with ammonium bicarbonate (NH_4HCO_3) forms insoluble calcium carbonate sludge that settles at the bottom and soluble ammonium sulphate which is a well-known fertilizer (US2640757 A). The settled calcium carbonate has also found many applications like in chalk making industries, cement industries and others. It took around three days (72 hours) time for complete disintegration of POP idols inside the tanks. Continuous monitoring was done at the time of the entire process. It was found during the reaction the layers of paints over the idols were removed like peel off separately.

$$2(NH_4)HCO_3 + CaSO_4.½H_2O \longrightarrow (NH_4)_2SO_4 + CaCO_3 + H_2O + CO_2$$

Ammonium Bicarbonate — POP — Ammonium Sulphate — Calcium Carbonate — Water — Carbon dioxide



Fig 2: Major immersion ghats in Bhopal city.

To study various chemical parameters and alteration in these characteristics after chemical reaction initial sample of freshwater was taken from the tank. Then after the completion of reaction when all the POP idols have disintegrated the sludge of calcium carbonate was allowed to settles at the bottom of tanks and sample was collected from the supernatant water. The collected samples were preserved as per the standard methods and brought in the laboratory of Central Pollution Control Board (Central), Regional Directorate, Bhopal for the analysis. To explore the utility of by-product ammonium sulphate formed after the disintegration of POP various dilutions viz. 2 times, 10 times and 20 times of supernatant water after reaction were also prepared for the analysis. The samples were coded as POP 01, POP 02, POP 03, POP 04 and POP 05.

The collected samples were analysed for important parameters including Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total suspended Solids (TSS), Total Dissolved solids (TDS), Specific conductivity (Cond.), Total hardness (TH), Calcium hardness (Ca-H), Magnesium hardness (Mg-H), sulphate (SO_4^{-2}) and Ammonical nitrogen (NH_3-N) following the standard procedures (APHA 1995).

The analysis of the majority of heavy metals including cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel

Table 2: Parameters under study before and after the disintegration of POP Ganesh idols by 20% ABC solution.

S. No.	Parameters Analysed	POP 01	POP 02	POP 03	POP 04	POP 05
		Before immersion	After immersion	After 2 times Dilution	After 10 times Dilution	After 20 times Dilution
1	pH	6.3	9.7	9.5	8.7	7.9
2	COD (mg/L)	4	115.1	49.79	11.08	3.24
3	BOD (mg/L)	<1	7.14	4	1.58	1.36
4	TSS (mg/L)	8	454	297	38	6
5	TDS (mg/L)	600	56308	34380	7044	485
6	Sp. Cond. (µmho/cm)	184	67500	42900	11700	1160
7	Ca-hardness (mg/L)	59.13	BDL	BDL	BDL	BDL
8	Mg-hardness (mg/L)	17.39	BDL	BDL	BDL	BDL
9	Total hardness (mg/L)	76.52	BDL	BDL	BDL	BDL
10	SO_4^{2-} (mg/L)	9.56	143603	32305	7080	1198.97
11	NH_3-N (mg/L)	0.439	0.637	0.546	0.1128	0.0528

*BDL (Below Detection Limits)

Table 3: Heavy metal analysis of samples before and after the disintegration of POP Ganesh idols by 20 % ABC solution.

S. No.	Heavy Metals	POP 01	POP 02	POP 03	POP 04	POP 05
		Before immersion	After immersion	After 2 times Dilution	After 10 times Dilution	After 20 times Dilution
1	Cadmium (Cd), mg/L	BDL	0.053	0.033	BDL	BDL
2	Chromium (Cr), mg/L	BDL	0.189	0.056	0.010	BDL
3	Copper (Cu), mg/L	BDL	0.172	0.085	0.020	BDL
4	Lead (Pb), mg/L	BDL	0.380	0.215	0.056	BDL
5	Nickel (Ni), mg/L	BDL	0.1018	0.0749	0.0255	BDL
6	Iron (Fe), mg/L	0.12	0.193	0.083	0.058	BDL
7	Manganese (Mn), mg/L	0.0015	0.0641	0.0379	0.0078	BDL
8	Arsenic (As), µg/L	BDL	0.402	BDL	BDL	BDL
9	Mercury (Hg), µg/L	BDL	BDL	BDL	BDL	BDL

*BDL (Below Detection Limits)

(Ni), iron (Fe), manganese (Mn), arsenic (As) and mercury (Hg) was done using flame Atomic Absorption Spectroscope (AAS) by GBC.

RESULTS AND DISCUSSION

The environmental impact of chemical disintegration of POP Ganesh idols was assessed substantially through analysis of characteristics physicochemical parameters of the water used in the method before and after immersion. This is an excellent indicator for discussing the utility of the used water without impact on the environment. The heterogeneity and conspicuous changes in studied variables during pre immersion and post immersion phases along with the dilutions prepared are depicted in Table 2 and Fig. 3. During the study period, the pH of the water showed a reasonable range of variation from 6.3 to 9.7 before and after immersion respectively. The pH value was acidic during pre immersion and became alkaline in post immersion phase. The highest dilution of 20 times brought it at a value of 7.9. The rise in the pH value is because of the dissolution of ammonium bicarbonate which made it basic. Moreover, large amounts of synthetic chemicals of POP idols get mixed with water during chemical disintegration making pH to vary.

As the method adopted for the disintegration of POP idols was chemical, COD is an important parameter for influencing the quality of water. COD increased sharply from 4 mg/L in pre immersion phase to 115.1 mg/L after disintegration. This reduced to 3.24 mg/L on 20 times dilution in POP 05 sample which can be used in plantation. Due to substantial increase of organic load contributed by some of the straw, flowers and other materials used in idol making and decoration respectively, increase in BOD concentration was observed from < 1 mg/L to 7.14 mg/L in POP 02 sample collected after immersion. With 20 times diluted sample POP 05, the BOD reduced to 1.36 mg/L and is safe to be reused. The analysis results of samples for total hardness, Ca-hardness and Mg-hardness were all found Below Detection Limits (BDL) except for the pre immersion water sample. This could be because of the chemical reaction of ammonium bicarbonate with POP all dissolved $CaSO_4$ and $MgSO_4$ salts responsible for hardness in water in water also get precipitated out as $CaCO_3$ sludge and settled at the bottom.

For the parameters TDS, specific conductivity and sulphate an extreme sharp upswing in value was observed post chemical disintegration of POP idols. The TDS which was determined as 600 mg/L in sample POP 01 pre immersion raised to 56308 mg/L in sample POP 02 collected after disintegration. An abrupt percentage increase of 9284.6% was observed which might be resulted from the increased concentration of dissolved compounds in water after the chemical disintegration of idols. A significant positive correlation with the correlation coefficient, r = 1 was observed between TDS and specific conductivity. Specific conductivity got increment from 184 µmho/cm to 67500 µmho/cm after immersion. However, after 20 times dilution values of the same, in sample POP 05, were determined as 485 mg/L and 1160 µmho/cm respectively for TDS and specific conductivity which are satisfactory. The TSS calculated as 8 mg/L in pre immersion sample POP 01 was raised to 454 mg/L in the post immersion sample POP 02 due to the release of paints and other materials used for decoration of idols during the chemical process.

Ammonical nitrogen (NH_3-N) concentration did not show much variation in the values. It was determined as 0.439 mg/L in POP 01 before disintegration and 0.637 mg/L in non-diluted sample POP 02 post immersion. However, sulphate concentration exhibited rapid appreciation in the value after the chemical breakdown of POP idols by ammonium bicarbonate solution (Table 2). In the first phase before immersion, sulphate (SO_4^{2-}) was 9.56 mg/L which shoot up to 143603 mg/L after reaction in non-diluted sample POP 02. The increased level of sulphate is evident from the reaction of ammonium bicarbonate with POP i.e. calcium sulphate to ammonium sulphate and calcium carbonate where carbon dioxide was released in the form of bubbles, which was confirmed by lime water test (Liu et al. 2009).

In the specific dilution, this ammonium sulphate can be used as an important nitrogen-sulphur fertilizer in fields and green belt areas for plant growth. Both the elements nitrogen and sulphur are important for the growth of plants as involved in different biological pathways and protein synthesis (Yu et al. 2018). This fertilizer is suitable for alkaline soil and where the requirement of nitrogen and sulphur is needed (Craswell et al. 1981). This is also having utility in pharmaceutical, baking, textile fire extinguishing powder and wood pulp industries. While the by-product obtained, calcium carbonate can be used as a raw material in chalk manufacturing, construction industry, metallurgy, chemical industry etc. it can also be used in the removal of environmental pollutants (Declet 2016).

Nowadays POP idols are also being decorated with a variety of materials including synthetic colours and paint which contains many hazardous chemicals and heavy metals which plays a significant role in water quality characteristics and posing serious impacts on water system as well as on human health. Considering this the water samples pre and post disintegration were analysed for heavy metals including cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), iron (Fe), manganese (Mn), arsenic (As) and mercury (Hg). In the first phase before chemical reaction in the sample

POP 01, except for Iron and Manganese, all other metals have concentration Below Detection Limits (BDL). However in the second phase after chemical disintegration of POP idols by ammonium bicarbonate solution in the sample POP 02 of supernatant water with no dilution the concentration of most of the heavy metals were determined as Cadmium (Cd)= 0.053 mg/L; Chromium (Cr)= 0.189 mg/L; Copper (Cu)= 0.172 mg/L; Lead (Pb)= 0.380 mg/L; Nickel (Ni)= 0.1018 mg/L; Iron (Fe)= 0.193mg/L; Manganese (Mn)= 0.0641 mg/L; Arsenic (As)= 0.402 µg/L and Mercury (Hg)= BDL.

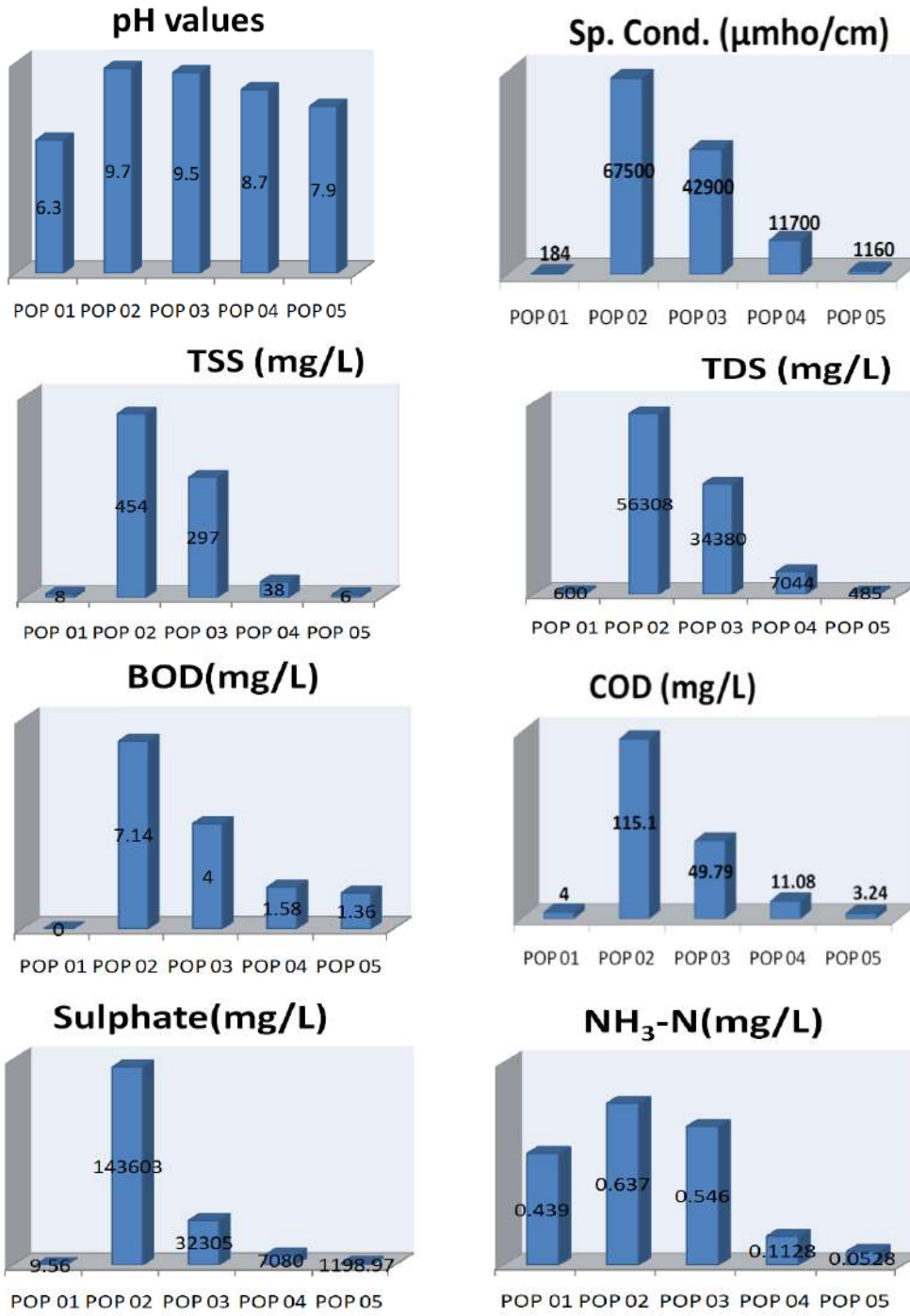


Fig. 3: Changes in studied variables during pre immersion and post immersion phases along with the dilutions prepared.

The results of Heavy Metal analysis of samples before and after disintegration of POP Ganesh idols are depicted in Table 3. The results expressed difficulty in the utility of this water because of heavy metal contents in it. But, after 20 times dilution in sample POP 05, their concentration reduces to satisfactory limits.

CONCLUSION

Plaster of Paris (POP) idol immersion in natural water bodies has immense contribution to water pollution which raised the concern for a safe and eco-friendly way to deal it. Complete chemical disintegration of POP idols by ammonium bicarbonate solution gives value-added products having reasonable practical utilities with no hostile effects. The post disintegration chemical water after specific dilutions can be effectively used as a fertilizer in agriculture. However, the method is not found so cost-effective as 99.99% purity ammonium bicarbonate is mandatory which costs high and the process requires a specific set up with tanks, mesh, overhead showers, continuous mixing, proper PPEs, supervision etc. but, in the transition stage from POP to complete clay idols, this chemical method seems to be a better and environmentally safe alternative with feasibility to arrest water pollution. But a complete ban on the use of POP idols is always preferable and should be adopted in such festivals.

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REFERENCES

APHA 1995. Standard Methods For The Examination of Water and Wastewater. 19th edition, American Public Health Association, Washington D.C.

Bhattacharya, S., Bera, A., Dutta, A. and Ghosh, U.C. 2014. Effects of idol immersion on the water quality parameters of Indian water bodies: environmental health perspectives. International Letters of Chemistry, Physics and Astronomy, 20.

Craswell, E.T., Datta, S.K., Obcemea, W.N. and Hartantyo, M. 1981. Time and mode of nitrogen fertilizer application to tropical wetland rice. Fertil. Res., 2: 247-259. https://doi.org/10.1007/BF01050197.

Declet, A. 2016. Calcium carbonate precipitation: a review of the carbonate crystallization process and application in bio-inspired composites. Rev. Adv. Mater. Sci., 44:87-107.

Liu, F., Wang, S. and Zhang, X. 2009. Study on ammonium bicarbonate decomposition after CO_2 sequestration by ammonia method. Huanjing Kexue Xuebao/Acta Sci. Circumst., 29: 1886-1890.

Navale, G.R., Gohil, K.N., Puppala, K.R., Shinde, S.S., Umbarkar, S. and Dharne, M.S. 2018. Rapid and greener method for utilization of POP waste generated from biomedical samples. Int. J. Environ. Sci. & Tech. https://doi.org/10.1007/s13762-018-2070-7.

Ram, A. and Sen, S. 1959. Plaster of Paris. Trans Indian Ceram Sic., 18: 35-54. https://doi.org/10.1080/037150X.1959.11011909.

Reddy, V.M. and Kumar, V.A. 2001. Effects of Ganesh idol immersion on some water quality parameters of Hussain Sagar. Curr. Sci., 81: 1412.

Upadhyaya, A. and Bajpai, A. 2010. Comparison of physico-chemical parameters of various water bodies in and around Bhopal (M.P.). Asian J. Chem. Environ. Res., 3: 20-26.

Vekinis, G., Ashby, M.F. and Beaumont, P.W.R. 1993. Plaster of Paris as a model material for brittle porous solids. J. Mater. Sci., 28: 3221-3227. https:// doi.org/10.1007/BF00354239.

Vyas, A., Bajpai, A., Verma, N. and Dixit S. 2008. Heavy metal contamination cause of idol immersion activities in urban lake Bhopal, India. J. Appl. Sci. Ad Environ. Mang., 11(4): 37-39.

Yu, Z., Juhasz, A. and Islam, S. 2018. Impact of mid-season sulphur deficiency wheat nitrogen metabolism and biosynthesis of grain protein. Sci. Rep., 8: 2499. https://doi.org/10.1038/s41598-018-20935-8.

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Monitoring Impacts of Human Activities on Bouskoura Stream (Periurban of Casablanca, Morocco): 3. Bio-Ecology of Epilithic Diatoms (First Results)

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ABSTRACT

The epilithic diatoms of a periurban watercourse of Casablanca city (Bouskoura stream) were studied from samples taken in 8 stations (distributed in the upstream-downstream direction) for two years (August 2015-July 2017). The recorded diatomic flora consists of 54 species corresponding to 27 genera and belonging to 5 main families: Monoraphids, Naviculaceae, Nitzschiaceae, Araphids and Surirellaceae. This flora is predominantly alkaliphile and characterizes β-mesosaprobe to polysaprobes and eutrophic to hypereutrophic media. Responses assemblage to natural and anthropogenic disturbances were analyzed. Diatom assemblages structure analysis shows that downstream of this watercourse, where pollution is intense, the abundances of pollosensitive taxa such as *Achnanthes minutissima*, *Cymbella affinis* are low or even nulls and we are witnessing the appearance of polysaprobe forms such as *Nitzschia palea*, *Nitzschia capitellata* and *Nitzschia frustulum* that tolerate rich environments in organic matter or highly polluted. Spatial variation in species diversity could not highlight changes in water quality at the prospected sites; on the other hand, the change in the percentage of pollutant-tolerant taxa (PTV) revealed the full extent of the alteration due to gradual nutrient and organic matter inputs into the Bouskoura watercourse. In addition, the correlation obtained between this index and the organic pollution index (IPO) is highly significant. Principal component analysis (PCA) highlighted taxonomic differences between stations. The results obtained in this work have emphasized the importance of diatoms as a bioindicator of the health status of this periurban watercourse.

INTRODUCTION

The problem of the deterioration of aquatic ecosystems and the water quality of watercourses in particular, is an increasingly important issue in the world and more particularly in countries with an arid and semi-arid climate such as than Morocco. These concerns are intimately related to the uses that man makes of them.

Different tools can be used to evaluate the lotic environments quality and the disturbances they undergo. The majority of the work on monitoring the quality of these environments in Morocco relies on physicochemical and microbiological methods of water samples taken occasionally in different sampling stations as well as on the use of macroinvertebrates as bioindicators of health of these hydrosystems. The first studies on diatoms in Morocco targeted the flora of stagnant waters (Hariot 1909, Gauthier 1930, Gattefosse 1935, Gayral 1954 etc). Subsequently, there has been little work on diatoms as a biological tool for assessing the quality of Moroccan lotic media (Werner 1949, Maiffi-Rassat 1988 and Cazaubon & Badri 1994 on the Tensift River, Fekhaoui et al. 1988 and Jaghror et al. 2017 on the Sebou River in addition to the work of Fawzi et al. 2001 and Fawzi et al. 2002 on Hassar stream).

This study is part of a research program aimed at characterizing the aquatic environment and water quality in the Moroccan Chaouia coastal watershed. It consists of evaluating the ecological status of the Bouskoura stream (suburban of Casablanca) by using diatom communities as bioindicators, and this, in relation to the physicochemical quality of the water. To date, studies on this hydrosystem have focused on macroinvertebrate, physicochemical and bacteriological components (Tazi et al. 2001, Mounjid et al. 2014, Benhassane et al. 2019) or the modelling of this watercourse (Stoffnerr 2013, Goury & Chelhaoui 2013, Boudaoud & Hadine 2013). The evaluation of its biological quality using diatoms has been omitted.

The choice of benthic diatoms in this study is justified by the fact that the quality of running waters is reflected in the composition of their assemblages (Round 1991). In fact, the different diatom species have a high sensitivity to the conditions of their aquatic environment (Fièvet et al. 2003, Stenger-Kovácsa et al. 2013, Kollár et al. 2015). These aquatic organisms are in constant physical, chemical and biological interaction with their ecosystem. They are thus able to integrate environmental evolutions in the short term as well as in the long term, but also the antagonistic or synergistic effects of the different types of contaminants, impossible to demonstrate by physicochemical measurements (Whitton 1991). In order to have a better appreciation of the real biological quality in a river, Torrisi et al. (2006) recommended that epilithic rather than epiphytic diatoms be preferentially sampled for the following reasons:

- Epilithic communities are more representative and more diverse than strictly epiphytic communities.
- In addition, stones are a frequent, inert, stable and homogeneous natural substrate in streams.

Finally, this work will also test the importance of using diatoms as a practical tool for estimating the overall quality of Bouskoura stream.

MATERIALS AND METHODS

Study Area and Sampling Stations

Bouskoura stream, a 20 km long and 10 L/s average flow, is located 10 km south of Casablanca city. This watercourse, fed by five main sources which drain the waters of the coastal Chaouia groundwater (Fig. 1). After 11 km, this stream receives the waters of its left tributary (Ain Joumaa). Bouskoura stream flows from south to north of the Casablanca city while irrigating 20 ha of the area of its catchment area estimated at 255 km^2 (Stoffnerr 2013).

This hydrosystem crosses the central zone of Casablanca where its natural bed is completely urbanized and the watercourse becomes channelled.

From the upstream to downstream, 8 sampling stations (including 2 stations belonging to the control network of the Bouregreg-Chaouia ABHBC Hydraulic Basin Agency) were positioned on the main course of Bouskoura and its tributary (Ain Joumaa) to target the sections of this hydrosystem that are the most degraded in terms of ecological integrity (Fig. 1). The Lambert coordinates for these stations are shown in Table 1.

The choice of these stations is based on the nature of the water (wastewater, natural waters), the morphological

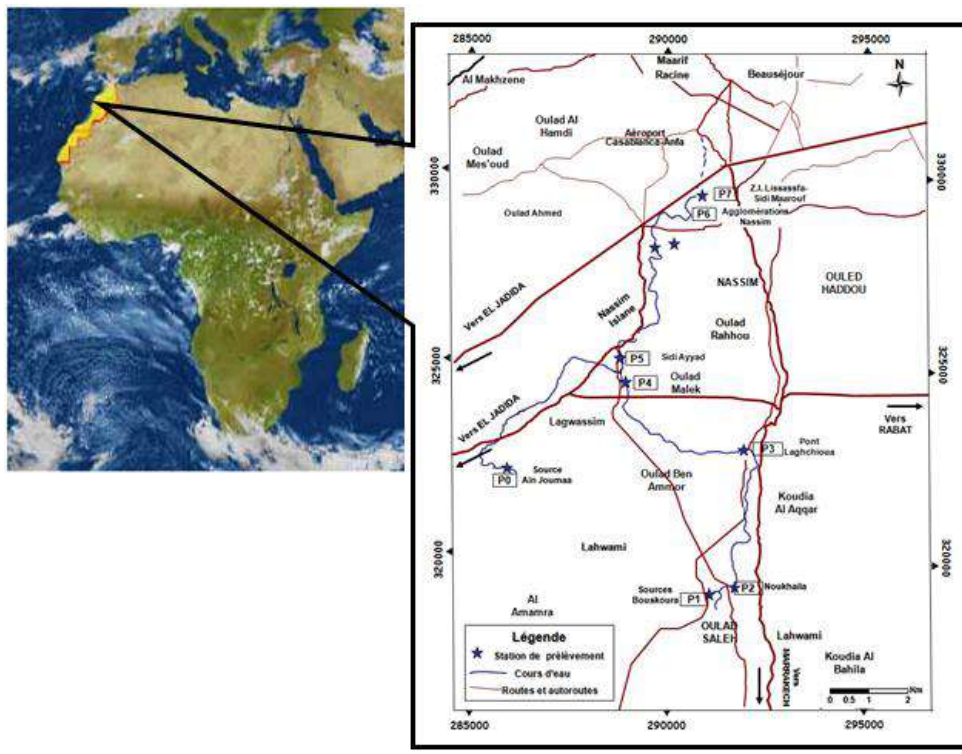


Fig. 1: Study area and sampling stations in the Bouskoura stream.

Table 1: Coordinates, characteristics and type of activities in monitoring stations in Bouskoura stream.

Stations	Lambert Coodinates		Distance between station (km)	Total distance (km)	Type of pollution
	X	Y			
P0 (Aïn Joumaa Source)	286190	322244	-	-	Bathing activity
P 1 (Bouskoura Source)	291150	318980	-	0	Reference station
P 2 (Noukhayla)	291364	319230	0.353	0.353	Domestic and industrial pollution
P 3 (Laghchiwa Bridge)	291933	322716	4.726	5.079	Agricultural pollution
P 4 (Oulad malek)	288948	323808	3.91	8.989	Agricultural pollution domestic pollution
P 5 (Sidi Ayyad)	288830	325255	2.44	11.429	Agricultural pollution
P 6 (Nassim Agglomerations)	289582	327110	3.006	14.435	Domestic pollution
P 7 (Lissasfa-Sidi Maârouf Industrial Zone)	289733	328117	1.112	15.547	Industrial pollution

structure of the Bouskoura watercourse, the nature of the bed and substrate, the proximity of urban and rural population centres and the possibilities of access and sampling offered.

Physico-Chemical Analyses

Between August 2015 and July 2017, 11 to 12 water samples have been carried out at the above-mentioned stations. The water samples taken were subjected to physico-chemical analyses to measure pH, temperature, dissolved oxygen (O_2), electrical conductivity, suspended matter (SM), ammonium ions (NH_4^+), nitrites (NO_2^-), nitrates (NO_3^-), orthophosphates (PO_4^{3-}), total phosphorus (TP), total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), biochemical oxygen demand for five days (BOD_5) and chlorophyll a (Chl a).

The samples of water to be analyzed were directly carried out in polyethene bottles of 500 mL, stored in a cooler at 4°C and then transported to the laboratory for analysis. Analyses of different chemical parameters were carried out, in accordance with French standard (NF T 90-354, 2000), at the Laboratory of Ecology and Environment of the Faculty of Sciences Ben M'Sik -Casablanca (Morocco).

All equipment was calibrated and verified with certified standards to ensure reliable results.

Sampling, Treatment and Determination of Diatoms

Samples of epilithic diatoms were made on a mineral substrate according to the French standard (NF EN 13946, 2003). After cleaning with concentrated hydrogen peroxide, the diatoms are mounted in the naphtha resin (IR = 1.74) for observation under a microscope according to the procedure described by the French standard (NF T90 354, 2003). According to this standard, each microscopic preparation has been counted to a minimum of 400 valves.

The determinations were made at the specific or even infra-specific level according to Krammer & Lange-Bertalot (1986, 1988, 1991a and 1991b), Round et al. (1990) and Bey et al. (2013).

Structural Indices of Diatomic Communities

Specific diversity (H') of Shannon and Weaver (1963): The diatom specific diversity was determined at the different Bouskoura stream sites by the Shannon and Weaver index (1963). This index makes it possible to measure the heterogeneity of the biodiversity of an environment; it was calculated using the following formula:

$$H' = -\sum_{i=1}^{s} \frac{n_i}{N} \log_2\left(\frac{n_i}{N}\right) \quad \ldots(1)$$

Where,

ni: Number of individuals of the species i

n: Total number of individuals in the sample

H': Expressed in bits (information unit)

S: Total number of species

Wilhm (1975) gave a water quality classification based on the values of the Shannon-Weaver Diversity Index (1963) as shown in Table 2:

Pielou evenness (1975): The Pielou index (1975) is the ratio of the calculated diversity to an optimal theoretical diversity, H_{max}, obtained by considering that each of the S species in the sample is represented by a single individual.

$$H_{max} = \text{Log}_2 S \quad \ldots(2)$$

$$\text{Evenness } E = (H' / \log_2 S) \quad \ldots(3)$$

Jaccard similarity coefficient (1901): The similarity coefficient of Jaccard (1901) allows a comparison between two sites, by evaluating the similarity between two records and by comparing the species common to both records and those

Table 2: Interpretation of the Shannon-Weaver Diversity Index H' (after Wilhm 1975).

H'	water quality
< 3	Little or no polluted
1 < H' < 3	Moderately polluted
H' < 1	Very polluted

specific to each survey.

From the presence-absence matrix, the Jaccard similarity index can be calculated easily according to the following formula:

Jaccard similarity coefficient $I_J = N_c/(N_a + N_b - N_c)$ …(4)

Where,

$\mathbf{N_c}$: Number of taxa common to stations a and b

$\mathbf{N_a}$ et $\mathbf{N_b}$: Number of taxa present in stations a and b, respectively

Organic pollution index (OPI): This index is calculated by integrating the concentrations of 4 chemical parameters related to organic pollution: Biological Oxygen Demand (BOD_5), ammonium ions (NH_4^+), nitrites (NO_2^-) and phosphates (PO_4^{3-}). Each of them is distributed in 5 classes (Table 3) which have a biological significance corresponding to typical diatom changes (Leclercq & Vandevenne 1987). After Leclercq & Maquet (1987), OPI is the mean value of the classes found for each parameter and gives a five-level pollution index (Table 4).

The percentage of pollution tolerant valves (% PTV): The impact of anthropogenic activities on the structure of the diatomic community has been estimated from the percentage of tolerant species to organic pollution (%PTV). The selection of these species was made according to Van Dam et al. (1994) who took into consideration in his work the preferences of 948 species of freshwater and brackish diatoms in terms of pH, nitrogen, oxygen, salinity, humidity, saprobity and trophic status.

% PTV was developed by Kelly (1998) and Kelly & Whitton (1995) to monitor sewage effluent (orthophosphate-phosphorus concentrations) and not the overall quality of watercourses. The presence of more than 20% of PTV shows significant organic impact (Table 5). This index has been used by many authors around the world (Bellinger et al. 2006, Walsh & Wepener 2009, Menye et al. 2012).

Table 3: Classes of the parameters for calculating the Organique Pollution Index (OPI).

Paramètres / Classes	BOD_5 (mg O_2/L)	ammonia (mg N/L)	nitrite (µg-N/L)	phosphates (µg-P/L)
5	< 2	< 0.1	5	15
4	2-5	0.1-0.9	6-10	16-75
3	5.1-10	2.4	11-50	76-250
2	10.1-15	2.5-6.0	51-150	251-900
1	> 15	> 6	> 150	> 900

Table 4: OPI classes and corresponding pollution levels.

Average class number of parameters	Level of organic pollution
5.0-4.6	No pollution
4.5-4.0	Low
3.9-3.0	Moderate
2.9-2.0	Strong
1.9-1.0	Very strong

Table 5: Interpretation of % PTV scores (after Kelly 1998).

% PTV	Interpretation
< 20%	Free from significant organic pollution
21% - 40%	Some evidence of organic pollution
41% - 60%	organic pollution to contribute significantly to eutrophication at site
> 61%	Site heavily contaminated with organic pollution

Statistical treatment: Results data were analyzed and statistically processed using Principal Component Analysis (PCA) to characterize the Diatom assemblages structure of Bouskoura stream. The PCA was performed using the STATISTICA software (v. 6.0).

For diatoms, the database used for the diatomic community typology of this hydrosystem is the average relative abundance of taxa (with a cumulative relative abundance greater than or equal to 5%) and 8 stations.

The degree of binding between physicochemical and biological variables was assessed using the Spearman rank correlation test using the same STATISTICA software.

RESULTS AND DISCUSSION

Physicochemical Quality of Water

Spatial evolution of physicochemical parameters in Bouskoura stream: The results of physicochemical analyses of Bouskoura stream water are given in Table 6. Water temperature varies according to the sites and sampling periods. Average water temperature for the stream would be of the order of 20.88°C. These data indicate that the temperature of the study area is favourable to aquatic life (Rodier 2009). The pH is around an average of about 7.69. In general, the waters of the studied hydrosystem will be slightly basic. The constancy of the pH can therefore be attributed, in the sense of Golterman (1971), to the buffering phenomenon of the carbonate-bicarbonate complex.

Electrical conductivity shows an upward trend along an upstream-downstream gradient. The average for all stations in the watercourse would be 3964.68 µS/cm. These results are comparable to those reported by several authors in most Mediterranean watercourses (Oubraim 2002, Morin 2006, Mouni et al. 2009, Mounjid et al. 2014, Nehar et al. 2015, Quevedo et al. 2018 etc.).

Dissolved oxygen shows decay from upstream to downstream. The most notable falls are those recorded in P2, P6 and P7 where there is a deficit in oxygen (respectively 1.73; 1.29 and 0.64 mg O_2/L). Indeed, these stations permanently

receive the wastewater from the Lissassfa-Sidi Maarouf industrial zone and the Nassim agglomerations and they are therefore loaded with organic matter whose degradation by the microorganisms consumes oxygen.

The nitrite averages evolution in the different stations of the Bouskoura lotic medium shows no particular tendency. The spatial variation of this parameter clearly shows significant differences between the stations testifying to the variability of the sources of pollution along this lotic ecosystem.

The analysis of the ammonium, phosphorus compounds, BOD_5, COD and suspended solids profiles shows an upward trend.

The increase in phosphorus compounds may be of domestic and industrial origin but also of agricultural origin, especially since the highest values are recorded during the rainy season when leaching of phosphorus elements is important.

The average BOD_5 and COD contents depend on the typology discharges in the different stations of Bouskoura stream. However, it is important to note that in the P2 station heavily impacted by wastewater from the Oulad Saleh industrial zone, the average value of BOD_5 remains low. In fact, in the field, we notice that the wastewater of this station contains fats, oils, detergents and probably heavy metals. These pollutants are capable of inhibiting the self-purifying power of aerobic bacteria present in the water.

The average levels of chlorophyll a are low P0 station (i.e. 0.37 µg/L) and then increase until reaching the value of 36.94 µg/L in the P3 station. This increase is followed by a gradual decrease towards downstream stations; the low concentration (i.e. 6.94 µg/L) is observed at the Sidi Maars ouf-Lissassfa industrial zone (P7 station).

The ANOVA statistical test followed by the Tukey HSD posterior test applied to the means of the physicochemical parameters, shows that the differences observed between the upstream and the downstream stations are significant ($P < 0.05$) for all the physicochemical parameters (at the exception of the temperature). Indeed, the evolution of these parameters illustrates a gradient of mineralization and organic pollution increasing from upstream to downstream of Bouskoura stream where the effect of human activities is significant. The strong standard deviations observed show significant intra-site variations.

Epilithic Diatoms of Bouskoura stream.

Global analysis of the diatom assemblage inventory: A total of 54 species and varieties of diatoms corresponding to 27 genera and belonging to 5 main families have been identified in the 8 stations of this hydrosystem (Table 7). Many of these taxa and varieties are cosmopolitan. Overall, this diatomic assemblage consists of species adapted, for some of them, to a high mineralization, and for others, significant eutrophication of the waters.

The taxonomic richness of diatomic assemblage in the Bouskoura watercourse is quite similar to that of several Moroccan and Mediterranean periurban rivers and streams (Table 8). Nevertheless, diatomic floral inventory of Hassar, Ul (Portugal) and Felent (Turkey) watercourses seems to be superior than in Bouskoura stream.

- The composition of diatom communities varies from upstream to downstream of Bouskoura stream. We go from assemblage characterized by species sensitive to alterations of the environment (monoraphidae, araphid and naviculaceous) to others that are composed of pollution-resistant species (polysaprobes) characterizing the disturbed environments (the Nitzschiaceae in particular).
- The main families represented are:
  - Monoraphids are represented by 4 genera and 4 species (i.e. 7.4% of total species richness). This family is composed mainly of pioneer and colonizing species that are generally sensitive to environmental alterations and characterize undisturbed rivers (Prygiel & Coste 2000).
  - Naviculaceae are represented by 14 genera and 33 species (i.e. 61% of total species richness). Naviculaceae contains the largest number of very different types of ecology.
  - Nitzschiaceae is composed of 4 genera that group together 11 species (i.e. 20% of the total species richness). Nitzschiaceae contains a large number of usually saprophilous or N-heterotrophic species. However, there are some sensitive and alkaliphilic forms.
  - Araphideae is composed of 3 genera corresponding to 4 species representing 7.4% of total species richness.
  - Surirellaceae is composed of 2 genera corresponding to 2 species representing 3.7% of the total species richness. It is very little represented in the inventories of the study stations.
  - Other Brachyraphideae, Centrophycideae and Epithemia families are absent in the diatomic communities of Bouskoura.

Table 6: Averages of the different physico-chemical parameters measured in the 8 stations of Bouskoura stream and results of the ANOVA test followed by the Tukey test.

Stations		pH	T°	EC	DO	PO_4^{3-}	CDO	BDO_5	TKN	NH_4^+	TP	NO_2^-	SM	Chla
		-	(°C)	(µS/cm)	(mg O_2/L)	(mg P/L)	(mg O_2/L)	(mg O_2/L)	(mg N/L)	(mg N/L)	(mg P/L)	(µg N/L)	(mg/L)	(µg/L)
P0	mean	7.38	21.33	1302	7.88	0.22	3.11	2.63	0.03	0.08	0.36	3.75	0.12	0.21
	SD	0.21	3.59	892.84	0.31	0.07	1.06	0.29	0.02	0.06	0.1	6.59	0.06	0.15
P1	mean	7.24	20.54	1804.75	8.07	0.28	2.37	3.19	0.57	0.07	0.35	0.66	0.12	3.52
	SD	0.23	3.75	55.56	0.2	0.07	0.37	0.73	0.31	0.08	0.11	0.25	0.06	1.54
P2	mean	7.62	20.45	3668.91	1.72	3.2	40.17	2.6	25.41	0.18	5.1	46	14.63	35.69
	SD	0.32	3.89	424.95	1.5	0.94	6.42	0.7	9.68	0.14	1.96	30.29	6.03	7.53
P3	mean	7.3	21.1	4164.91	5.59	1.82	31.98	8.53	27.54	3.79	3.37	10.53	13.33	21.45
	SD	0.31	3.99	1248.34	0.51	0.85	6.25	2.06	5.26	1.31	1.63	2.61	3.09	6.63
P4	mean	8.05	20.99	6088.64	4.54	1.61	35.82	10.82	27.54	0.55	2.17	6.37	19.53	25.7
	SD	0.1	3.57	1287.3	0.63	1.01	6.6	2.62	5.26	0.31	1.1	1.32	4.28	10.83
P5	mean	7.93	20.46	4339.18	5.11	2.87	49.72	2.41	4.86	0.72	2.79	0.4	15.23	9.72
	SD	0.14	4.67	584.55	1.66	1.44	27.99	4.72	8.14	2.93	1.54	10.82	5.78	4.42
P6	mean	7.94	21.19	4260.45	1.29	3.94	122.09	27.07	27.54	10.61	5.82	25.06	19.73	14.73
	SD	0.16	4.16	1264.1	0.44	1.05	13.13	4.4	5.26	5.64	1.46	15.65	3.8	3.73
P7	mean	8.05	20.99	6088.64	0.64	3.97	153.45	37.38	27.54	15.68	5.82	46.02	19.3	6.17
	SD	0.1	3.33	1226.34	0.4	1.29	31.11	13.27	5.43	4.69	1.54	12.02	4.58	1.86
	F	36.3983	0.0905	50.0537	135.7999	12.0848	80.0624	53.7081	30.2015	55.1007	19.2340	27.8845	13.2158	53.1899
	Pr > F	< 0.0001	0.9987	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Significatif	Oui	Non	Oui	Oui	Oui	Oui	Oui	Oui	Oui	Oui	Oui	Oui	Oui

P0 : Ain Joumaa Source; P1 : Bouskoura Source; P2 : Noukhayla; P3: Laghchiwa bridge; P4 : Oulad Malek; P5 : Sidi Ayyad; P6: Nassim Agglomeration; P7: Z.I Lissassfa-Sidi Maârouf zone. //SD: Standard deviatio

Table 7: Taxonomic list and relative abundance of diatom species and varieties inventoried in the Bouskoura watercourse.

Family	Genera	Species	Acronym	P 0	P 1	P 2	P 3	P 4	P 5	P 6	P 7
Monoraphideae	Achnanthidium	*Achnanthes minutissima* Kützing var. minutissima	AMIN	29.7	24.6	2.59	2.48	16.8	11.5	1.8	
	Planotihidium	*Achnanthes lanceolata* (Breb.) Grunow	ALAN	7.2							
	Achnanthes	*Achnanthes lanceolata* (Breb.) Grunow var. *elliptica Cleve*	ALAE		3.2		0.45				
	Coconieis	*Cocconeis placentula* Ehrenberg var. *placentula*	CPLA	4.65	17.9				5.9		
Araphideae	Diatoma	*Diatoma problematica* Lange-Bertalot	DPRO				5.7		1.2		1.2
		Diatoma tenuis C. Agardh	DITE						0.8		
	Fragilaria	*Fragilaria ulna* (Nitzsch.) Lange-Bertalot	FULN		2.05			5.2			
	Ctenophora	*Fragilaria pulchella* (Ralfs ex Kütz.) Lange-Bertalot (Ctenophora)	FPUL	0.45			5.2				
Naviculaceae	Cembella	*Cymbella affinis* Kützing	CAFF	13.46	7.28			9.8	22.9		
	Reimeria	*Cymbella sinuata* Gregory	CSIN		5.9						
	Cistula	*Cymbella cistula* (Ehrenberg) Kirchner	CCIS				1.2				
	Cembopleura	*Cymbella cuspidata* Kützing	CCUS	6.73	0.5				0.6		
	Cymbella	*Cymbella amphicephala* Naegeli ex Kützing	CAPH		0.5				8.6		
	Amphora	*Amphora pediculus* (Kützing) Grunow	APED	6.03				18.4	7.9		
		Amphora veneta Kützing	AVEN		0.45	0.56		3.2	3.4	5.8	
		Amphora coffeaeformis (Agardh) Kützing	ACOF							4.03	
		Amphora ventricosa Gregory	AVNT			0.4					
	Gomphonema	*Gomphonema parvulum* (Kützing) Kützing	GPAR					11.6	11.5		3.3
		Gomphonema angustum Agardh	GANT				2.9	3.2			
		Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	GPUM						0.9		
		Gomphonema tergestinum (Grunow in Van Heurck) Schmidt in Schmidt & al.	GTER					1.2			
		Gomphonema gracile Ehrenberg	GGRA		2.8		1.3				
		Gomphonema gracile Ehrenberg	GMIN	0.15	0.5				0.4		
	Navicula	*Navicula cryptocephala* Kützing	NCRY		12.6						
		Navicula radiosa Kützing	NRAD				0.45				
		Navicula tenelloides Hustedt	NTEN					2.9			
		Navicula recens (Lange-Bertalot) Lange-Bertalot	NRCS				10.4			3.2	
		Navicula cincta (Ehr.) Ralfs in Pritchard	NCIN					5.7			
		Navicula hungarica Grunow	NHUN			6.26					
		Navicula capitata Ehrenberg (=Hippodonta)	NCAP				10.17			12.5	
		* *Navicula radiosa*	NRAD		0.35						
		Navicula tripunctata (O.F.Müller) Bory	NTPT	0.43							
		Navicula rhynchocephala Kützing	NRHY								
		Navicula schroeteri Meister	NSHR				0.13			4.1	
		Navicula concentrica Carter in Carter & Bailey-Watts	NCCT								1.8
	Reimeria	*Reimeria sinuata* (Gregory) Kociolek & Stoermer	RSIN	5.5			8.4		5		
	Diplonieis	*Diploneis ovalis* (Hilse) Cleve	DOVA					5.2			

Table Cont....

Family	Genera	Species	Acronym	P 0	P 1	P 2	P 3	P 4	P 5	P 6	P 7
	Amphipleura	*Amphipleura pellucida* Kützing	APEL						2.5		
	Gyrosigma	*Gyrosigma attenuatum* (Kützing) Rabenhorst	GYAT					0.8	3.6		
	Caloneis	Caloneis amphisbaena (Bory de Saint Vincent) Cleve	CAMP		5.6						
	Rhoicosphenia	*Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot	RABB	0.93		3.8					
Surirellaceae	Cymatopleura	*Cymatopleura solea* (Brebisson) W.Smith	CSOL		1.2						
	Surirella	Surirella terricola Lange-Bertalot & Alles in Lange-Bertalot & al.	STER			0.3					1.6
Nitzschiaceae	Bacillaria	*Bacillaria paradoxa* Gmelin in Linneaeus	BPAR			0.3				1.7	
	Tryblionella	*Nitzschia constricta* (Kützing) Ralfs in Pritchard	NCOT	5.8	2.43			0.6			3.8
	Nitzschia	*Nitzschia frustulum* (Kützing) Grunow	NIFR		0.43		12.6	0.2		6.9	8.3
		Nitzschia dissipata (Kützing) Grunow ssp.dissipata	NDIS	25.99	0.49						
		Nitzschia palea (Kützing) W.Smith	NPAL			27			3.1	30	33.7
		Nitzschia capitellata Hustedt in A.Schmidt & al.	NCPL			17.4	23.3			20.4	22.9
		Nitzschia amphibia Grunow	NAMP			7.78	9.6				5.6
		Nitzschia microcephala Grunow in Cleve & Moller	NMIC			1.2				1.6	12.6
		Nitzschia hungarica Grunow	NIHU						0.4		
		Nitzschia inconspicua Grunow	NINC								3.89
		Nitzschia denticula Grunow in Cleve & Grunow var. denticula	NDEN						2.7		

P0: source Ain Joumaa, P1: sources Bouskoura, P2: Mounkhaila, P3: Pont Laghchiwa, P4: Oulad Malek, P5: SidiAyyad, P6: Agglomérations Nassim P7: Zone Industrielle Lissassfa-Sidi Maârouf.

- The specific composition varies from one station to another and also within each family of diatomic flora. Nevertheless one can note a clear dominance of the species belonging to the Naviculaceae in the majority of the stations of the Bouskoura stream.

Diversity, structure and ecology of the diatom assemblage: The values of the average species richness S (Table 9) show an uptrend from sources P0 and P1 up to P5 (Sidi Ayyad). Beyond this, one notes a fall of this index. The average species richness S varies from 10 to 18 species. The highest value is noted at the P1 station (Bouskoura source) and the lowest at the P2 and P6 stations which are characterized by an untreated domestic pollutant load. It is also in these two stations that we noted the total absence of Araphidae. Similar results have been reported in the work of Slim et al. (2013) on the Hasbani watercourse (South Lebanon).

The mean values of the Shannon-Weaver H' diversity index range from 2.63 at the P6 station to 3.49 at the P5 station.

With the exception of the two stations P6 and P7, where the averages H' are the lowest (respectively 2.63 bits and 2.76 bits), this index is greater than 2.9 bits for most of the study stations, thus reflecting a homogeneous distribution of individuals within the species that make up the diatomic flora of Bouskoura stream. The values of the Shannon-Weaver diversity index H' in the Bouskoura stream are similar to those obtained by Letàkova et al. (2018) in 13 watercourses of the Morava basin (Czech Republic), by Gomà et al. (2005) in the upper Segre basin (Spain) but remain higher than those obtained by Nehar et al. (2015) at El-Hammam stream (Algeria) where H' does not exceed 2.5 bits and to those obtained by Timizel et al. (2017) in Pazarsuyu stream (Turkey) where H' varied from 0.81 to 0.85 bits.

Referring to Wilhm's classification (1975), given in Table 2, stations P2, P6 and P7 would be moderately polluted while P0, P1, P3, P4 and P5 would be little or even unpolluted.

In Chícamo Stream (S-E Spain), peak diversity and species richness of the diatom communities were linked to discharge, depth, dissolved oxygen and sulfate concentrations (Ros et al. 2009). According to Patrick (1971), algal species richness and diversity increase, in general, with temperature, although this is modulated by the climatic characteristics of the basins.

In general in Bouskoura stream, it can be noted that the spatial variation of the specific diversity H' did not underline the full extent of the deterioration of the water quality, because it is noted that despite a significant inflow of raw wastewater into the P3 station (Oulad Ben Ammor) the diversity index H' showed a marked increase.

As for the diversity index, evenness (E) shows an increasing evolution from upstream (stations P0 and P1) until to station P5 (where it reaches 0.92) and then decreases downstream of this station to reach the value of 0.79 in P6 and P7. It is often accepted that evenness values equal or greater than 0.8 are synonymous with a structured and balanced population (Daget 1976). From the results of the present study, it can thus be said that globally the diatomic

Table 8: Taxonomic richness of diatomic assemblage in some Mediterranean rivers and streams.

Watercourse	Country	Taxonomic richness	Reference
Verdon river	France	38 taxa	Millerioux et al. (1981) in Cazauban & Badri (1991)
Upstream of Sebou river	Morocco	47 species	Fekhaoui et al. (1988)
Boufekrane stream	Morocco	5 orders, 8 families et 53 species	Hammada et al. (1996)
Tensift river	Morocco	73 taxa	Cazauban & Badri (1991)
Hassar stream	Morocco	130 taxa	Fawzi et al. (2002)
Akçay stream	Turkey	78 taxa	Solak (2007)
Ul river	Potugal	94 taxa (27 genera)	Resende et al. (2010)
Upper Porsuk Creek	Turkey	57 taxa	Solak (2011)
Felent creek	Turkey	117 taxa	Solak et al. (2012)
Murat stream	Turkey	75 taxa	Tokatlı & Dayıoğu (2014)
El-Hammam stream at Mascara	Algeria	44 taxa	Nehar et al. (2015)
Clarà stream	Spain	59 to 74 species	Burfeid Castellanos (2018)
Salada stream	Spain	15 to 58 species	
Araban-Yavuzeli catchment	Turkey	75 taxa	Çelekli & Bilgi (2019)
Tohma stream	Turkey	70 taxa	Yildirim & Baran (2019)
Bouskoura stream	Morocco	5 Orders, 5 Families and 57 Species	Present study

Table 9: Values of ecological indicators in Bouskoura stream stations (data derived from OMNIDA software).

stations		P0	P1	P2	P3	P4	P5	P6	P7
Diversity and structure of diatom assemblage	Species richness (S)	13	18	10	15	14	17	10	11
	Shannon-Weaver diversity index (H ‘)	2.99	3.27	2.9	3.29	3.42	3.49	2.63	2.76
	Evenness (E)	0.85	0.86	0.87	0.92	0.92	0.92	0.79	0.79
Ecology of dominant diatom taxa according to Van Dam et al. (1994)	pH	4-Alkaliphilous mainly occurring at pH >7	4-Alkaliphilous mainly occurring at pH >7	4-Alkaliphilous mainly occurring at pH >7	4-Alkaliphilous mainly occurring at pH >7	4-Alkaliphilous mainly occurring at pH >7	4-Alkaliphilous mainly occurring at pH >7	4-Alkaliphilous mainly occurring at pH >7	4-Alkaliphilous mainly occurring at pH >7
	Salinity	2-Fresh to brackish	2-Fresh to brackish	2-Fresh to brackish	2-Fresh to brackish	2-Fresh to brackish	2-Fresh to brackish	2-Fresh to brackish	2-Fresh to brackish
	Nitrogen Uptake metabolism (N)	2-Nitrogen autotrophic taxa tolerating elevated levels of organically bound nitrogen	2-Nitrogen autotrophic taxa tolerating elevated levels of organically bound nitrogen	4-Obligately nitrogen-heterotrophic taxa, needing continuously elevated concentration of organically bound nitrogen	4-Obligately nitrogen-heterotrophic taxa, needing continuously elevated concentration of organically bound nitrogen	2-Nitrogen autotrophic taxa tolerating elevated levels of organically bound nitrogen	2-Nitrogen autotrophic taxa tolerating elevated levels of organically bound nitrogen	4-Obligately nitrogen-heterotrophic taxa, needing continuously elevated concentration of organically bound nitrogen	4-Obligately nitrogen-heterotrophic taxa, needing continuously elevated concentration of organically bound nitrogen
	Oxygen Requirement	O_2 continuously high	moderate (50% saturation)/ low (30% sat.)	moderate (50% saturation)/ low (30% sat.)	moderate (50% saturation)/ low (30% sat.)	O_2 continuously high	O_2 continuously high	very low (>10% sat.)	very low (>10% sat.)
	Saprobity	2-β-mesosaprobous	2-β-mesosaprobous	3-α-mesosaprobous	3-α-mesosaprobous	2-β-mesosaprobous	2-β-mesosaprobous	5-polysaprobous	5-polysaprobous
	Trophic state	5-eutrophentic	5-eutrophentic	5-eutrophentic	5-eutrophentic	5-eutrophentic	5-eutrophentic	6-hypereutrophentic	6-hypereutrophentic

flora of the Bouskoura hydrosystem is poorly structured to well structured and balanced.

According to Van Dam (1994), the diatom communities of the Bouskoura stream indicate mainly alkaliphilic, fresh to brackish conditions. The trophic state is eutrophic (in P, P1 to P5) to hypereutrophic in P6 and P7 (Table 9). Thus,

- In stations close to sources P0 and P1, they are of the N-autotrophic type tolerant of high nitrogen, β-mesosaprobous and eutrophic concentrations. The floristic procession of station P1 is dominated by forms with low ecological weight euryece: *Achnanthes minutissima, Cocconeis placentula*, *Navicula cryptotenella*, *Cymbella affinis*. While at station P0 the most represented taxa are: *Achnanthes minutissima, Nitzschia dissipata, Cymbella affinis* and *Achnanthes lanceolata*.
- At the P2 and P3 stations, the diatomic community is of the obligatory N-heterotrophic type needing continuously elevated concentration of organically bound nitrogen, eutrophic β-mesosaprobous and requiring moderate oxygenation. The diatomic assembly is dominated mainly by the taxa *Nitzschia palea*, *Navicula capitata*, *Nitzschia frustulum* with also (in station P3) *Navicula recens* and *Nitzschia amphibia*.
- In the intermediate stations (P4 and P5) the community of epilithic diatoms is dominated by *Amphora pediculus, Achnanthes minutissima, Gomphonema parvulum, Cymbella affinis* to which the taxon *Cymbella amphicephala* is added in station P5. These assemblies would be the result of a relative improvement in the ecological conditions in these two stations following the self-cleaning phenomenon in the two stations in addition to the phenomenon of dilution by the waters of the tributary of the Bouskoura stream Ain Joumaa. Thus, we go from a mandatory N-heterotrophic diatomic community needing continuously elevated concentration of organically bound nitrogen, eutrophic, β-mesosaprobous and requiring moderate oxygenation. in station P3 to N-autotrophic communities tolerant of high levels of organic nitrogen compounds, dissolved oxygen continuously high, and β-mesosaprobous.
- In stations P6 and P7 (impacted by discharges from Nassim urban agglomerations and from the Lissas-sfa-SidiMaarouf industrial zone), the Nitzschiaceae family represent more than 80% of the individuals surveyed. The benthic diatom communities of these stations are composed of the following taxa: *Nitzschia palea*, *Nitzschia capitellata*, *Nitzschia frustulum*, in addition to *Navicula capitata* in station P6 and *Nitzschia microcephala* in station P7. The diatomic communities is therefore of obligatory N-heterotrophic type needing continuously elevated concentration of organically bound nitrogen, hypereutrophic, polysaprobous and very little oxygen requirement.

Jaccard similarity coefficient (1901): Overall, the values of this coefficient taken as the index of similarity between pairs of readings are low (Table 10); they do not exceed 30%. The high values of this index can be observed either between the P0, P1, P4 and P5 stations which are not very polluted (or showing a certain recovery of their physicochemical quality) or between polluted stations with highly polluted (P2, P3, P6 and P7). This result confirms the relatively high degree of differentiation of the diatomic communities between these two groups. In the sense of De Bello et al. (2007), we can say in this case that, for each pair of habitats compared, the species are almost completely different indicating different environmental conditions that determine a turnover of important species.

Diatomic community and water quality: The evolution of the % PTV index along Bouskoura stream seems to respond to changes in organic pollution in this environment (Fig. 2). Thus, in the P0, P1, P4 and P5 stations, the values of this index are less than 20%, which suggests that organic pollu-

Table 10: Similarity of the diatom assemblage between the different stations of the Bouskoura stream according to the Jaccard index (1902) expressed as a percentage.

	P0	P1	P2	P3	P4	P5	P6	P7
P0								
P1	29.17							
P2	4.55	7.41						
P3	7.70	10.00	13.04					
P4	17.39	23.08	8.70	11.54				
P5	24.00	24.14	11.54	6.45	23.08			
P6	4.35	11.54	29.41	30.00	13.64	11.54		
P7	7.60	7.41	29.41	18.18	13.64	11.54	22.22	

tion would be absent (especially in the first two stations) at very low especially in P4 and P5 stations where the effect of a self-purification phenomenon is noted. On the other hand, the % PTV values in P2 and P3 stations indicate (according to the Kelly 1998 classification) that the organic pollution generated respectively by the untreated wastewater from the Oulad Saleh (P2) industrial zone and the Oulad Malek and Oulad Ammor (P3) is likely to contribute significantly to eutrophication. In the stations located downstream of Bouskoura stream (S6 and S7) the contamination of water by organic pollution is very high and the % PTV is of the order of 80% at station S6 because of the inputs of wastewater from urban agglomerations of the Nassim city. This index is close to 100% in the Lissassfa-Sidi Maârouf industrial zone (station S7). The pollutant-tolerant diatomic community is composed mainly of polysaprobic species belonging mainly to the family of Nitzschiaceae.

The results of this study are consistent with those of Jarvie et al. (2002) who observed increases in the % PTV index downstream of wastewater treatment facilities indicating that organic pollutants contributed significantly to the observed eutrophic conditions. Fore & Grafe (2002) found that the number of eutrophic species (% PTV) correlated significantly with human disturbance.

In addition, changes in the %PTV index appear to be in good agreement with the results of organic load analyses (in particular BOD_5, COD and dissolved oxygen) in Bouskoura stream. The correlation test between the organic pollution index (OPI) and the four biological indices [species richness S, specific diversity H', regularity or equitability E and the percentage of tolerant valves (%PTV)] of Table 11 shows that the best correlation is obtained between %PTV and the IPO index. This correlation is highly significant ($p < 0.01$). This result means that changes in diatom communities would be the result of changes in nutrient and organic inputs.

Typology of diatomic communities in Bouskoura stream: Principal Component Analysis (PCA), applied to a matrix of 28 diatom taxa (with a cumulative relative abundance greater than or equal to 5%) and 8 stations, allowed to visualize the main features of the spatial distribution of these taxa (Fig. 3). The first two factor axes of the PCA represent nearly half of the total information expressed by the data matrix.

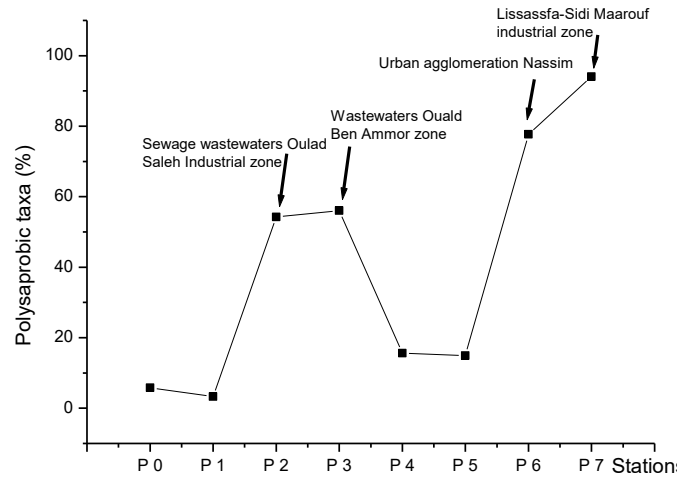


Fig. 2: Evolution of the percentage of tolerant valves (%PTV) at the Bouskoura stream stations.

Table 11: Spearman rank correlation coefficient calculated between diatom specific richness (S), specific diversity (H'), Pielou index (E) and the per cent pollution-tolerant valves (%PTV) along the Bouskoura stream during the study period.

	Richness (S)	Diversity (H')	Eveness (E)	% PTV	OPI
Richness (S)	1.00	-	-	-	-
Diversity (H')	0.85**	1.00	-	-	-
Eveness (E)	0.62	0.91**	1.00	-	-
% PTV	-0.72*	-0.72*	-0.60	1.00	
OPI	0.59	0.55	0.45	-0.94**	1.00

*significatif Correlations at $p < 0.05$
**significatif Correlations at $p < 0.01$

Axis 1 alone accounts for 30.84% of the total information. The stations are distributed along this axis according to an increasing pollution gradient (from left to right), with, at one end, the unpolluted stations (P0 and P1 stations) or reflecting the sewerage effect (P4 and P5) and at the other end the stations subjected to the direct influence of the discharges of human activities (P2, P3, P6 and P7).

The 28 species of epilithic algae are distributed in the F1x F2 plane according to a sensitivity (or resistance) gradient to pollution, represented by the axis 1. Thus, three groups of taxa become individualized in this plane (Fig. 3).

Group 1 contains the diatomic species and varieties of the sources (P0 and P1 stations) characterized by well-oxygenated waters and with low organic and trophic pollution. The diatomic communities of these stations are dominated by pollution-sensitive taxa. These are *Achnanthese minutissima* (AMIN), *Cocconeis placentula* (CPLA), *Nitzschia constricta* (NCOT), *Achnanthes lanceolata* (ALAN), *Cymbella cuspidata* (CCUS), *Navicula cryptotenella* (NCRY), *Cymbella sinuata* (CSIN), *Amphora ventricosa (*AVNT), *Ntizschia dissipata (*NDIS). Among these taxa, AMIN and CPLA show the strongest correlations with factorial axis 1 (0.87 and 0.59 respectively). Walsh & Wepener (2009) reported that along the Crocodile and Magalies Rivers, reference or natural sites showed strong associations of *A. minutissima* with *C. placentula*.

The results of the work on certain taxa of this group such as *Achnanthes lanceolata*, *Navicula cryptotenella* and *Ntizschia dissipata* by Juttner et al. (1996), *C. placentula* by Juttner et al. (1996), Sabanci (2014), Becer et al. (2017) corroborate with those obtained in this work.

Group 2 is located on the "polluted" side of the gradient and is composed of species characteristic of environments strongly impacted by organic matter and showing an oxygen deficit. It is in this group that most of the taxa belonging to the family Nitzschiaceae are found. *Nitzschia capitellata* (NCPL), *Nitzschia frustulum* (NIFR), *Nitzschia amphibia* (NAMP) and *Navicula recens* (NRCS) have the largest contributions to the factorial axis 1 and the strongest correlations to it (i.e. 0.97, 0.86, 0.81 and -0.72 respectively). In Mediterranean streams, NPAL and NIFR are described as type-specific for river sections affected by intensive agricultural and industrial activities (Tornés et al. 2007) and as highly tolerant and resistant to organic pollution (Vidal & Gentili 2000, Fawzi et al. 2001, Fawzi et al. 2002, Tornés et al. 2007).

In addition to these species, group 2 includes *Nitzschia microcephala* (NMIC), *Reimeria sinuata* (RSIN)*, Navicula hungarica* (NHUN), *Navicula capitata* (NCAP), *Diatoma problematica* (DPRO), *Fragilaria pulchella* (FPUL) and *Nitzschia palea* (NPAL). The last 4 species have correlations with the F1 axis equal to -0.67; -0.65; -0.60 and -0.54 respectively. These pollution-tolerant species characterize the stations where there is strong organic pollution of domestic and/or industrial origin, materialized by low concentrations in oxygen but very high in COD, BOD_5, NO_2^-, NH_4^+, PO_4^{3-} and total phosphorus.

The *Nitzschia capitellata* (NCPL), *Nitzschia frustulum* (NIFR), *Nitzschia amphibia* (NAMP) and *Navicula recens* (NRCS) taxa have the largest contributions to the factorial axis 1 as well as the strongest correlations to it (i.e. 0.97, 0.86, 0.81 and -0.72 respectively).

The same results were obtained for *Navicula recens* by Ponsati et al. (2016), *Nitzschia capitellata* by Szczepocka et al. (2018); *Nitzschia frustulum* by Taylor et al. (2007a), Gomà et al. (2004), Ponsati et al. (2016) and Demir (2019); *Nitzschia palea* by Juttner et al. (1996), Gomà et al. (2004), Rimet et al. (2004), Bere et al. (2014), Nehar et al. (2015), Ponsati et al. (2016), Szczepocka et al. (2018), Letáková (2018); and *Reimeria sinuata* by Jakovljeviǧet al. (2016) and Ponsati et al. (2016).

The assembly of group 3 taxa reflects the environmental remediation effect observed in the P4 and P5 stations following the self-purification and/or water dilution of the Bouskoura mainstream by the waters of its tributary Ain Joumaa.

The group 3 is composed of the following taxa: *Cymbella affinis* (CAFF), *Amphora pediculus* (APED), *Fragilaria ulna* (FULN). These 3 taxa show the strongest correlations with the F1 axis (0.74, 0.63 and 0.55 respectively). In this group, we also find the following taxa: *Cymbella amphicephala* (CAPH), *Amphora veneta* (AVEN), *Gomphonema parvulum* (GPAR) as well as *Navicula cincta* (NCIN) and *Diploneis ovalis* (DOVA).

The work of Kivrak & Uygun (2012) showed that in the Turkish Akarçay stream (highly polluted), *Amphora pediculus, Amphora veneta* and *Cocconeis placentula* are dominant upstream of the polluted area. Becer et al. (2017) reported that *Cymbella affinis* is frequently developed as benthic, especially in rivers and is dominant in clean waters.

Name of diatom taxa: AMIN: *Achnanthese minutissima*, CPLA: *Cocconeis placentula*, NCOT: *Nitzschia constricta*, ALAN: *Achnanthes lanceolata*, CCUS: *Cymbella cuspidate*, NCRY: *Navicula cryptotenella*, CSIN: *Cymbella sinuata*, AVNT: *Amphora ventricosa*, NDIS: *Ntizschia dissipata*, NCPL: *Nitzschia Capitellata*, NIFR: *Nitzschia frustulum*, NAMP: *Nitzschia amphibia*, NRCS: *Navicula recens***,** NMIC: *Nitzschia microcephala*, RSIN: *Reimeria sinuata*, NHUN: *Navicula hungarica*, NCAP: *Navicula capitata*, DPRO: *Diatoma problematica*, FPUL: *Fragilaria*

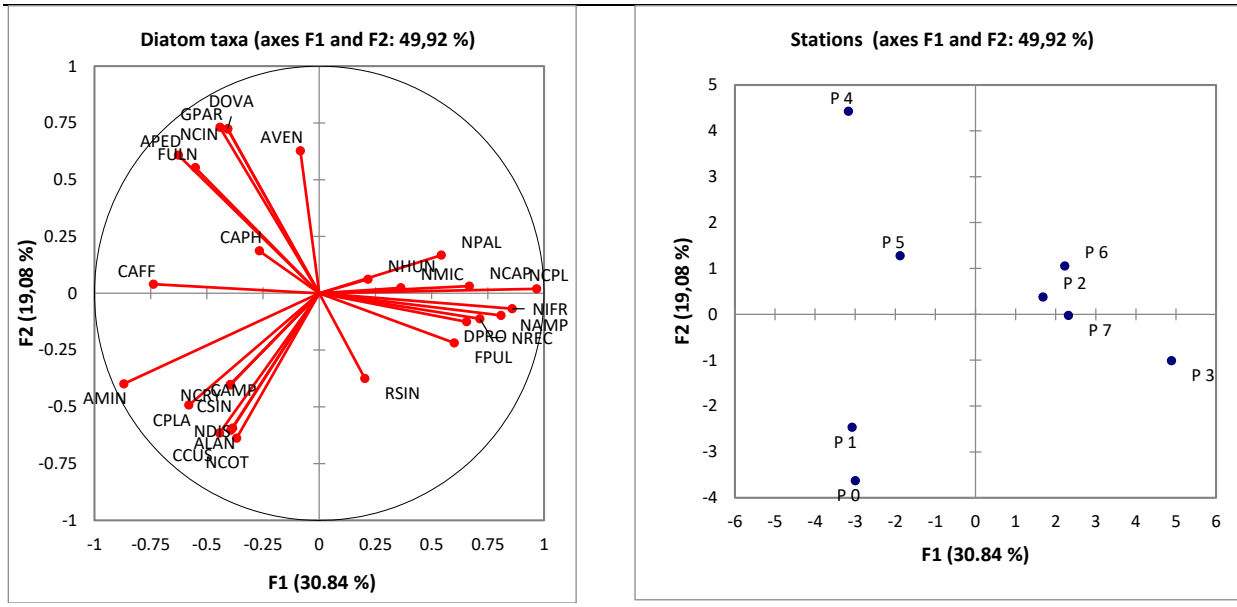


Fig. 3: projections on the factorial plane F1xF2 of the ACP of the species and varieties of diatoms (ǧ5% of cumulative relative abundance) (on the left) and of the 8 stations prospected in the Bouskoura stream (on the right).

pulchella, NPAL: *Nitzschia palea*, CAFF: *Cymbella affinis*, APED: *Amphora pediculus*, FULN: *Fragilaria ulna*, CAPH: *Cymbella amphicephala*, AVEN: *Amphora veneta*, GPAR: *Gomphonema parvulum*, NCIN: *Navicula cincta*, DOVA: *Diploneis ovalis*

Station names: P0: Ain Joumaa Source, P1: Bouskoura Sources, P2: Noukhayla, P3: Laghchiwa, P4: Oulad malek, P5: Sidi Ayyad P6: Nassim Agglomerations, P7: Lissasfa Sidi maarouf Industrial Zone.

Correlation between diatomic flora and physicochemical parameters of water: Table 12 presents the Spearman rank correlation coefficient (for $p < 0.05$ and $p < 0.01$) calculated between some physicochemical variables of water and the average relative abundance of taxa (with a cumulative relative abundance greater than or equal to 5%)

The *Achnanthese minutissima* (AMIN), *Cocconeis placentula* (CPLA) and *Cymbella affinis* (CAFF) taxa appear to develop better when pollution parameters are low. Indeed, these three species are negatively correlated with all the pollution parameters, in particular the KTN, with which they have significant correlations ($p < 0.05$).

In the Bouskoura stream, the relative abundance of the diatom *A. minutissima* shows highly significant ($p < 0.01$) and positive correlations with dissolved oxygen but negative correlations with phosphorus elements. Moreover, the correlations of this species with the three pollution indicators (SM, COD and nitrites) are significant ($p < 0.05$). In accordance with the works of Prygiel & Coste (1993), Leclercq et al. (1996) and Abd El-Karim (2014), this species is therefore low organic matter content indicator.

C. placentula can tolerate mesotrophic to eutrophic conditions (Van Dam et al. 1994, Taylor et al. 2007b). The findings of this study are in agreement with the findings of Kivrak & Uygun (2012), who found that in Akarçay stream (Turkey), *C. placentula* is prevalent at upstream with clean, freshwaters and well-oxygenated sites.

In contrast, the relative abundance of *N. palea* (NPAL) shows highly significant ($p < 0.01$) and negative correlation with oxygen, but positive with total phosphorus, orthophosphate, COD and nitrites. The same species shows a positive correlation and significant ($p < 0.05$) for BOD_5 and ammonium. Kivrak & Uygun (2012) have shown that in the Akarçay stream, the abundance of this taxon is strongly and positively correlated with BOD_5, COD and nutrients. In the Seydisuy stream basin (Turkey), *N. palea* is highly correlated ($p < 0.01$) with nitrites (Atici et al. 2018).

Nitzschia capitellata (NCPL) shows a significant ($p < 0.05$) and negative correlation with dissolved but positive oxygen with three KTN pollution parameters, nitrites and total phosphorus. The correlation with this last parameter is highly significant ($p < 0.01$). According to Krammer & Lange-Bertalot (1986), Van Dam et al. (1994) and Rimet et al. (2004) this taxon is α-meso-polysaprobic to polysaprobic and eutrophic.

Nitzschia microcephala (NMIC) also has a negative but insignificant correlation with dissolved oxygen. The

correlations of the abundances of this taxon with the pollution parameters are positive, in particular the COD and the BOD_5 with which these correlations are significant ($p < 0.05$) and the ammonium NH_4^+ with which this correlation is highly significant ($p < 0.01$). According to Van Dam et al. (1994), *N. microcephala* (NMIC) characterizes eutrophic and α-mesosaprobic media. NMIC also shows a negative correlation with dissolved oxygen. The correlations of the abundances of this taxon with the pollution parameters are positive, in particular, the DOC and BOD_5 with which these correlations are significant ($p < 0.05$) and the ammonium NH_4^+ with which this correlation is highly significant ($p < 0.01$).

CONCLUSIONS

This work is a first ecological study conducted on the diatomic communities of Bouskoura stream which are subjected to natural and anthropogenic disturbances. At the end of this study, the following conclusions can be drawn.

- The evolution of the average environmental descriptors has shown that the waters of this ecosystem are characterized by significant mineralization and an increasing gradient (upstream-downstream) of organic pollution linked to discharges of untreated liquid discharges from domestic, industrial and agricultural sources. In view of the physicochemical analyses, the situation would

Table 12: Spearman rank correlation coefficient calculated between diatom taxa and physicochemical parameters of Bouskoura stream waters.

Taxa	pH	T	CE	O_2	SM	PO_4^{3-}	DCO	BOD_5	NTK	NH_4^+	PT	NO_2^-	Chl. a
AMIN	-0.44	0.06	-0.66	0.86**	-0.73*	-0.89**	-0.73*	-0.58	-0.81*	-0.64	-0.94**	-0.72*	-0.35
ALAN	-0.36	0.52	-0.62	0.49	-0.66	-0.55	-0.38	-0.28	-0.54	-0.26	-0.52	-0.28	-0.44
CPLA	-0.53	-0.43	-0.64	0.69	-0.65	-0.62	-0.51	-0.44	-0.80*	-0.41	-0.69	-0.54	-0.53
DPRO	-0.32	0.18	0.17	0.09	0.18	0.02	-0.02	-0.00	0.29	0.11	0.11	-0.09	0.04
FULN	0.21	-0.03	0.29	0.23	0.15	-0.37	-0.29	-0.13	0.10	-0.33	-0.39	-0.36	0.49
FPUL	-0.49	0.30	-0.01	0.22	-0.01	-0.16	-0.20	-0.13	0.26	-0.03	-0.02	-0.17	0.10
CAFF	0.08	-0.25	-0.22	0.55	-0.13	-0.38	-0.43	-0.53	-0.71*	-0.54	-0.61	-0.70	-0.27
CSIN	-0.52	-0.40	-0.50	0.52	-0.56	-0.53	-0.39	-0.26	-0.52	-0.26	-0.52	-0.35	-0.35
CCUS	-0.38	0.45	-0.66	0.55	-0.69	-0.58	-0.42	-0.33	-0.62	-0.31	-0.57	-0.35	-0.49
CAPH	0.26	-0.52	0.06	0.14	0.24	0.14	-0.06	-0.30	-0.42	-0.24	-0.11	-0.38	-0.21
APED	0.42	0.09	0.32	0.23	0.27	-0.27	-0.28	-0.24	-0.04	-0.41	-0.39	-0.47	0.41
AVEN	0.61	0.06	0.33	-0.33	0.47	0.42	0.34	0.23	0.21	0.14	0.29	-0.13	0.21
GPAR	0.65	-0.25	0.57	-0.01	0.59	0.10	0.02	-0.08	0.00	-0.18	-0.11	-0.32	0.29
NCRY	-0.52	-0.40	-0.50	0.52	-0.56	-0.53	-0.39	-0.26	-0.52	-0.26	-0.52	-0.35	-0.35
NREC	-0.37	0.36	0.07	0.04	0.10	0.03	-0.02	0.05	0.40	0.13	0.17	-0.09	0.12
NCIN	0.42	0.13	0.49	0.03	0.37	-0.17	-0.14	-0.03	0.30	-0.23	-0.19	-0.23	0.64
NHUN	-0.07	-0.50	-0.07	-0.37	-0.03	0.26	-0.11	-0.28	0.24	-0.26	0.34	0.60	0.53
NCAP	-0.07	0.47	0.09	-0.24	0.18	0.31	0.30	0.33	0.46	0.37	0.41	0.04	0.05
RSIN	-0.46	0.26	-0.27	0.49	-0.20	-0.32	-0.39	-0.40	-0.25	-0.27	-0.31	-0.47	-0.23
DOVA	0.42	0.13	0.49	0.03	0.37	-0.17	-0.14	-0.03	0.30	-0.23	-0.19	-0.23	0.64
CAMP	-0.52	-0.40	-0.50	0.52	-0.56	-0.53	-0.39	-0.26	-0.52	-0.26	-0.52	-0.35	-0.35
NCOT	-0.25	0.41	-0.42	0.34	-0.57	-0.44	-0.08	0.10	-0.49	0.11	-0.41	-0.06	-0.66
NIFR	-0.03	0.47	0.36	-0.34	0.34	0.38	0.51	0.59	0.59	0.67	0.51	0.29	-0.07
NDIS	-0.37	0.51	-0.63	0.50	-0.67	-0.56	-0.39	-0.29	-0.55	-0.27	-0.53	-0.29	-0.45
NPAL	0.50	0.00	0.39	-0.91**	0.42	0.85**	0.84**	0.73*	0.56	0.75*	0.89**	0.92**	0.06
NCPL	0.11	0.23	0.39	-0.72*	0.40	0.68	0.66	0.63	0.76*	0.71	0.84**	0.75*	0.15
NAMP	-0.23	-0.06	0.23	-0.35	0.20	0.31	0.14	0.10	0.56	0.20	0.48	0.57	0.33
NMIC	0.46	0.13	0.51	-0.62	0.40	0.56	0.80*	0.82*	0.37	0.85**	0.58	0.69	-0.24

* : significatif Correlations at $p < 0.05$
** : significatif Correlations at $p < 0.01$
Name of diatom taxa abbreviated as per Table 3

be alarming in the stations located downstream of the watercourse (in particular stations P6 and P7). However, there is a tendency to improve the quality of the water downstream of the P4 site and upstream of the P5 site following the dilution of the pollutant load by the waters of the Bouskoura tributary (Ain Joumaa) as well as the water treatment process self-purification;

- The diatomic flora is predominantly alkaliphilic and characterizes β-mesoseprobes to polysaprobes, eutrophic to hypereutrophic media. It has a total of 54 species and varieties corresponding to 27 genera and belonging to 5 main families (the Monoraphids, the Naviculaceae, the Nitzschiaceae, the Aripidae and Surirellaceae) and have no particularity with respect to that observed in the Mediterranean area.
- The change in the percentage of pollutant-tolerant taxa was the best indicator of the magnitude of the impact of environmental stress and human activities on the natural waters of the Bouskoura watercourse.
- The exploitation of the data of this study confirms that the diatoms are excellent indicators of the overall water quality of Bouskoura stream especially with regard to saprobia and trophic status and this study represents a contribution to a future database on the study of diatoms in Moroccan rivers.

REFERENCES

Becer, Z.A., Yuce, A.M. and Kalyoncu, H. 2017. Epilithic diatom composition and some physico-chemical parameters of the streams in the Çoruh Basin (Turkey). International Journal of Contemporary Research and Review. 8(11): 20252-20257.

Bellinger, B. J., Cocquyt, C. and O'Reilly C.M. 2006. Benthic diatoms as indicators of eutrophication in tropical streams. Hydrobiologia, 573: 75-87.

Benhassane, L., Loudiki, M., Fadlaoui, S., Belhouari A. and Oubraim S. 2019. Monitoring impacts of human activities on Bouskoura stream (periurban of Casablanca, Morocco): 1. Physico-chemistry. Indian Journal of Applied Research, 9(1): 41-49.

Bere, T., Mangadze, T. and Mwedzi, T. 2014. The application and testing of diatom-based indices of stream water quality in Chinhoyi Town, Zimbabwe. Water SA, 40(3): 503-512.

Bey, M.Y. and Ector, L. 2013. Atlas des diatomées des cours d'eau de la région Rhône-Alpes. Direction régionale de l'Environnement, de l'Amenagement et du Logement Rhone-Alpes (ed.), Lyon, pp. 1182.

Boudaoud, Y. and Haddine, A. 2013. Collecte des eaux pluviales du bassin de Bouskoura. Mémoire de projet de fin d'études pour l'obtention du diplôme d'Ingénieur d'Etat en Génie Civil de l'EHTP, pp. 127.

Burfeid Castellanos, A. M. 2018. Ecological Factors and Diatom Diversity at Rivers of the Iberian Mediterranean River Basins: Macro-scale, Meso-scale and Micro-scale. PhD Thesis of Barcelona University, pp. 233.

Cazaubon, A. and Badri, A. 1994. Influence des variations hydrodynamiques de l'oued Tensift (Maroc) sur les peuplements de Diatomées. Ecologia Mediterranea, XX (112): 97-108.

Çelekli, A. and Bilgi, F. 2019. Bioassessing ecological status of surface waters in the Araban-Yavuzeli catchment (Turkey): application of diatom indices. Turkish Journal of Botany, 43: 597-607.

CEMAGREF 1982. Étude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport Division qualité des eaux Cemagref Lyon. Agence de l'eau Rhône-Méditerranée-Corse, Lyon: 218 p.

Daget, J. 1976. Les modèles mathématiques en écologie. In Masson (ed.), Paris, pp. 172.

De Bello, F., Lepš, J., Lavorel, S. and Moretti, M. 2007. Importance of species abundance for assessment of trait composition: An example based on pollinator communities. Community Ecology, 8(2): 163-170.

Demir, C.T. 2019. The use of phytobenthos for the ecological status assessment in upper Sakarya Basin, Turkey (Turkey). Applied Ecology and Environmental Research, 17(4): 10155-10172.

Ector, L. and Rimet, F. 2005. Using bioindicators to assess rivers in Europe: An overview. In Lek, S., Scardi, M., Verdonschot, P.F.M., Descy, J.P. and Park, Y.S. (ed.) Modelling Community Structure in Freshwater Ecosystems, pp. 7-19, Springer Verlag, Berlin Heidelberg.

Fawzi, B., Chlaida, M., Oubraim, S., Loudiki, M., Sabour, B. and Bouzidi, A. 2001. Application de certains indices diatomiques à un cours d'eau marocain: Oued Hassar. Revue des Sciences de l'Eau/Journal of Water Science, 14(1): 73-89.

Fawzi, B., Loudiki, M., Oubraim, S., Sabour, B. and Chlaida, M. 2002. Impact of wastewater effluent on the diatom assemblages structure of a brackish small stream: Oued Hassar (Morocco). Limnologica, 32: 54-65.

Fekhaoui, M., Hamada, S. and Dakki, M. 1988. Fonctionnemnt de l'Oued Sebou à l'aval de la ville de Fès: étude du peuplement d'algues benthiques. Bulletin de l' Institut Scientifique Rabat, 12: 59-68.

Fièvet, E., Prompt, P. and Martinez, P.J. 2003. Utilisation des indices biologiques pour évaluer l'état écologique des cours d'eau au sens de la Directive Cadre sur l'Eau. Rapport final de l'étude Interagences. Agence de l'eau (ed.), pp.128 + 2 annexes.

Fore, L. S. and Grafe, C. 2002. Using diatoms to assess the biological condition of large rivers in Idaho (USA). Freshwater Biology, 47: 2015-2037.

Gattefosse, J. 1935. Contribution à l'étude de la flore cryptogamique du Maroc (Bryophytes, Algae, Lichens et Fungi). Bull. Soc. Hist. Nat. Afr. N., 26(3): 72-85.

Gauthier, L. 1930. Note d'algologie marocaine. Bulletin de la Société d'histoire naturelle d'Afrique du Nord, 21: 189-192.

Gayral, P. 1954. Recherches phytolimnologique au Maroc. Travaux de l' Institut Scientifique Chérifiènne, série Botanique, 4, pp. 305 + 14 plates.

Golterman H.L. 1971. Methods for Chemical Analysis of Freshwaters. International Biological Programme N° 8, Handbook, Blackwell Scientific (ed.), London, Oxford, pp. 166.

Gomà, J., Rimet, F., Cambra, J., Hoffmann, L. and Ector L. 2005. Diatom communities and water quality assessment in Mountain Rivers of the upper Segre basin (La Cerdanya, Oriental Pyrenees). Hydrobiologia, 551: 209-225.

Goury, M. and Chelhaoui, Y. 2013. Evaluation des scénarios des changements climatiques locaux via le modèle SDSM pour une zone de montagne et une zone de plaine. Mémoire de projet de fin d'études pour l'obtention du diplôme d'Ingénieur d'Etat de l'EHTP, pp. 162.

Hariot, R.L. 1909. Sur une collection d'Algues recueillies au Maroc par Buchet. Bulletin Museum de Paris,15: 128-130.

Jaccard, P. 1901. Etude Comparative de la distribuition florale dans une portion des Alpes et des Jura. Bulletin de la Société Vaudoise des Sciences Naturelles, 7: 547-579.

Jaghror, H., Serieyssol, K., Beauger, A., Loukili, K. and Fadli, M. 2017. Diatom diversity and ecology of the Sebou watershed (Morocco). Biolife, 5(4): 564-577.

Jakovljeviğ O.S., Popoviğ S.S., Vidakoviğ D.P, Stojanoviğ K.Z. and Krizmaniğ2016. The application of benthic diatoms in water quality assessment (Mlava River, Serbia). Acta Botanica Croatica, 75(2): 199-205.

Jarvie, H. P., Lycett, E., Neal, C. and Love, A. 2002. Patterns in nutrient concentrations and biological quality indices across the upper Thames river basin, UK. The Science of the Total Environment, 282-283: 263-294.

Juttner, I., Rothfritz, H. and Ormerod, S.J. 1996. Diatoms as indicators of river quality in the Nepalese Middle Hills with consideration of the effects of habitat-specific sampling. Freshwater Biology, 36: 475-486.

Kelly, M.G. 1998. Use of the trophic diatom index to monitor eutrophication in rivers. Water Research, 32(1): 236-242.

Kelly, M.G. and Whitton, B.A. 1995. The Trophic Diatom Index: A new index for monitoring eutrophication in rivers. Journal of Applied Phycology, 7: 433-444.

Kivrak, E. and Uygun, A. 2012. The structure and diversity of the epipelic diatom community in a heavily polluted stream (the Akarçay, Turkey) and their relationship with environmental variables. Journal of Freshwater Ecology, 27(3): 443-457.

Kollár, J., Fránková, M., Hašler, P., Letáková, M. and Poulíčková, A. 2015. Epiphytic diatoms in lotic and lentic waters – Diversity and representation of species complexes. Fottea, 15(2): 259-271.

Krammer, K. and Lange-bertalot, H. 1986. Bacillariophyceae. Tome 1: Naviculaceae. Semper Bonis Artibus, pp. 876.

Krammer, K. and Lange-bertalot, H. 1988. Bacillariophyceae. Tome 2: Bacillariaceae, Epithemiaceae et Surirellaceae. Semper Bonis Artibus, pp. 596.

Krammer, K. and Lange-bertalot, H. 1991a. Bacillariophyceae. Tome 3: Centrales, Fragilariaceae, Eunotiaceae. Semper Bonis Artibus, pp. 576.

Krammer, K. and Lange-bertalot, H. 2000. Bacillariophyceae (Tome 5). Semper Bonis Artibus. English and French translation of the Keys, pp. 311.

Krammer, K. and Lange-bertalot, H.1991b. Bacillariophyceae. Tome 4: Achnanthaceae. Semper Bonis Artibus, pp. 437.

Lange-Bertalot, H. 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedw., 64: 285-304.

Leclercq, L. and Maquet, B. 1987. Deux nouveaux indices chimiques et diatomiques de la qualité d'eau courante. Application au Samson et à ses affluents (Bassin de la Meuse Belge). Comparaison avec d'autres indices chimiques, biologiques et diatomiques. Inst. Roy. Sci. Nat. Belgique. Document de travail 38: pp. 98.

Leclercq, L. and Vandevenne, L. 1987. Impact d'un rejet d'eau chargée en sel et d'une pollution organique sur les peuplements de diatomées de la Gander (Grand-Duché du Luxembourg). Cahiers de Biologie Marine, 28: 311-317.

Leclercq, L., Rosillon, F. and Van Der Borght, P. 1996. Qualité chimique et biologique du bassin de la Semois. Bulletin français de la pêche et de la pisciculture. 341/342: 81-108.

Letáková, M., Fránková, M. and Poulíčková, A. 2018. Ecology and applications of freshwater epiphytic diatoms – review. Cryptogamie, Algologie, 39(1): 3-22.

Maiffi-Rassat, M. 1988. La flore algale de l'Oued Tensift : Impact des eaux usées de la ville de Marrakech (Maroc). Les diatomées, indicateurs biologiques de la qualité des eaux. Thèse 3èrne Cycle, Univ. Paris, pp. 234.

Menye, E.D., Togouet, S.H.Z., Menbohan, S. F., Kemka, N., Nola, M., Boutin, C., Nguetsop, V.F,. Moussa D. and Njiné, T. 2012. Bio-écologie des diatomées épilithiques de la rivière Mfoundi (Yaoundé, Cameroun): diversité, distribution spatiale et influence des pollutions organiques. Revue des sciences de l'eau / J. Water Sci., 25(3): 203-218.

Morin, S. 2006. Bioindication des effets des pollutions métalliques sur les communautés de diatomées benthiques approches in situ et expérimentales. Thèse de Doctorat de L'Université de Bordeaux 1: 306p.

Morin, S. and Coste, M. 2006. Atlas des Diatomées du Bassin Versant du Riou-Mort. Cemagref: Bordeaux, pp.109.

Mouni, L., Merabet, D., Arkoub, H. and Moussaceb, K. 2009. Etude et caractérisation physico-chimique des eaux de l'oued Soummam (Algérie). Sécheresse, 20 (4): 360-366.

Mounjid, J., Cohen, J., Fadlaoui, S. and Oubraim, S. 2014. Study of physicochemical and microbiological quality of Oued Bouskoura Peri-Urbain of Casablanca, Morocco. Int. Res. J. Environment Sci. International Science, 3(5): 60-66.

Nehar, B., Blanco, S. and Hadjadj-Aoul, S. 2015. Diversity and ecology of diatoms in northwest of Algeria: Case of el-hammam stream and estuary of Cheliff river. Applied Ecology and Environmental Research, 13(1): 37-52.

NF EN 13946 2003. Qualité de l'eau. Guide pour l'échantillonnage en routine et le prétraitement des diatomées benthiques des rivières. AFNOR (ed.), pp. 16.

NF T 90-354 2000. Détermination de l'Indice Biologique Diatomées (IBD). AFNOR (ed.), pp. 64.

Oubraim, S. 2002. Qualité physico-chimique et biologique des cours d'eau du réseau hydrographique de la Meseta Occidentale Marocaine: Cas de l'Oued Mellah. Thèse de doctorat d'état. Faculté des Sciences Ben M'Sik.

Patrick, R. 1971. The effects of increasing light and temperature in the structure of diatoms communities. Limnology and Oceanography, 16: 405-421.

Pielou, E.C. 1975. Ecological Diversity. John Wiley and Sons, New York, pp. 165.

Ponsati, L., Corcoll, N., Petrovic, M., Pico, Y., Ginebreda, A., Tornes, E., Guasch, H., Barcelo, D. and Sabater, S. 2016. Multiple-stressor effects on river biofilms under different hydrological conditions. Freshwater Biology, 61: 2102-2115.

Prygiel, J. and Coste, M. 2000. Guide méthodologique pour la mise en œuvre de l'Indice Biologique Diatomées. NF T 90- 354. Agences de l'Eau-Cemagref, Bordeaux, 134 p.

Prygiel, J., Coste, M. and Bukowska, J. 1999. Review of the major diatom-based techniques for the quality assessment of rivers – State of the art in Europe. In: Use of Algae for Monitoring Rivers III, Prygiel J., Whitton B. A., Bukowska J. (eds.), Agence de l'Eau Artois-Picardie, pp. 224-238.

Quevedo, L., Ibáñez, C., Caiola, N., Cid, N. and Hampel, H. 2018. Impact of a reservoir system on benthic macroinvertebrate and diatom communities of a large Mediterranean river (lower Ebro river, Catalonia, Spain). Limnetica, 37(2): 209-228.

Resende, P.C., Resende, P., Pardal, M., Almeida, S. and Azeiteiro, U. 2010. Use of biological indicators to assess water quality of the Ul River (Portugal). Environ. Monit. Assess., 10p.

Rimet, F., Cauchie H.M., Hoffmann, L. and Ector L. 2005. Response of diatom indices to simulated water quality improvements in a river. Journal of Applied Phycology, 17: 119-128.

Rimet, F., Ector, L., Cauchie, H.M. and Hoffmann, L. 2004. Regional distribution of diatom assemblages in the headwater streams of Luxembourg. Hydrobiologia, 520: 105-117.

Rimet, F., Heudre, D., Matte, J.L. and Mazuer, P. 2006. Communauté de Diatomées des Bassins Moselle, Meuse et Sarre- Correspondance avec les Hydro- Ecorégions. Diren Lorraine, 51pp.

Rimet, F., Matte, J.L. and Mazuer, P. 2006. Analyse de l'Application de Deux Indices Diatomées sur les Cours d'Eau Lorrains. I.B.D. et I.P.S.: Diren Lorraine; 1-21.

Rodier, J. and Legube, B. 2009. L'Analyse de l'eau. 9ème édition, Dunod, Paris, 1526p.

Ros, M.D., Marín-Murcia, J.P. and Aboal, M. 2009. Biodiversity of diatom assemblages in a Mediterranean semiarid stream: Implications for conservation. Marine and Freshwater Research, 60: 14-24.

Round, F.E. 1991. Diatoms in river water-monitoring studies. Journal of Applied Phycology, 3: 129-145.

Round, F.E., Crawford, R. and Mann, D. 1990. The Diatoms. Biology and Morphology of the Genera. Cambridge Univ. Press (ed.), pp. 747.

Sabanci, F.Ç. 2014. Phytoplankton distribution and its relationship to the physico-chemical environment in a coastal lagoon. Ekoloji, 23(90): 61-72.

Shannon, C. E. and Weaver, W. 1963. The Mathematical Theory of Communication, Univ. Illinois Press, pp. 117.

Slim Kamal, Ali Atoui and Mirvat Temsah 2013. Impact des rejets de margines sur la qualité des eaux du Nahr Hasbani (Sud Liban) par référence spéciale aux indices diatomiques. Nature & Technologie, 9: 2-12.

Solak, C.N. 2011. The application of diatom indices in the upper Porsuk Creek Kütahya-Turkey. Turkish Journal of Fisheries and Aquatic Sciences, 11: 31-36.

Solak, C.N., Barinova, S., ÁCS, E. and Dayioğu, H. 2012. Diversity and ecology of diatoms from Felent creek (Sakarya river basin), Turkey. Turk. J. Bot., 36: 191-203.

Solak, C.N., Fehér, G., Barlas, M. and Pabuçcu, K. 2007. Use of epilithic diatoms to evaluate water quality of Akçay stream (Büyük Menderes River) in Mugla/Turkey. Large Rivers, 17(3-4) (Archiv Hydrobiol. Suppl.161(3-4): 327-333).

Srivastava Prateek, Sarika Grover, Jyoti Verma and Ambrina Sardar Khan 2017. Applicability and efficacy of diatom indices in water quality evaluation of the Chambal River in Central India. Environ. Sci. Pollut. Res., 1-22p.

Stenger-Kovácsa, C., Lengyela, E., Crossettib, L.O., Üvegesa, V. and Padisáka, J. 2013. Diatom ecological guilds as indicators of temporally changing stressors and disturbances in the small Torna-stream, Hungary. Ecological Indicators, 24: 138-147.

Stoffnerr, F. 2013. Modélisation du bassin de Bouskoura avec STORM. Méthodologie du développement d'un modèle hydrologique. GIZ AGIRE. http://www.swim-sustain-water.net/fileadmin/resources/5-2013-06-21-SWIM-Modelisation-du-bassin-de-Bouskoura-avec-STORM-version-finale-FS-NH.pdf.

Szczepocka, E., Nowicka-Krawczyk, P. and Kruk, A. 2018. Deceptive ecological status of urban streams and rivers-evidence from diatom indices. Ecosphere, 9(7):e 02310. 10.1002/ecs2.2310, 1-17.

Taylor, J.C., Harding, W.R. and Archibald, C.G.M. 2007b. An illustrated guide to some common diatom species from South Africa. WRC Report No TT 282/07. Water Research Commission, Pretoria, South Africa, 225pp.

Taylor, J.C., Prygiel, J., Vosloo, A., De la Rey, P. A. and Van Rensburg, L. 2007a. Can diatom-based pollution indices be used for biomonitoring in South Africa? A case study of the Crocodile West and Marico water management area. Hydrobiologia, 592: 455-464.

Tazi, O., Fahde, A. and El Younoussi, S. 2001. Impact de la pollution sur l'unique réseau hydrographique de Casablanca, Maroc. Sécheresse, 12(2): 129-134.

Temizel, B., Soylu, E.N. and Marağıoğu, F. 2017. Water quality assessment of the Pazarsuyu Stream based on epilithic diatom communities. Fundam. Appl. Limnol., 190(3): 189-197.

Tokatl, C. and Dayıoğu, H. 2014. Use of biological diatom index to evaluate the water quality of lotic ecosystems: A case study of Murat Stream (Kütahya, Turkey). 4th International Conference on Environment Science and Engineering IPCBEE, IACSIT Press, Singapore, 68: 19-23.

Tornés, E., Cambra, J., Gomà, J., Leira, M., Ortiz, R. and Sabater, S. 2007. Indicator taxa of benthic diatom communities: A case study in Mediterranean streams. Ann. Limnol. Int. J. Lim., 43 (1): 1-11.

Torrisi, M., Rimet, F., Cauchie, H.M., Hoffmann, L. and Ector, L. 2006. Bioindication par les diatomées épilithiques et épiphytes dans la rivière sûre (Luxembourg). Belgian Journal of Botany, 139(1): 39-48.

Van Dam, H., Mertens, A. and Sinkeldam, J. 1994. A coded checklist and ecological indication values of freshwater diatoms from The Netherlands. Netherlands Journal of Aquatic Ecology, 28: 117-133.

Vidal, H. and Gentili, R. 2000. Evolution de la qualite d'un petit cours d'eau mediterraneen, la Bouillide, après rehabilitation d'une station d'epuration. In: Compte rendu du 18ème colloque de l'Association des diatomistes de langue francaise (Cryptogamie Algol.). Ector L., Compère P., Vidal H., Semprini M. & Gentili R. (eds.), 245-246.

Walsh, G. and Wepener, V. 2009. The influence of land use on water quality and diatom community structures in urban and agriculturally stressed rivers. Water SA, 35(5): 579-594.

Werner, R.G. 1949. Contribution à l'étude algologique de quelques rivières marocaines. Le Botaniste, 34: 367-374.

Whitton, B.A. 1991. Aims of monitoring. In: Whitton, B.A., Rott, E. et Friedrich, G. (eds.), Use of Algae for Monitoring Rivers, pp.5-8.

Wilhm, J.L. 1975. Biological indicators of pollution. In: Whitton, B. A. (ed.) River Ecology, Studies in Ecology, Vol. 2, Blackwell Sci. Publ., Oxford, London, Edinburgh, Melbourne, pp. 725.

Yildirim, V. and Baran, N. 2019. The relationship between epilithic diatom communities and water quality variations across Tohma stream (Malatya-Turkey). Fresenius Environmental Bulletin, 28(4 A): 3043-3049.

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Remazol Effluent Treatment in Batch and Packed Bed Column Using Biochar Derived from Marine Seaweeds

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ABSTRACT

The release of textile effluents into the biosphere is a serious threat to the environment and promotes several health issues. Although several studies have been carried out in the remediation of textile effluents using adsorbents, the continuous mode of operation (packed bed) to treat effluent generated from the cotton-based textile industry using biosorbent is seldom reported. Here, one such investigation is made to remediate the Remazol effluent solution in batch and continuous mode of operation. A maximum decolourization efficiency of 77.5% and 49.66% was obtained for *Ulva lactuca* derived biochar in batch and continuous study. Column data parameters such as overall sorption time zone, breakthrough time, exhaustion time and volume of effluent treated were also calculated. Regeneration studies showed that 0.01 M sodium hydroxide can be utilized for sorption-elution up to three regeneration cycles.

INTRODUCTION

Due to an increase in population and rapid industrialization, pollutants are becoming one of the emerging problems. Of the different resources, water is one of the important natural resources which is very essential for day to day activities in human life. But in recent years the water demand is continuously increasing because of groundwater depletion and surface water contamination. Surface water is polluted mainly due to industrial activities. These industries are not having effective treatment methods and in turn, discharging their pollutants directly into the nearby water bodies (Abdolali et al. 2014). This pollutant when mixed with the surface water, it affects the quality of the water and it is becoming unfit for domestic purpose. Even a very less concentration (1 mg/L) of pollutant if mixed with the water it will change the physical, chemical and biological characteristics of the water. If this water is consumed by the human it will result in many health issues like skin allergy, typhoid, fever, cholera, cancer, kidney failure, respiratory problems and several other health issues (Salleh et al. 2011). So, the effluent generated from the industry must be completely treated and then should be disposed to the nearby water bodies.

Many treatment methods are available for the removal of pollutants from the wastewater. These treatment methods are broadly classified as physical, chemical and biological (Robinson et al. 2001). Physical methods namely membrane filtration, adsorption, electrokinetic coagulation, ion-exchange and irradiation are the commonly used methods for the removal of pollutants (Yagub et al. 2014). The chemical method used for the removal of pollutants from wastewater includes ozonation, oxidation and photochemical methods. The chemical method utilizes one form of chemical to convert the pollutants into another form. So chemical methods will produce secondary pollutants to the environment. In recent years, much research has been carried out using biological methods for the remediation of pollutants (Safa et al. 2011, Kaushik & Malik 2009). These include biosorption, phytoremediation, bioventing, biodegradation, bioleaching bioaccumulation, bio-sparging, bio-augmentation and bio-stimulation. But biosorption is the mainly used biological methods for the remediation of pollutants. It involves the utilization of active or dead biomass for the remediation of the pollutants. Biosorption treatment is based on the combination of physical adsorption, micro-precipitation, ion exchange, and chelation process (Veglio & Beolchini, 1997). The biosorbents are generally derived from bacteria, fungi, industrial wastes, agricultural wastes and seaweeds (Vijayaraghavan & Yun 2008).

In recent years biochar (a carbon-rich material produced in the absence of oxygen) is used for the biosorption process (Chen et al. 2019, Gunarathne et al. 2019). It is also reported that biochar, a green adsorbent is capable of remediating heavy metals (Sahmoune et al. 2019). In the past research, biochar has been effectively utilized for soil enrichment (Xu et al. 2018). But very limited work has been carried out for the utilization of biochar in the biosorption process. The current investigation is to produce biochar from green marine seaweeds. The utilization of these marine seaweeds will produce effective sorbent at very low cost or nil cost. These seaweeds are considered as a secondary pollutant to the environment since it affects the self-purification process and also it affects photosynthesis process of the water bodies (Zaharia et al. 2009). Textile industries are the fast-growing industries that use different types of dyes for colouring and utilize an enormous amount of water that leads to water pollution. The current investigation attempts the treatment of real Remazol effluent that is generated from cotton-based textile industries in batch and continuous mode of operation (packed bed). This is the first attempt made to produce biochar from marine seaweeds for the remediation of real Remazol effluent solution in batch and continuous process.

MATERIALS AND METHODS

Seaweeds and Biochar Preparation

Three green seaweeds (marine algae) namely *Ulva lactuca*, *Ulva reticulata* and *Caulerpa scalpelliformis* collected from Rameswaram (Tamilnadu, India) are used for the current investigation. The collected biomass was grounded and sieved to obtain a particle size of 0.75 mm. A measured quantity of 50 g is taken and it is rinsed with deionized water and dried under natural conditions for 24 hours. Then the seaweeds are dried under 103°C for 12 hours to remove the moisture content present in the biomass. Now biochar is produced by thermal pyrolysis at 300°C under oxygen-free environment (Gokulan et al. 2019a).

Preparation of Remazol Effluent

The Remazol dyes and other analytical grade chemical agents used in the current investigation were procured from Sigma-Aldrich (India). Remazol effluent that is employed in the textile industry is prepared based on the composition suggested by Alaton et al. (2002a & b). According to this, the effluent comprises of Remazol brilliant blue R (369 mg), Remazol brilliant orange 3R (6 mg), Remazol brilliant violet 5R (194 mg) and Remazol black B (768 g), sodium hydroxide (510 mg), sodium carbonate (13000 mg), sodium chloride (41000 mg) and acetic acid (790 mg). To confirm the 100% composition of the real Remazol effluent, the mixture was boiled for 180 min in deionized water. After cooling the effluent for 12 h, the effluent characteristics such as pH, initial colour and total dissolved solids were measured. The effluent pH was estimated to be 10.7 and effluent was purple, whereas total dissolved solids were determined to be 840 mg/L. The absorbance of the Remazol effluent was measured at 579 nm using UV-vis spectrophotometer (Shimadzu UV-1800).

Batch Experiments

Batch studies were carried out to find the optimum parameters for the maximum dye removal and it is achieved by varying biochar dosage, pH, temperature and initial concentration. A 0.25-litre conical flask with an operating volume of 0.1-litre was used for the batch studies. The equilibrium time for the batch study was fixed for 8 hours and the content of the conical flask was completely mixed by placing in in an orbital shaker which rotates at 160 rpm. A centrifuge with an operating speed of 3000 rpm for 5 minutes was used to separate the supernatant and pellet and the dye concentration was measured using a spectrophotometer. Desorption study was carried out to find the reusability of the spent biochar and to find the regeneration cycles to which the biochar can efficiently has the sorption process. Based on the previous study 0.01 M of Sodium Hydroxide is used as the elutant with an S/L ratio of 5 and the three sorption- elution cycle is carried out.

Column Experiments

A packed bed column setup is used to find the ability of the biochar to continuously remediate the effluent solution. The packed column is made up of acrylic glass with 19 mm as the internal diameter of 330 mm as the total height of the column. On the other hand, a stainless sieve of 0.5 mm was placed at the bottom of the column followed by glass wool. A 1.5 mm glass circular beads were positioned at a height of 20 mm from the base of the column to maintain a steady flow of the effluent solution into the column. The column data analysis is carried out and it is discussed in detail in our previous study (Gokulan et al. 2019b). The schematic presentation of the packed bed column is given in Fig. 1.

To examine the applicability of the sorbent in the real wastewater scheme, it is important to conduct continuous-flow trials. So, a packed bed column was used to explore the possibility of the sorbent to remediate the Remazol effluent continuously. Packed bed column was found to be one of the best models to continuously remediate the effluent solution in real-time effluent treatment (El Messaoudi et al. 2016, Diniz et al. 2008, Barquilha et al. 2017). It was also reported that when compared to all other methods, the packed bed is having several advantages in effluent treatment (Vijayaraghavan & Yun 2008, Chen et al. 2012). In the current investigation,

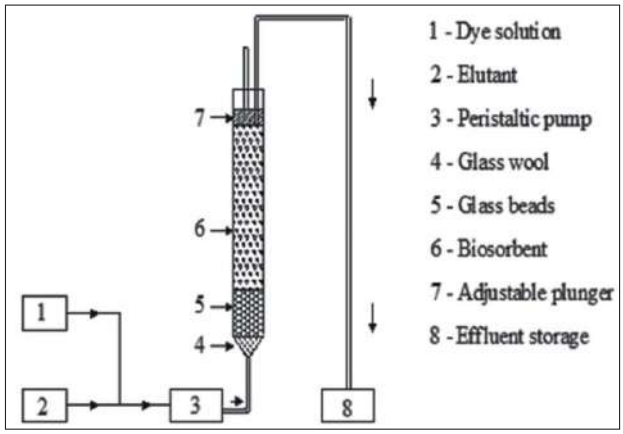


Fig.1: Packed bed for dye removal using biochar.

continuous trails were carried out by pumping the effluent solution from bottom to top and that allows the sorbent to be in contact with the sorbent where the sorption occurs. The exit effluent solution was collected and analysed for effluent concertation based on the absorbance value. The S-shaped curve is obtained when a plot is done for time versus effluent concentration. This S-shaped curve is due to the mass transfer between the sorbent and the effluent (Hatzikioseyian et al. 2001). Based on the previous study, an up-flow packed column with the sorbent bed depth of 25 cm and a flow rate of 0.3 L/hr was used for the continuous remediation of effluent solutions.

RESULTS AND DISCUSSION

Batch Studies

The batch study was carried out to find the optimum conditions for the maximum uptake capacity of the biochar and the maximum removal efficiency of different Remazol dyes with different biochar. The batch results indicated that that the optimum condition for all dye loaded biochar is dosage 2 g/L, pH 2, temperature 30°C and initial concentration of 0.05 mmol/L with a contact time of 300 minutes (Gokulan et al. 2019c). These optimum conditions are used for the remediation of complex Remazol effluent solution in batch mode operation.

Remazol Effluent Treatment in Batch Mode of Operation

Effluent treatment studies are carried out to understand the potential of the adsorbent in the remediation of real-time effluent that is having industrial applications. In real dye effluents, along with the auxiliary chemicals, many other chemicals will be present and that is the biggest challenge for the sorbent in the remediation of the effluent solution. The influence of pH on the remediation of the Remazol effluent is studied and represented in Fig. 2a. Since Remazol effluent is complex with many dyes and other chemicals it is tough to express the concentration the effluent solution. So, the removal efficiency is calculated based on the absorbance value of the effluent solution and similar studies

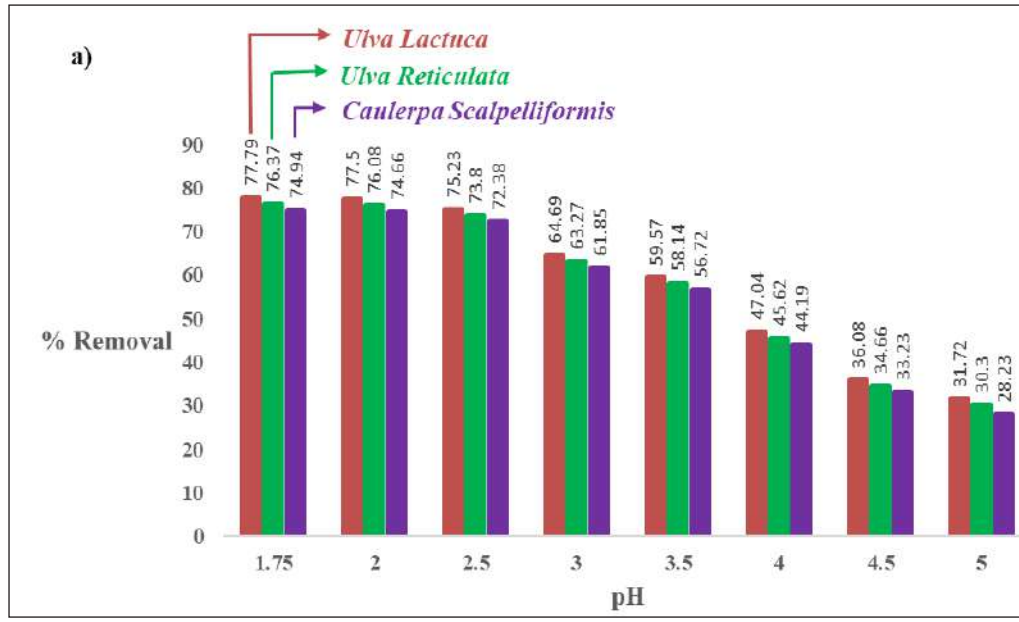


Fig.Cont....

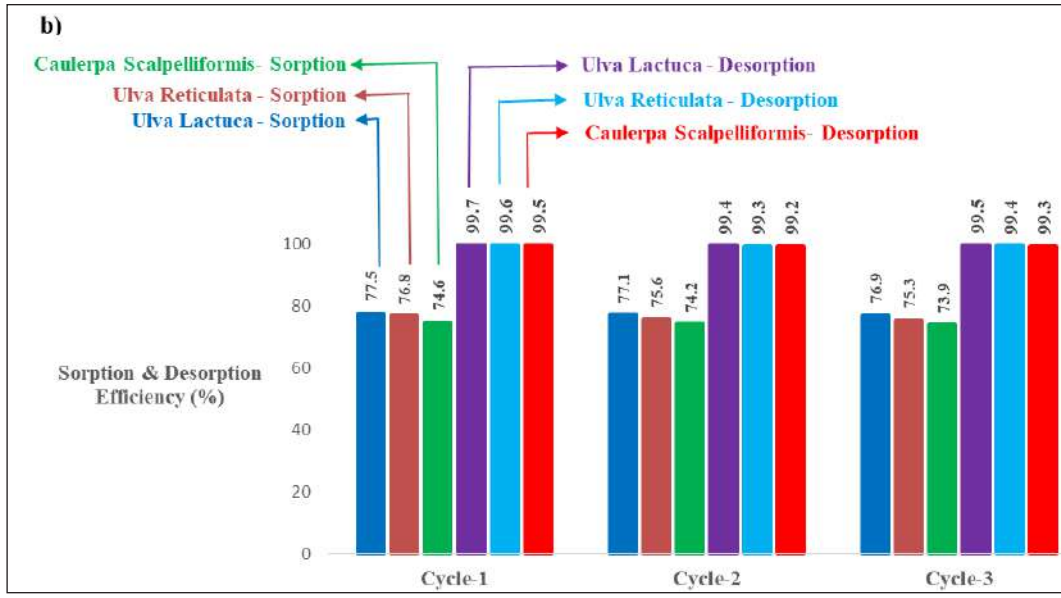


Fig. 2: a) Effect of pH on the sorption and b) Effect of sorption and desorption in regeneration cycles.

were carried out by many researchers (Alaton et al. 2002a, Vijayaraghavan et al. 2008a). The results showed that % colour removal efficiency decreased with an increase in solution pH. For instance, at pH 2.0, *U. lactuca*, *U. reticulata* and *C. scalpelliformis* exhibited 77.5%, 76.8%, 74.6% removal efficiency compared to only 31.7%, 30.30% and 28.23% at pH 5.0. when compared to pH 2. % removal efficiency is more for pH 1.75 but the difference in efficiency is less than 0.3% for all the concentrations of biochar. So, based on the economic aspect, pH 2 can be considered as the optimum conditions for the maximum decolourization efficiency.

In the final set of effluent batch studies, the regeneration potential of different biochar towards remediation of Remazol effluent was attempted in successive three sorption-desorption cycles. Based on the previous batch studies 0.01 M sodium hydroxide is considered as the best elutant for the regeneration studies. The results indicated that in all three sorption-elution cycles, sodium hydroxide exhibited a desorption efficiency higher than 99.5%, 99.4%, 99.3%.

Remazol Effluent Treatment in Continuous Mode of Operation

The breakthrough curve obtained during remediation of Remazol dye effluent by different biochar is presented in Figs. 2a, 2b and 2c. The bed depth and flow rate were fixed at 25 cm and 0.3 L/h. The performance of biochar bed in the remediation of dye effluent was very good, with total dye decolourization efficiency of 49.66 %, 48.86 % and 47.93 %, with a column exhaustion time of 765, 750, and 735 minutes and breakthrough occurred at 120 minutes for biochar derived from *Ulva lactuca, Ulva reticulata* and *Caulerpa scalpelliformis.* Table 1 demonstrates the overall sorption zone, breakthrough time, exhaustion time and decolourization efficiency of the different biochar's. An effort has been made to regenerate the column bed using 0.01 M sodium hydroxide at a flow rate of 0.6 L/h. Figs. 3a, 3b and 3c explore the dye effluent elution curve, which increased sharply at the beginning and decreased gradually to reach equilibrium. The elution procedure produced a highly concentrated solution with elution efficiency of 99.14%, 99.08%, 99.22%.

CONCLUSION

Based on the experimental results the following observations were made:

- A maximum decolourization efficiency of 77.5%, 76.08%, 74.68% for *Ulva lactuca*, *Ulva reticulata, Caulerpa scalpelliformis* is obtained at pH 2.0, biochar dosage of 2 g/L, the temperature of 30°C and initial absorbance of 3.5125.
- Desorption studies indicated that in all three sorption-elution cycles sodium hydroxide exhibited a desorption efficiency higher than 99.5%, 99.4%, 99.3%.
- Continues studies reviled that the decolourization efficiency of 49.66%, 48.86% and 47.93% with a column exhaustion time of 765, 750, and 735 minutes is obtained for biochar derived from *Ulva lactuca, Ulva reticulata and Caulerpa scalpelliformis*

Table 1: Column parameters for effluent treatment using different biochar.

Biochar	Breakthrough time (hours)	Exhaustion Time (hours)	Overall Sorption Zone (hours)	The volume of Effluent treated (litres)	Total dye removal (%)
Ulva lactuca	01	12.75	11.75	3.82	49.66
Ulva reticulata	01	12.50	11.50	3.75	48.86
Caulerpa scalpelliformis	01	12.25	11.25	3.67	47.93

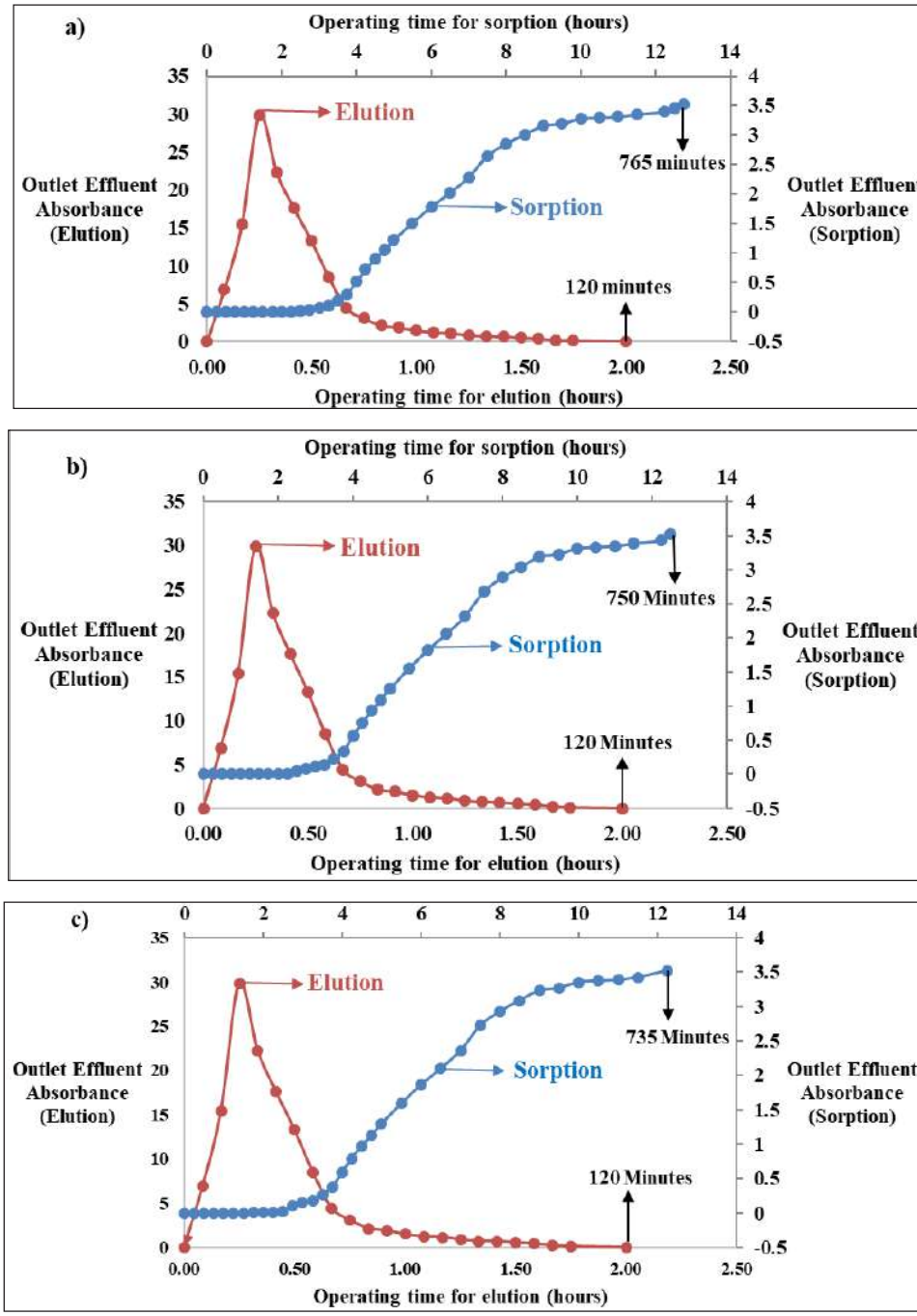


Fig. 3: Sorption and desorption of effluent by (a) *Ulva Lactuca,*(b) *Ulva reticulata*, and (c) *Caulerpa scalpelliformis* derived biochar.

- Regeneration studies of the continues process explored that the elution has been completed within 120 minutes for all the biochar with elution efficiency of 99.14%, 99.08 %, 99.22%.

REFERENCES

Abdolali, A., Guo, W.S., Ngo, H.H., Chen, S.S., Nguyen, N.C. and Tung, K.L. 2014. Typical lignocellulosic wastes and by-products for biosorption process in water and wastewater treatment: a critical review. Bioresour. Technol., 160:57-66. doi:10.1016/j.biortech.2013.12.037.

Alaton, I.A., Balcioglu, I.A. and Bahnemann, D.W. 2002a. Advance oxidation of a reactive dyebath effluent: comparison of O_3, H_2O_2/UV-C and TiO_2/UV-A processes. Water Res., 36: 1143-1154. doi: 10.1016/S0043-1354(01)00335-9.

Alaton, I.A., Kornmuller, A. and Jekel, M. 2002b. Ozonation of spent reactive dye-baths: effect of HCO_3^-/CO_3^{2-} alkalinity. J. Environ. Eng., 128: 689-696. doi: 10.1061/(ASCE)0733-9372(2002)128:8(689).

Barquilha, C.E.R., Cossich, E.S., Tavares, C.R.G. and Silva, E.A. 2017. Biosorption of nickel (II) and copper (II) ions in batch and fixed-bed columns by free and immobilized marine algae Sargassum sp. **J. Clean. Prod.,** 150:58-64. doi: 10.1016/j.jclepro.2017.02.199

Chen, L., Li, F., Wei, Y., Li, G., Shen, K. and He, H.J. 2019. High cadmium adsorption on nanoscale zero-valent iron coated *Eichhorniacrassipes* biochar. Environ. Chem. Lett., 17: 589-594. doi:10.1007/s-10311-0811-018y

Chen, S., Yue, Q., Gao, B., Li, Q., Xu, X. and Fu, K. 2012. Adsorption of hexavalent chromium from aqueous solution by modified corn stalk: a fixed-bed column study. Bioresour. Technol., 113: 114-120. doi: 10.1016/j.biortech.2011.11.110

Diniz, V., Weber, M.E., Volesky, B. and Naja, G. 2008. Column biosorption of lanthanum and europium by Sargassum. Water Res., 42: 363-371. doi: 10.1016/j.watres.2007.07.027

El-Messaoudi, N., Elkhomri, M., Dbik, A., Bentahar, S., Lacherai, A. and Bakiz, B. 2016. Biosorption of Congo red in a fixed-bed column from aqueous solution using jujube shell: Experimental and mathematical modelling. J. Environ. Chem. Eng., 4: 3848-3855. doi: 10.1016/j.jece.2016.08.027

Gokulan, R., Raja Murugadoss, J., Jegan, J. and Avinash, A. 2019c. Comparative desorption studies on remediation of remazol dyes using biochar (sorbent) derived from green marine seaweeds. Chemistryselect, 4: 7437-7445. doi: 10.1002/slct.201901348

Gokulan, R., Ganesh Prabhu, G. and Jegan, J. 2019b. A novel sorbent *Ulva lactuca*-derived biochar for remediation Remazol brilliant orange 3R in packed column. Water Environ. Res., 91: 642-649. doi: 10.1002/wer.1092

Gokulan, R., Ganesh Prabhu, G. and Jegan, J. 2019a. Remediation of complex remazol effluent using biochar derived from green seaweed biomass. Int. J. Phytoremediat., 21: 1179-1189. doi: 10.1080/15226514.2019.1612845

Gunarathne, V., Ashiq, A., Ramanayaka, S., Wijekoon, P. and Vithanage, M. 2019. Biochar from municipal solid waste for resource recovery and pollution remediation. Environ. Chem. Lett., 17: 1225-1235. doi:10.1007/s0-00866-019-10311

Hatzikioseyian, A., Tsezos, M. and Mavituna, F. 2001. Application of simplified rapid equilibrium models in simulating experimental breakthrough curves from fixed bed biosorption reactors. Hydrometallurgy, 59: 395-406. doi: 10.1016/S0304-386X(00)00169-9

Kaushik, P. and Malik, A. 2009. Fungal dye decolourization: Recent advances and future potential. Environ. Int., 35: 127-141. doi:10.1016/j.envint.2008.05.010

Robinson, T., McMullan, G., Marchant, R. and Nigam, P. 2001. Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. Bioresour. Technol., 77: 247-255. doi:10.1016/S0960-8524(00)00080-8.

Safa, Y. Bhatti, H.N. Bhatti, I.A. and Asgher, M. 2011. Removal of direct Red-31 and direct Orange-26 by low cost rice husk: Influence of immobilisation and pre-treatments. Can. J. Chem. Eng., 89: 1554-1565 doi:10.1002/cjce.20473

Sahmoune, M.N. 2019. Evaluation of thermodynamic parameters for adsorption of heavy metals by green adsorbents. Environ. Chem. Lett., 17: 697-704. doi:10.1007/s-00819-018-10311z

Salleh, M.A.M., Mahmoud, D.K., Karim, W.A.W.A. and Idris, A. 2011. Cationic and anionic dye adsorption by agricultural solid wastes: A comprehensive review. Desalination, 280: 1-13. doi:10.1016/j.desal.2011.07.019.

Veglio, F. and Beolchini, F. 1997. Removal of metals by biosorption: A review. Hydrometallurgy, 44: 301-316. doi: 10.1016/S0304-386X(96)00059-X

Vijayaraghavan, K. and Yun, Y.S. 2008. Bacterial biosorbents and biosorption. Biotechnol. Adv., 26: 266-291. doi: 10.1016/j.biotechadv.2008.02.002

Vijayaraghavan, K. Lee, M.W. and Yun, Y.S. 2008a. A new approach to study the decolorization of complex reactive dye bath effluent by biosorption technique. Bioresour. Technol., 99:5778-5785. doi: 10.1016/j.biortech.2007.10.012.

Xu, M. Wu, J. Yang, G. Zhang, X. Peng, H. Yu, X. and Qi, H. 2018. Biochar addition to soil highly increases P retention and decreases the risk of phosphate contamination of waters. Environ. Chem. Lett., 17:533-541. doi:10.1007/s-0802-018-10311z

Yagub, M.T. Sen, T.K. Afroze, S. and Ang, H.M. 2014. Dye and its removal from aqueous solution by adsorption: A review. Adv. **Colloid Interface.** Sci., 209: 172-184. doi: 10.1016/j.cis.2014.04.002

Zaharia, C. Suteu, D. Muresan, A. Muresan, R. and Popescu A. 2009. Textile wastewater treatment by homogenous oxidation with hydrogen peroxide. Environ. Eng. Manag. J., 8:1359-1369. doi: 10.30638/eemj.2009.199.

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Growth and Reproduction of *Perionyx excavatus* (Perrier) During Vermicomposting of Different Plant Residues

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ABSTRACT

The data on growth and reproduction of composting earthworms may be beneficial for large scale earthworm production. The growth and reproduction of *Perionyx excavatus* were assessed in limited supplies of four experimental diets-cow dung alone and its mixture with acacia (*Acacia auriculiformis*) leaf litter, bamboo (*Bambusa polymorpha*) leaf litter and terrestrial weed *Mikania micrantha* under laboratory conditions to select suitable diet from our locally available resource for vermiculture. Growth performance of *Perionyx excavatus* was significantly ($P<0.05$) higher in cow dung (maximum weight mg worm^{-1} 629 and growth rate mg worm^{-1} day^{-1} 22.91). The rate of reproduction (0.3 cocoons worm^{-1} day^{-1} and 3.31 juveniles adult^{-1} week^{-1}) was significantly higher ($P<0.05$) in acacia-cow dung mixture. The cocoon production was maximum on the 30th day in all experimental diets (cow dung, acacia-cow dung, bamboo-cow dung, mikania-cow dung). All the diets showed a maximum peak of juvenile production on the 45th day. The lowest rate of biomass increase as well as the rate of reproduction were observed in mikania-cow dung mixture. Present result indicates that cow dung and acacia leaf litter can be used as vermiculture substrate for *P. excavatus*.

INTRODUCTION

Vermicomposting is the process of conversion of organic wastes into organic manure called vermicompost through the joint activities of epigeic earthworms and microorganisms. Selection of earthworm species for vermicomposting technology is mainly based on their reproductive potential, growth rate, the range of tolerance to ecological factors and nutrient contents of organic wastes etc. (Hallatt et al. 1992, Reinecke et al. 1992, Singh 2006, Suthar & Ram 2008). Growth and reproduction of *Eisenia fetida* and *Eudrilus eugeniae* have been investigated by various workers (Suthar & Ram 2008, Edwards et al. 1998, Gunadi & Edwards 2003, Garg et al. 2005). Besides earthworm species such *as Perionyx sensibaricus*, *Perionyx bainii*, *Perionyx ceylanensis* and *Perionyx nainianous* have been tested for degradation of organic wastes (Julka et al. 2009). Animal dung (cattle, sheep, horse) have been widely used as vermiculture substrates (Edwards et al. 1998). Other vermiculture substrates include organic wastes from industry such as spent brewery yeast (Butt 1993) and agricultural wastes such as roots of barley (Bostrom & Holmin 1986), ground corn (*Zea mays*) residue (Cook & Linden 1996) and alfalfa leaves (Shipitalo et al. 1988). Native epigeic species *Perionyx excavatus* is a potential candidate for the conversion of organic wastes into useful plant growth media (Edwards et al. 1998, Kale et al. 1982, Chaudhuri 2005). Based on the above facts we aimed to investigate the effects of some locally available organic wastes such as terrestrial weed, *Mikania micrantha*, leaf litter of *Acacia auriculiformis*, leaf litter of *Bambusa polymorpha* mixed with cow dung on the growth and reproduction of oriental epigeic earthworm species, *Perionyx excavatus* in mass culture. The data on growth and reproduction of native species *Perionyx excavatus* may be useful to design vermiculture for large scale production of earthworms using different waste residues. It is mentionable that earthworms are used as fish baits worldwide and earthworm tissue is a good source of protein (vermitin) for poultry, pig and aquarium (Lowe & Butt 2005).

MATERIALS AND METHODS

A study on growth and reproduction of Indian vermi-composting earthworm, *Perionyx excavatus* was carried out in a well-aerated room on four different diets such as cow dung (C), cow dung mixed with terrestrial climbing weed, *Mikania micrantha* (M) (often found to form hazardous bush around the roadside lamp posts forming shades against the light in Tripura), leaf litter of *Acacia auriculiformis* (A) and bamboo (*Bambusa polymorpha*) (B). Urine free cow dung, *M. micrantha*, leaf litter of *Acacia* and *Bambusa* were collected from Tripura University campus and its neighbouring areas. The leaf litter and plant biomass of *M. micrantha* were air-

dried. The air-dried plant substrates were crushed to get small fractions. Four vermicomposting treatments were established by using earthen pot (2.5 L capacity, diameter 25 cm and depth 15 cm) having 300 g of dry feed mixtures in each pot. One treatment contained only C (300 g) as control and rest three experimental treatments were prepared by mixing the different plant substrates (A, B and M) with C in 7:3 ratios (dry weight), i.e. 210 g A and 90 g C (AC) in 7:3 ratio, 210 g B and 90 g C (BC) in 7:3 ratio, 210 g M and 90 g C (MC) in 7:3 ratio. Before setting up the experiment, the authors made pilot studies using different ratios of C with weed (M) and leaf litters (A and B) on a dry weight basis. It was observed that weeds (M) and leaf litter (A and B) Vs. C when mixed with C at 7:3 ratio, were suitable for earthworm inoculation and their subsequent activity. *P. excavatus* generally inhabits cow dung pits and prefers cow dung as its best food of choice compared to other diets. Each treatment had three replicas. Among these diets mixtures, AC was unpalatable to earthworms up to 21 days. So these feed mixtures (C, AC, BC, and MC) were pre-composted for 3 weeks to make palatable to earthworms. After pre-composting each culture pot of the four vermicomposting sets was inoculated with 30 adults of *P. excavatus* [cumulative weight 9.99±0.09 g (Mean ± SE)]. The containers were covered tightly with jute cloth to prevent the escape of earthworms and entry of light inside. The taxonomic identification of the earthworm species was confirmed at Zoological Survey of India, Solan. The moisture content of the incubation media was maintained at 50 to 60% (Chaudhuri 2007) by using hand sprayer at maximum room temperature ranging in between 29°C to 31°C. The earthworms were not supplied with additional food during 45 days experiment.

Cocoon and juvenile produced were collected fortnightly from the culture media with the aid of a hand lens. The live biomass of earthworms (mg worm^{-1}), net weight gain (mg worm^{-1}), growth rate (mg worm^{-1}day^{-1}) and the rate of reproduction in terms of the cocoon (worm^{-1}day^{-1}) and juvenile generation (adult^{-1}week^{-1}) were measured at 15 days interval by hand sorting of the culture media. The rate of growth (mg worm^{-1}day^{-1}), the rate of cocoon production (worm^{-1}day^{-1}) and rate of juvenile production (adult^{-1}week^{-1}) were calculated by the following formulae-

i. Rate of growth (mg worm^{-1}day^{-1}) $= \dfrac{\text{Maximum worm weight – Initial worm weight}}{\text{No. of days to attain maximum weight}}$

ii. Rate of cocoon production (worm^{-1} day^{-1})

$$= \frac{\text{Maximum number of cocoons}}{\text{No. of adult at '0' day} \times \text{Total number of days to attain maximum number of cocoons}}$$

iii. Rate of juvenile production (adult^{-1} week^{-1})

$$= \frac{\text{Maximum number of juveniles}}{\text{No. of adult at '0' day} \times \text{Total number of days to attain the maximum number of juvenile population}} \times 7$$

Initial substrates materials were analysed for pH (1:2.5 suspensions of the material and distilled water), electrical conductivity (distilled water suspension of each waste mixture in the ratio of 1:10 (W/V), total carbon (Walkley & Black 1934), total Nitrogen (Jackson 1975), available phosphorus (Kuo 1996) and available potassium (Jones 2001). The data were subjected for analysis of variance (ANOVA) followed by Tukey's post hoc test using OriginPro 2016. All the results reported in the text are the mean of three replicates.

RESULTS

Table 1 shows the composition of food substrates provide to earthworms. The pH values of the food substrates were alkaline side of neutral (6.3 - 7.63). The highest electrical conductivity (990 µMho/cm), nitrogen (3.08%), available phosphorus (163.71 mg/100g) and available potassium (7321.33 mg/100g) was recorded in MC. The maximum carbon content (20.37%) was in MC and minimum (17.14%) was in C.

The differential growth (mg worm^{-1}) response of *P. excavatus* on various experimental diets over a period of 45 days is represented in Fig. 1. *P. excavatus* had the highest individual live weight (629 mg worm^{-1}) in C with a net weight gain of 296.18 mg worm^{-1} and growth rate of 22.91 mg worm^{-1} day^{-1} (Table 2). In fact, maximum weight gain, net weight gain and growth rate were significantly higher ($P < 0.05$) in C compared to the other diets (Table 2). Highest growth rate (worm^{-1} day^{-1}) in C was followed by that in BC, MC, and AC. In AC, lowest net weight gain (52.02 mg worm^{-1}) and lowest growth rate (3.47 mg worm^{-1} day^{-1}) were recorded. *P. excavatus* had early biomass gain in C, BC, and AC on the 15th day, whereas the highest individual biomass was achieved on the 30th day in MC (Table 2). After attaining highest biomass a gradual decline was observed up

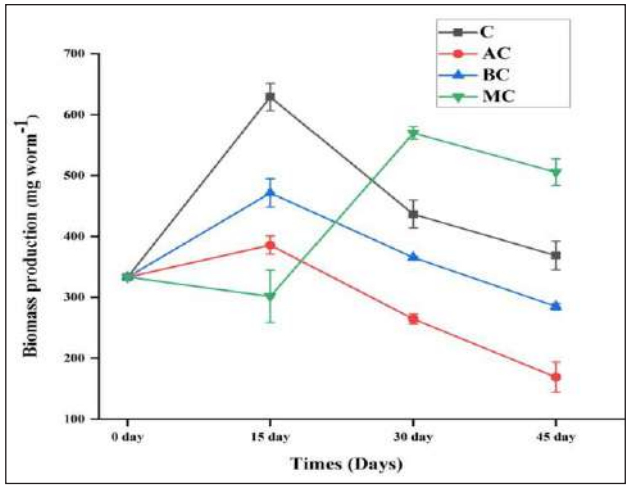


Fig. 1: Changes in biomass production of *P. excavatus* in different experimental diets; Abbreviation: C- cow dung, AC- acacia-cow dung, BC- bamboo- cow dung, MC- mikania- cow dung.

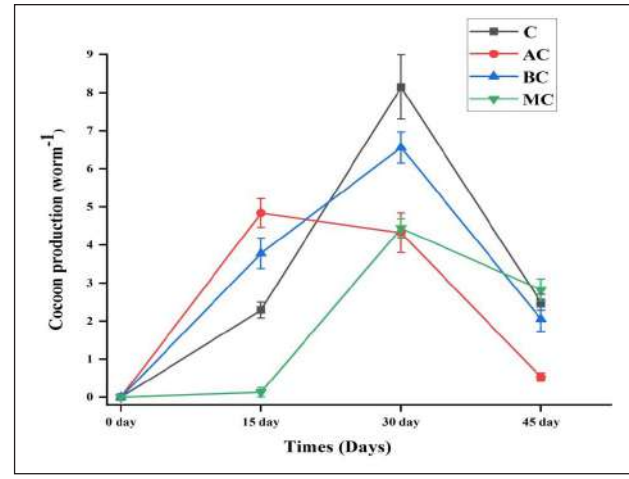


Fig. 2:Dynamics of cocoons production of *P. excavatus* in different experimental diets; Abbreviation: C- cow dung, AC- acacia-cow dung, BC- bamboo- cow dung, MC- mikania- cow dung.

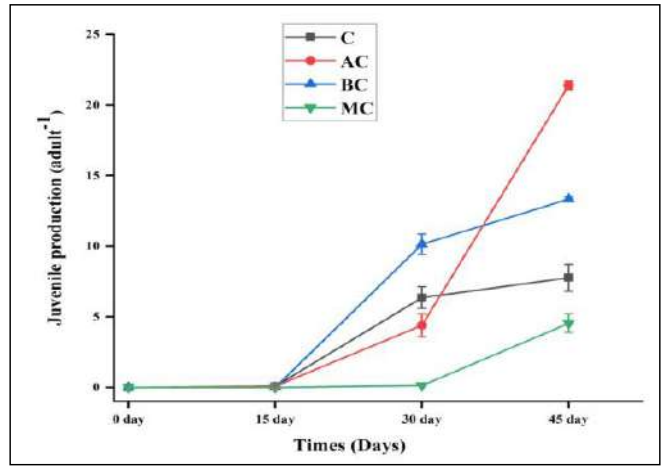


Fig. 3: Patterns juveniles production of *P. excavatus* in different experimental diets; Abbreviation: C- cow dung, AC- acacia-cow dung, BC- bamboo- cow dung, MC- mikania- cow dung.

Table 1: Some analytical characteristics of experimental diets (Mean±SE).

	C	AC	BC	MC
pH	7.42 ± 0.14	6.3 ± 0.005	6.75 ± 0.003	7.63 ± 0.11
Electrical Conductivity(µMho/cm)	580.66 ± 0.66	570 ± 0.00	720 ± 0.00	990 ± 0.01
Organic Carbon (%)	17.14 ± 0.58	19.83 ± 0.04	19.52 ± 0.76	20.37 ± 0.54
Available Phosphorus (mg/100g)	147.39 ± 5.08	26.15 ± 0.17	48.97 ± 0.8	163.71 ± 2.5
Available Potassium (mg/100g)	1000 ± 14.43	1087 ± 7.21	937.33 ± 7.21	7321.33 ± 14.2
Total Nitrogen (%)	1.26 ± 0.006	1.82 ± 0.003	1.62 ± 0.003	3.08 ± 0.05

Abbreviation: C- cow dung, AC- acacia-cow dung, BC- bamboo- cow dung, MC- mikania-cow dung.

to 45 days in all diets (Fig. 1). At the end of the experiment, the maximum mean biomass of *P. excavatus* was achieved in MC followed by those in C, BC, and AC (Fig. 1).

The reproduction potential of *P. excavatus* in terms of their cocoon production (worm^{-1} day^{-1}) and juvenile production (worm^{-1} week^{-1}) among different experimental diets are

summarized in Table 3 (a, b). The rate of cocoon production was highest ($P < 0.05$) in AC followed by C, BC and least in MC (Table 3a). *P. excavatus* had an earlier peak of cocoon production (4.84 worm^{-1}) on the 15th day in AC (Fig. 2). However, cocoon production (worm^{-1}) in general was highest in all the experimental diets on the 30th day (Fig. 2). The cocoon production was ceased after 30th day in all experimental diets (Fig. 2). The rate of juvenile production (adult^{-1} week^{-1}) also differed ($P < 0.05$) among the four different food substrates (Table 3b). All the treatments (C, AC, BC, and MC) showed a maximum peak of juvenile production on the 45th day (Fig. 3) i.e. 15 days after the peak of cocoon production (Fig. 2). The appearance of the juveniles was first encountered in C and AC (i.e. on the 15th day) and later in BC and MC (i.e. 30th day) (Table 3b). The highest number (total number) and rate of juvenile production (adult^{-1} week^{-1}) were on the 45th day and were in the following order AC > BC > C > MC (Fig. 3). Growth and reproduction rate of some epigeic earthworm species are given in Table 4.

DISCUSSION

The palatability of earthworms for organic wastes is influenced by its physico-chemical characteristics and nutrient contents that affect the efficiency of earthworms in the decomposition process and their growth in the organic wastes (Chaudhuri 2002, Suthar 2007a). Individual live weight (629 mg worm^{-1}), growth rate (22.91 mg worm^{-1} day^{-1}) and net weight gain (296.18 mg worm^{-1}) of *P. excavatus*, were maximum in C. The highest growth rate of *P. excavatus* in C was because a minimum of only 15 days was required to attain its highest biomass and net weight gain. According to Loh et al. (2005) cow dung is a most preferred diet of earthworms irrespective of the species which could be due to easily assimilable organic matter and presence of low growth retarding factors in it (Suthar 2007a). Maximum individual biomass of *P. excavatus* (600 mg worm^{-1}) in animal dung was reported by Hallat et al. (1990) supports our observation of maximum mean individual live weight of *P. excavatus* (629 mg worm^{-1} on the 15th day) in C. Nitrogen-rich diets promote

Table 2: Growth of *P. excavatus* in different experimental diets (Mean±SE).

Substrates	Mean initial weight (mg worm^{-1})	Mean maximum weight (mg worm^{-1})	Maximum weight achieved on	Net weight gain (mg worm^{-1})	Growth rate (mg worm^{-1} day^{-1})
C	333.31 ± 0.12^{a}	629± 22.31^{a}	15th day	296.18 ± 23.11^{a}	22.91 ± 1.93^{a}
AC	333.33 ± 0.30^{a}	385.86 ± 14.87^{b}	15th day	52.02 ± 0.84^{b}	3.47 ± 0.99^{b}
BC	333.3 ± 0.1^{a}	471.52 ± 23.18^{c}	15th day	138.18 ± 23.18^{c}	12.6 ± 1.54^{c}
MC	333.37 ± 0.36^{a}	570 ± 10.32^{d}	30th day	236.66 ± 20.2^{d}	9.47 ± 1.13^{d}

Abbreviation: C- cow dung, AC- acacia-cow dung, BC- bamboo- cow dung, MC- mikania- cow dung.
Different letters correspond to a significant difference ($P < 0.05$).

Table 3: Rate of reproduction of *P. excavatus* in differential experimental diets (Mean±SE).

(a) Cocoons production of *P. excavatus* in different experimental diets.

Substrates	Cocoon production started in	Total no. of cocoons produced after 45 days	No. of cocoons produced (worm^{-1})	Rate of cocoon production (worm^{-1} day^{-1})
C	15th day	205 ± 32.5^{a}	6.84 ± 0.9^{a}	0.25 ± 0.008^{a}
AC	15th day	107 ± 22.8^{b}	3.05 ± 0.7^{b}	0.3 ± 0.25^{b}
BC	15th day	136 ± 21.9^{b}	3.9 ± 0.6^{b}	0.23 ± 0.11^{a}
MC	15th day	81 ± 21.3^{c}	2.2 ± 0.6^{c}	0.13 ± 0.01^{c}

(b) Juveniles production from the cocoons of *P. excavatus*.

Substrates	Emergence of juveniles on	Total no. of juvenile production after 45 days	Rate of juvenile production (adult^{-1} week^{-1})
C	15th day	156 ± 39.5^{a}	1.39 ± 0.07^{a}
AC	15th day	284 ± 23.2^{b}	3.31 ± 0.03^{b}
BC	30th day	387 ± 23.8^{c}	2.22 ± 0.027^{c}
MC	30th day	78 ± 10.2^{d}	0.66 ± 0.11^{d}

Abbreviation: C- cow dung, AC- acacia-cow dung, BC- bamboo- cow dung, MC- mikania- cow dung.
Different letters correspond to a significant difference ($P<0.05$).

Table 4: Growth and reproduction rate in some epigeic earthworm species.

Earthworm species	Culture materials	Growth rate (mg worm^{-1} day^{-1})	Reproduction rate		References
			Cocoon (worm^{-1} day^{-1})	Juvenile (adult^{-1} week^{-1})	
Perionyx excavatus	Cowdung	22.91	0.25	1.39	Present study
	Acacia leaf litter- Cowdung	3.47	0.3	3.31	Present study
	Bamboo leaf litter- Cowdung	12.6	0.23	2.22	Present study
	Mikania micrantha- Cowdung	9.47	0.13	0.66	Present study
	Cowdung	2.86	-	2.45	Chaudhuri & Bhattacharjee (2002)
	Cowdung-Kitchen wastes	2.47	-	1.37	Chaudhuri & Bhattacharjee (2002)
	Cowdung- Straw	4.75	-	14	Chaudhuri & Bhattacharjee (2002)
	Cowdung- Leaf litter	4.02	-	11.7	Chaudhuri & Bhattacharjee (2002)
	Rubber leaf litter	5.04	-	0.2	Chaudhuri et al. (2003)
	Pressmud-Cowdung	4.81	0.79	-	Birundha et al. (2013)
Perionyx ceylanensis	Cowdung	1.34	0.94	-	Karmegam & Daniel (2009)
Perionyx sansibaricus	Kitchen waste- *Mangifera indica* leaf litter	3.77	0.25	-	Suthar (2007a)
	Cattle solid wastes	8.00	0.22	-	Suthar (2009)
Eisenia fetida	Cowdung	16.3	0.39	-	Garg et al. (2005)
	Goat waste	16.5	0.32	-	Garg et al. (2005)
	Sheep wastes	26.2	0.44	-	Garg et al. (2005)
	Rubber leaf litter	6.2	-	1.3	Chaudhuri et al. (2003)
Eudrilus eugeniae	*Diospyrosa meanoxylon* leaf litter	68.00	0.54	-	Kadam (2015)
	Rubber leaf litter	28.8	-	1.4	Chaudhuri et al. (2003)
Lumbricus rubellus	Cowdung	8.02	0.77	-	Elvira et al. (1996)
Dendrobaena rubida	Cowdung	3.84	0.2	-	Elvira et al. (1996)
Dichogaster modiglianii	Pasture soil	-	0.19	-	Bhattacharjee & Chaudhuri (2002)

in rapid growth and reproduction in earthworms (Evans & Guild 1948). Despite high nitrogen (3.08 mg/100g) content in MC, *P. excavatus* showed a delay in the increase in biomass production in MC, probably due to its high pH (7.63) and electrical conductivity (990 µMho/cm). Presence of high content of soluble salts as indicated by the highest electrical conductivity in MC might harm earthworm feeding activity. Moreover, the antifungal and antimicrobial properties of essential oils of mikania (Baral et al. 2011) perhaps prevent the microflora to release the locked-up nutrients during the initial stage of waste decomposition. So during the first 15 days, earthworm growth was retarded in MC instead of a sudden rise as found in other diets. But with the progress of decomposition, those growth retarding substances in the wastes probably declined so that palatability and release of locked up nutrients gradually increased in MC at a later stage leading to highest weight gain by *P. excavatus* in MC on the 30th day. Lowest growth rate (3.47 mg worm^{-1} day^{-1}) with lowest net weight gain (52.02 mg worm^{-1}) of *P. excavatus* in the AC was probably due to high lignin and rich polyphenol content of *Acacia* that suppressed the growth rate of earthworms through their effects on their feeding activities (Ganesh et al. 2009). Polyphenol content in the leaf has an inverse relationship with the palatability of earthworms (Edwards & Bohlen 1996). Low biomass values of earthworms under 3-10 years old rubber plantations due to excess flavonoid, lignin, and polyphenol contents of rubber leaves as reported by Chaudhuri et al. (2013) support our present findings. The growth rate of *P. excavatus* in different experimental diets (C, AC, BC, and MC) ranging in between 3.47 mg worm^{-1} day^{-1} (AC) to 22.91 mg worm^{-1} day^{-1} (C) is much higher than those of *P. ceylanensis* (1.34-1.79 mg

worm^{-1} day^{-1}) and the growth rate recorded for *P. excavatus* and *P. sansibaricus* by various workers that ranged from 3.5 to 8.0 mg worm^{-1} day^{-1} (Reinecke et al. 1992, Suthar & Ram 2008, Karmegam & Daniel 2009, Suthar 2009) but lower than the growth rate of other vermicomposting species viz. *E. eugeniae* and *E. fetida* as mentioned in Table 4 (Garg et al. 2005, Kadam 2015).

Earthworms begin reproduction after attainment of a certain level of biomass (Chaudhuri et al. 2002). Thus, in all experimental diets (C, AC, BC and MC) following attainment of maximum biomass on the 15th day, the highest level of cocoon production and juvenile production was observed on the 30th day and 45th day respectively. The reason behind the occurrence of the peak of cocoon production earlier than the juvenile production was because after a certain period of incubation (13-14 days development time) hatchlings were produced. Thus cocoon development time of 15 days and more than one hatchling emergence per cocoon (Bhattacharjee & Chaudhuri 2002) were responsible for the dramatic rise in juvenile production on the 45th day in all of the diets (C, AC, BC, and MC). Interestingly, despite lowest biomass and growth rate of *P. excavatus* in AC, the rate of reproduction in terms of cocoon production (0.3 worm^{-1} day^{-1}) or juvenile production (3.31 adult^{-1} week^{-1}) in it was very high. The rate of reproduction in terms of cocoon production in AC was much higher than that in *P. sansibaricus* (0.22 cocoon worm^{-1} day^{-1} and 0.25 cocoon worm^{-1} day^{-1}) reared in cattle solid wastes and kitchen waste mixed with *M. indica* leaf litter respectively (Table 4). High biomass value with a lower rate of reproduction of *P. excavatus* in the kitchen wastes was earlier recorded (Table 4). Thus a good biomass supporting medium may not be a good medium for reproduction (Haimi 1990, Dominguez et al. 1997). Besides, the biochemical qualities of the food substrates, microbial biomass, and decomposition activities are some of the important factors that determine the onset of cocoon production in earthworms (Dominguez et al. 2003, Suthar 2006). Thus high polyphenol and lignin-containing substrates which exerted negative impacts on the growth rate of earthworm, *P. excavatus* in AC were probably not the inhibitory factor for the reproduction of *P. excavatus*. Juvenile production (3.31 juvenile adult^{-1} week^{-1}) in *P. excavatus* cultured in AC is higher than that in *E. fetida* (1.3 juvenile adult^{-1} week^{-1}) and 1.4 juvenile adult^{-1} week^{-1} in *E. eugeniae* reared in rubber leaf litter (Chaudhuri et al. 2003) (Table 4). According to Suthar (2007b), the nitrogen content of the culture media has a positive effect on the rate of cocoon production in earthworms and their further development through influencing dietary need of protein. Despite having rich nitrogen contents (3.08%) in MC, *P. excavatus* had the lowest rate of reproduction (rate of cocoon production 0.13 worm^{-1} day^{-1} and rate of juvenile production 0.66 adult^{-1}week^{-1}) in MC. Microbial communities act as a good food source of earthworms (Suthar 2008). The rich content of phytochemicals along with various essential oil having antifungal and antimicrobial activities (Baral et al. 2011, Rufatto et al. 2012) probably inhibited the rate of reproduction of *P. excavatus* in MC at the initial stage of the experiment. For this reason, no juveniles were produced on the 15th day and only a few juveniles (0.14 adult^{-1}) appeared on the 30th day in the MC. Suthar & Sharma (2013) also reported a negative effect of high phytochemicals on the cocoon production of *E. fetida*. However, 0.66 juvenile generation adult^{-1} week^{-1} in *P. excavatus* reared in MC was much lesser than 1.3 juvenile adult^{-1} week^{-1} and 1.4 juvenile adult^{-1} week^{-1} in *E. fetida* and *E. eugeniae* cultured in rubber leaf litter (Table 4). The lowest rate of cocoon production by *P. excavatus* (0.25 and 0.23 worm^{-1} day^{-1}) despite having highest mean numbers of cocoons per worm in C (6.84) and BC (3.05 worm^{-1}) was since the time required to achieve the maximum number of cocoons were longer.

CONCLUSION

The present work was conducted to assess the growth and reproduction of *P. excavatus* during vermicomposting of cow dung and its mixture with different plant residues (leaf litter of *Acacia auriculiformis*, *Bambusa polymorpha*, *Mikania micrantha*) under laboratory conditions. Maximum biomass gain ($P < 0.05$) and significantly higher ($P < 0.05$) growth rate of *P. excavatus* were observed in cow dung. Reproduction performance (rate of cocoon production worm^{-1}day^{-1} and juvenile production worm^{-1}week^{-1}) of *P. excavatus* was highest in ($P < 0.05$) acacia- cow dung mixture. Thus it can be concluded from the present study that cow dung and acacia leaf litter can be used as vermiculture substrate for *P. excavatus*.

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REFERENCES

Baral, B., Bhattarai, N. and Vaidya, G.S. 2011. Pharmacological and antagonistic potentials of *Mikania micrantha*. Nepal J. Sci. Technol., 12: 75-84.

Bhattacharjee, G. and Chaudhuri, P.S. 2002. Cocoon production, morphology, hatching pattern and fecundity in seven tropical earthworm species-a laboratory-based investigation. J. Biosci., 27: 283-294.

Birundha, M., Paul, J.A.J and Mariappan, P. 2013. Growth and reproduction of *Perionyx excavatus* in different organic wastes. Int. J. Curr. Microbiol. Appl. Sci., 2(2): 28-35.

Bostrom, U. and Lofs-Holmin, A. 1986. Growth of earthworms (*Allolobophora caliginosa*) fed shoots and roots of barley, meadow fescue and lucerne. Studies in relation to particle size, protein, crude fiber content and toxicity. Pedobiologia., 29: 1-12.

Butt, K.R. 1993. Utilisation of solid paper-mill sludge and spent brewery yeast as a feed for soil-dwelling earthworms. Bioresour. Technol., 44: 105-107.

Chaudhuri, P.S. and Bhattacharjee, G. 2002. Capacity of various experimental diets to support biomass and reproduction of *Perionyx excavatus*. Bioresour. Technol., 82: 147-150.

Chaudhuri, P.S., Pal, T.K, Bhattacharjee, G. and Dey, S.K. 2003. Rubber leaf litters (*Hevea brasiliensis,* var RRIM 600) as vermiculture substrate for epigeic earthworms, *Perionyx excavatus*, *Eudrilus eugeniae* and *Eisenia fetida*. Pedobiologia., 47: 796-800.

Chaudhuri, P.S. 2005. Vermiculture and vermicomposting as biotechnology for conversion of organic wastes into animal protein and organic fertilizer. Asian J. Microbiol. Biotechnol. Environ. Sci.,7: 359-370.

Chaudhuri, P.S. 2007. Vermicomposting as biotechnology for conversion of organic wastes into organic fertilizer and animal protein. In: Singh, S.M. (ed.) Earthworms for Solid Waste Management. International Book Distributing Co., Lucknow, pp. 75-88.

Chaudhuri, P.S., Bhattacharjee, S., Dey, A., Chattopadhyay, S. and Bhattacharya, D. 2013. Impact of age of rubber (*Hevea brasiliensis*) plantation on earthworm communities of West Tripura (India). J. Environ. Biol., 34: 59-65.

Cook, S.M.F. and Linden, D.R. 1996. Effect of food type and placement on earthworm (*Aporrectodea tuberculata*) burrowing and soil turnover. Biol. Fert. Soils., 21: 201-206.

Dominguez, J., Briones, M.J.I. and Mato, S. 1997. Effect of the diet on growth and reproduction of *Eisenia andrei* (Oligochaeta, Lumbricidae). Pedobiologia., 41(6): 566-576.

Dominguez, J., Parmelee, R.W. and Edwards, C.A. 2003. Interactions between *Eisenia andrei* (Oligochaeta) and nematode populations during vermicomposting. Pedobiologia., 47: 53-60.

Edwards, C.A. and Bohlen, P.J. 1996. Biology and Ecology of Earthworms. Chapman and Hall, London., pp. 426.

Edwards, C. A., Dominguez, J. and Neuhauser, E.F. 1998. Growth and reproduction of *Perionyx excavatus* (Perr.) (Megascolecidae) as factors in organic waste management. Biol. Fert. Soils., 27: 155-161.

Elvira, C., Dominguez, J. and Mato. S. 1996. The growth and reproduction of *Lumbricus rubellus* and *Dendrobaena rubida* in cow manure mixed cultures with *Eisenia andrei*. Appl. Soil. Ecol., 5: 97-103.

Evans, AC. and Guild, W.J.M. 1948. Studies on the relationships between earthworms and soil fertility. Ann. App. Biol., 35: 471-484.

Garg, V.K., Chand, S., Chhillar, A. and Yadav, A. 2005. Growth and reproduction of *Eisenia foetida* In various animal wastes during vermicomposting. App. Ecol. Env. Res., 3(2): 51-59.

Ganesh, P.S., Gajalakshmi, S. and Abbasi, S. 2009. Vermicomposting of the leaf litter of acacia (*Acacia auriculiformis*): Possible roles of reactor geometry, polyphenols, and lignin. Bioresour. Technol., 100: 1819-1827.

Gunadi, B. and Edwards, C.A. 2003. The effects of multiple applications of different organic wastes on the growth, fecundity and survival of *Eisenia fetida* (Savigny) (Lumbricidae). Pedobiologia., 47: 321-329.

Hallatt, L., Reinecke, A.J. and Viljoen, S.A. 1990. The life cycle of the oriental compost worm *Perionyx excavatus* (Oligochaeta). S. Afr. J. Zool., 25: 41-45.

Hallatt, L., Viljoen, S. and Reinecke, A. 1992. Moisture requirements in the life cycle of *Perionyx excavatus* (Oligochaeta). Soil Biol. Biochem., 24: 1333-1340.

Haimi, J. 1990. Growth and reproduction of the compost-living earthworms *Eisenia andrei* and *E. fetida*. Revue d´Ecologie et de Biologie du Sol., 27: 415-421.

Jackson, M.L. 1975. Soil Chemical Analysis. Prentice-Hall, New Delhi., pp.183-226.

Jones, J.B. 2001. Laboratory Guides for Conducting Soil Tests and Plant Analysis. CRC Press, Boca Raton., pp. 384.

Julka, J. M., Paliwal, R. and Kathireswari, P. 2009. Biodiversity of Indian earthworms – an overview. In: Edwards, C.A., Jayaraaj, R. and Jayraaj, I.A. (eds.) Proceedings of Indo-US Workshop on Vermitechnology in Human Welfare. Rohini Achagam, Coimbatore, India.

Kadam, D.G. 2015. Growth and reproduction of *Eudrilus eugeniae* in Tendu (*Diospyros melanoxylon Roxb.*) leaf residues as influenced by feed particle size. Int. J. Curr. Microbiol. Appl. Sci., 4: 206-210.

Kale, R.D., Bano, K. and Krishnamoorthy, R.U. 1982. Potential of *Perionyx excavatus* for utilizing organic wastes. Pedobiologia., 23: 419-425.

Karmegam, N. and Daniel, T. 2009. Growth, reproductive biology and life cycle of the vermicomposting earthworm, *Perionyx ceylanensis* Mich. (Oligochaeta: Megascolecidae). Bioresour. Technol., 100: 4790-4796.

Kuo, S. 1996. Phosphorus. In: Sparks, D.L. (ed.) Methods of Soil Analysis: Part 3 Chemical Methods, SSSA and ASA, W.I., Madison., pp. 869-920.

Lowe, C. and Butt, K.R. 2005. Culture techniques for soil dwelling earthworms: A review. Pedobiologia., 49: 401-413.

Loh, T.C., Lee, Y.C., Liang, J.B. and Tan, D. 2005. Vermicomposting of cattle and goat manures by *Eisenia foetida* and their growth and reproduction preference. Bioresour. Technol., 96: 111-114.

Reinecke, A., Viljoen, S. and Saayman, R. 1992. The suitability of *Eudrilus eugeniae*, *Perionyx excavatus* and *Eisenia fetida* (Oligochaeta) for vermicomposting in southern Africa in terms of their temperature requirements. Soil Biol. Biochem., 24: 1295-1307.

Rufatto, L.C, Gower, A., Schwambach, J. and Moura, S. 2012. Genus *Mikania*: chemical composition and phytotherapeutical activity. Revista Brasileira de Farmacognosia., 22(6): 1384-1403.

Shipitalo, M., Protz, R. and Tomlin, A. 1988. Effect of diet on the feeding and casting activity of *Lumbricus terrestris* and *L. rubellus* in laboratory culture. Soil Biol. Biochem., 20: 233-237.

Singh, N. 2006. Effect of Initial Substrate pH on Vermicomposting using *Perionyx excavatus* (Perrier, 1872). Appl. Ecol. Environ. Rese., 4: 85-97.

Suthar, S. 2006. Potential utilization of guargum industrial waste in vermicompost production. Bioresour. Technol., 97, 2474-2477.

Suthar, S. and Ram, S. 2008. Does substrate quality affect earthworm growth and reproduction patterns in vermicomposting systems? A study using three popular composting earthworms. Int. J. Environ. Waste. Manage., 2: 584-600.

Suthar, S. 2007a. Influence of different food source on growth and reproduction performance of composting epigeics: *Eudrilus eugeniae*, *Perionyx excavatus* and *Perionyx sansibaricus*. Appl. Ecol. Environ. Res., 5(2): 79-92.

Suthar, S. 2007b. Nutrient changes and biodynamics of epigeic earthworm *Perionyx excavatus* (Perrier) during recycling of some agricultural wastes. Bioresour. Technol., 98: 1608-1614.

Suthar, S. 2008. Microbial and decomposition efficiencies of monoculture and polyculture vermireactors, based on epigeic and anecic earthworms. World J. Microbiol. Biot., 24: 1471-1479.

Suthar, S. 2009. Growth and fecundity of earthworms: *Perionyx excavatus* and *Perionyx sansibaricus* in cattle waste solids. The Environmentalist, 29: 78-84.

Suthar, S. and Sharma, P. 2013. Vermicomposting of toxic weed-*Lantana camara* biomass: Chemical and microbial properties changes and assessment of toxicity of end product using seed bioassay. Ecotox. Environ. Safe., 95: 179-187.

Walkley and Black, I.A. 1934. Determination of organic carbon in soil. Soil Sci., 37: 29-38.

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The Impact of Climate Change on Agricultural and Livestock Production and Groundwater Characteristics in Abu Dhabi, UAE

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ABSTRACT

Agriculture is located at the crossing point between ecosystems and society, where changes in the global environmental conditions affect agricultural activities. The total agricultural area in Abu Dhabi Emirate in 2017 was 749,868 donums. This study had two main objectives; first, to understand how agricultural and livestock production has changed and how these changes are relevant to socioeconomic statuses; second, to assess climate change's impact on agricultural and livestock production through groundwater characteristics. We distributed and collected 301 surveys throughout the three main regions in the Abu Dhabi Emirate (Abu Dhabi City, Al-Ain City, and Al Dhafrah). The results indicated that approximately 68% of the respondents in Al-Ain agreed that it is currently much easier and more profitable to manage a farm than it was 20 years ago. Further, 39% of the farmers agreed that both product quality and quantity have improved over the past 20 years. About 51% of Emirati nationals agreed that production has changed over time. The farmers aged between 51-60 years also agreed that there has been a change in production over time. Half of the farm owners agreed that production has changed, while a majority of the workers provided neutral responses on this topic. While a number of both owners and workers agreed that both production and income levels changed, more respondents disagreed than agreed that these changes had occurred. Finally, the farmers aged between 51-60 years agreed more that the groundwater levels and quality had changed over the past 20 years.

INTRODUCTION

Agriculture is located at the crossing point between ecosystems and society (Olesen & Bindi 2002). Changes in the global environmental conditions affect agricultural activities, but these activities contribute nearly 20% of the greenhouse gas emissions (methane and nitrous oxide in particular) (Rosenzweig & Hillel 2000). Climate is the main determinant of agricultural productivity, and agriculture's processes of food and fibre production heavily burden the environment (Ibrahim et al. 2015). It is expected that climate change will affect agriculture in various ways across the globe (Parry et al. 1999). These effects are highly dependent on the current climatic and soil conditions, the pathways of change, and a region's ability (based on infrastructure and resources) to survive the change (Olesen & Bindi 2002). Based on Stocker et al. (2013) *Summary for Policymakers* in an Intergovernmental Panel on Climate Change Report, anthropogenic influences have been altering the global water cycle since 1960. Anthropogenic influences have magnified moisture content in the atmosphere, which led to global-level changes in the precipitation patterns over land. Currently, cultivated ecosystems have replaced natural vegetation in many areas of the world to grow crops. Unfortunately, many of these cultivated areas have been abandoned for cultural, economic and sustainability reasons (McGuire et al. 2001).

Past experiments have demonstrated that increasing temperatures will generally be beneficial for many field-grown vegetable crops, as this crop production can continue to expand northwards. Temperature increases in certain areas will allow for longer harvesting seasons, leading to continuous market supplies for longer periods throughout the year (Olesen & Bindi 2002). According to the Statistics Centre Abu Dhabi (SCAD)'s 2018 yearbook, horticultural crops covered approximately 5,118 donums of the total cultivated areas in Abu Dhabi Emirate in 2017 (SCAD 2018). The main effects of climatic warming, that is projected for the protected crops, would be changes in the greenhouses' heating and cooling requirements (Olesen & Bindi 2002). As time passes, scientists are becoming more confident that an increase in greenhouse gases will lead to a rise in global temperatures (Houghton et al. 1996). Developing countries have thus become more concerned about climate change's

economic impact on agriculture (Watson et al. 1996). Developing countries largely rely on climate-dependent agriculture; this dependence, in conjunction with poverty rates and rapid population growth, makes developing countries highly vulnerable to climate change (Porter et al. 2014).

Climate change will influence crop and livestock production, hydrologic balances, and several other agricultural system components. Nevertheless, the human responses to these biophysical effects are complex and undefined (Ibrahim et al. 2015, Al-Maamary et al. 2017). Livestock production, like any other production process, releases carbon gases as a result of the energy and fertilizer used in the production process. Cultivated soil, land-use change, and the animals themselves can also release carbon gases (Steinfeld et al. 2006, Gerber et al. 2010). The Ministry of Economy (MOE) reported that the amount of rainfall in the northern and eastern parts of the United Arab Emirates UAE increased from 31 mm in 2001 to 145.5 mm in 2006 (MOE 2007). For these regions, heavy rainfall periods may occur every 10 years (Rizk et al. 1997). In the Arab Gulf states, there is still a dilemma about specifying the water stress levels. However, both virtual water trading and desalination can lessen the many surrounding water stress issues that might occur (Al-Maamary et al. 2017).

The majority of studies have only focused on the local impacts of climate change on the rainfall that feeds agriculture (Al Shidi et al. 2016). However, climate change's impacts on human societies will vary according to numerous factors, such as the amount of low-lying or arid land they inhabit and/or their degree of dependence on agriculture or aquatic resources (Bruce et al. 1996). Water vitally links climate change and agriculture. Water is the base upon which agriculture rests, yet climate change induced temperatures are expected to cause a wider variability in rainfall and temperatures (Sanderson & Curtis 2016).

In the UAE, there has been both a reduction in groundwater resources and an increase in demand for water, leading to stressful situations and limited natural water resources (Murad 2010). Groundwater aquifers depend on precipitation, and precipitation is then highly dependent on the climate. Groundwater is the main freshwater source in the Arabian Gulf Countries' hydrological cycles, and this source (especially during droughts) provides water for daily human consumption, agriculture, industry, and other sectors (Kløve et al. 2014). Based on the above information, this study's research questions are the following: (1) Have agricultural and livestock production changed over the past 20 years in UAE? (2) Has climate change influenced the agricultural and livestock production in UAE? The research objectives of this study are twofold. First, this study aims to understand how agricultural and livestock production has changed and how these changes are relevant to socioeconomic statuses. Second, this study attempts to assess climate change's impact on agricultural and livestock production by analysing groundwater characteristics.

STUDY AREA

Abu Dhabi is the capital and the largest UAE emirate by area (67,340 km^2), accounting for approximately 87% of the country's total land area (excluding islands). This Emirate's topography is dominated by a low-lying sandy terrain dotted with sand dunes, many of which exceed 300 meters in height in certain southern areas. Eastern Abu Dhabi borders the western fringes of the Al-Hajar Mountains. The mountain with the highest elevation in Abu Dhabi is Hafeet Mountain, which is located south of Al-Ain city and reaches approximately 1,300 meters in height. The emirate is located in the tropical dry region. The climate is arid, and the region experiences high temperatures throughout the year with very hot summers. Abu Dhabi has warm winters and only occasionally experiences low temperatures. There are variations in air temperatures among the coastal strip in Abu Dhabi City, the desert interior in Al Dhafra and Al-Ain City, and the areas of higher elevation. These areas together comprise the Emirate's topography. The total agricultural area in Abu Dhabi Emirate in 2017 was 749,868 donums. Al-Ain City had the largest area of plant holdings with approximately 452.503 donums, Al Dhafra had the second-largest area with 207.686 donums, and Abu Dhabi had the third-largest area with 89.679 donums. Based on the SCAD's 2018 statistical yearbook, the total amount of field crop production in 2017 was 234,230 tons, with 16,381 tons in the Abu Dhabi region, 163,056 tons in the Al-Ain Region, and 54,793 tons in the AlDhafra Region.

DATA AND METHODOLOGY

Fig. 1 displays the content and coverage area included in the study's survey. The survey content includes general information, production, costs, and income.

The survey includes 38 questions that are grouped into three sections, questionnaires A, B, and C (see Appendix 1). Table 1 shows the categories and questions that are included in each questionnaire. Many survey questions (Q1, Q2, Q3, Q6…) are relevant to more than one category (production and cost of production and income).

We applied a principal component analysis (PCA) to convert the possibly correlated variables into a set of values of linearly uncorrelated variables. However, this analysis did not show a realistic connection between the variables within the set of values. We performed a pilot study to assure that the questions were clear and answerable. We received feed-

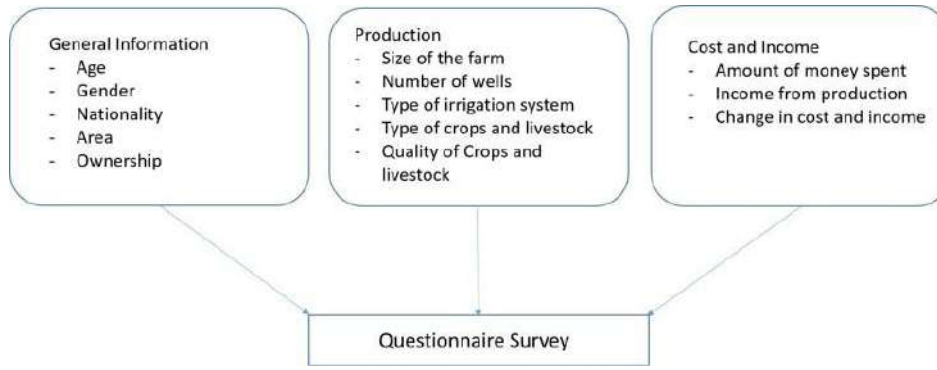


Fig. 1: Survey content and coverage areas.

Table 1: Questionnaire categories and survey questions.

Category	Survey questions
Production (quantity and quality)	Q1, Q2, Q6 to Q19, Q24, Q29, Q32, Q34, Q35, Q37, Q38
Wells and groundwater	Q4, Q5, Q23, Q24, Q25, Q26
Income and cost of production	Q1 to Q3, Q6 to Q22, Q24, Q28, Q30, Q31, Q33, Q36-Q38
Change in production over time	Q20, Q26, Q27, Q28, Q30 to Q36

back from a volunteer who is a colleague that owns a farm, and the survey was modified accordingly. We collected 301 questionnaires in the three cities of the study area.

We distributed the questionnaires according to the regions' production percentages. We used descriptive statistical methods, and used nonparametric tests to compare the quantitative data at different levels of the sociodemographic variables.

RESULTS

Of the 301 collected surveys, 300 of the respondents were male and one was female. The respondents included 162 Emiratis who own farms, 19 Arabs who work on farms, and 1120 Asians who also work on farms. We collected 171 surveys in Al-Ain, 80 in Al Dhafrah, and 50 in Abu Dhabi. Approximately 50% of the respondents were aged between 31 to 40 years old. Fig. 2 displays the pie charts that represent the survey's demographic and general information. We conducted a Cronbach's Alpha reliability test to measure the internal consistency of the questionnaire groupings. The results indicate that the Cronbach's Alpha values were more than 80% for questionnaires B and C, while it was 56% for Questionnaire A. The acceptable values are more than 70% yet for Questionnaire A, the questions are general and will not affect the results. These results might have occurred because the questions are nominal, but we still consider our data to be reliable (Table 2). The survey includes five farm sizes, ranging between 10,000 sqft as small size farms to the big size farms, which are more than 1,000,000 sqft. Above 80% of the farms in Al-Ain are larger than 1,000,000 sqft. In Al Dhafrah, 60% of the farms are between 10,000 to 50,000 sqft in size, while 22% of the farms in Abu Dhabi are between 55,000 to 100,000 sq ft in size.

Nearly 60% of the farmers in Al-Ain use sprinklers for their irrigation systems, followed by the Aflaj irrigation system (an old irrigation system used mainly for date palm planting) and the drip system. The majority of Abu Dhabi farmers use drip as irrigation system (25%), while 34% of farmers use the Aflaj system in Al Dhafrah.

Cows comprise the largest number of livestock in Al-Ain (67%). In AI Dhafrah and Abu Dhabi, the largest number of livestock are camels (31%) and goats (24%), respectively. Fig. 3. Since the category for *other* is undefined, we excluded this category from the analysis. Approximately 68% of the respondents in Al-Ain agreed that managing a farm nowadays is much easier and more profitable than it was 20 years ago. In Al Dhafrah, 29% of the respondents agreed with the profitability statement, while the majority of the Abu Dhabi respondents disagreed.

Table 2: Cronbach's Alpha values for each questionnaire group.

Questionnaire	Cronbach's Alpha
Questionnaire A	0.562
Questionnaire B	0.913
Questionnaire C	0.835



Fig. 2: Demographic and general information collected from the survey.

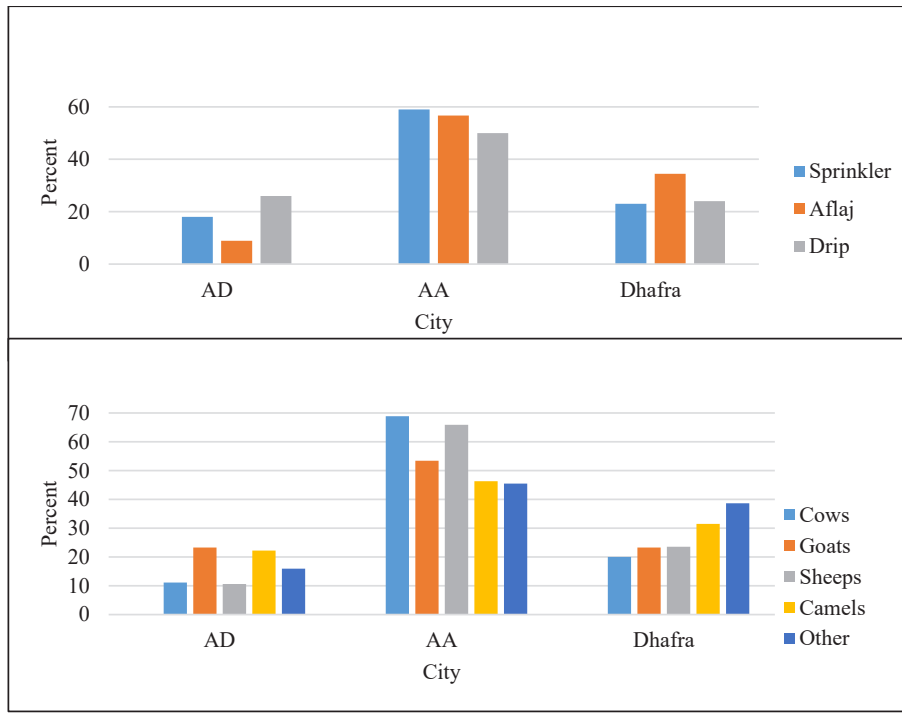
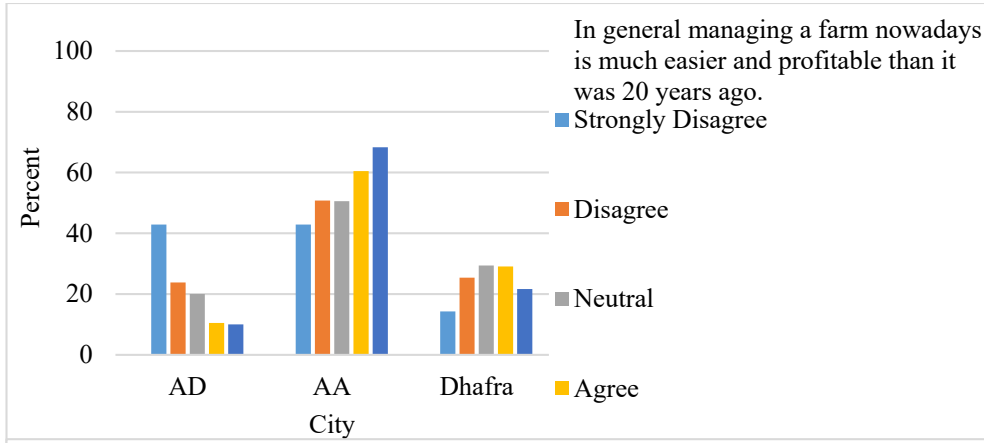
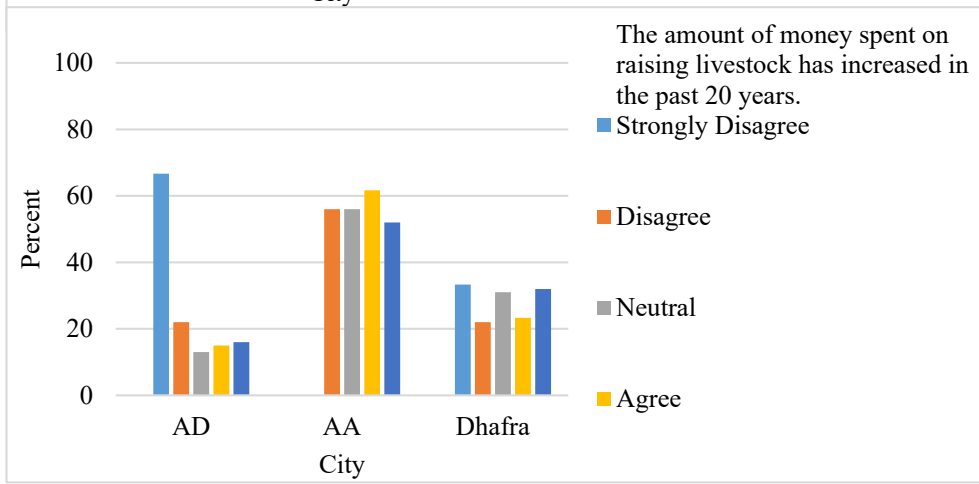


Fig. 3: Irrigation systems and livestock production.

In regard to the question that gauged whether farmers spent more money raising livestock over the past 20 years, the majority (60%) of Al-Ain respondents either disagreed or responded neutrally. Nearly 70% of the respondents in Abu Dhabi, and 34% of the respondents in AI Dhafrah, disagreed that raising livestock has been more expensive. More than 50% of the farm owners (UAE nationals) strongly agreed that their crop sales have increased over the past 20 years, while the majority of workers (Arabs and Asians) either disagreed or responded neutrally (Fig. 4). Approximately 65% of the farmers living in Al-Ain strongly agreed that the groundwater's salinity has increased over the past 20

years. In AI Dhafrah, 28% of the farmers agreed with the groundwater salinity statement, while 24% of the respondents in Abu Dhabi disagreed. The respondents in Abu Dhabi and AlDhafrah strongly disagreed that the depth of groundwater in the wells has decreased over the past 20 years, while 73% of the farmers in Al-Ain strongly agreed.





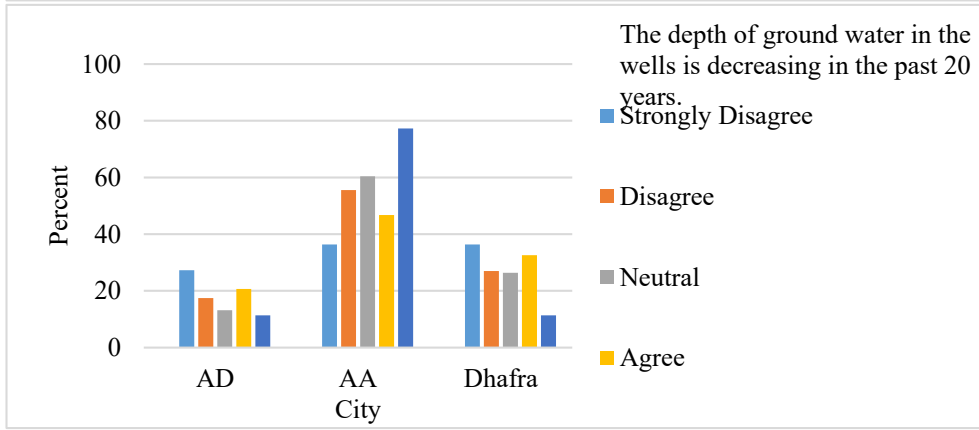


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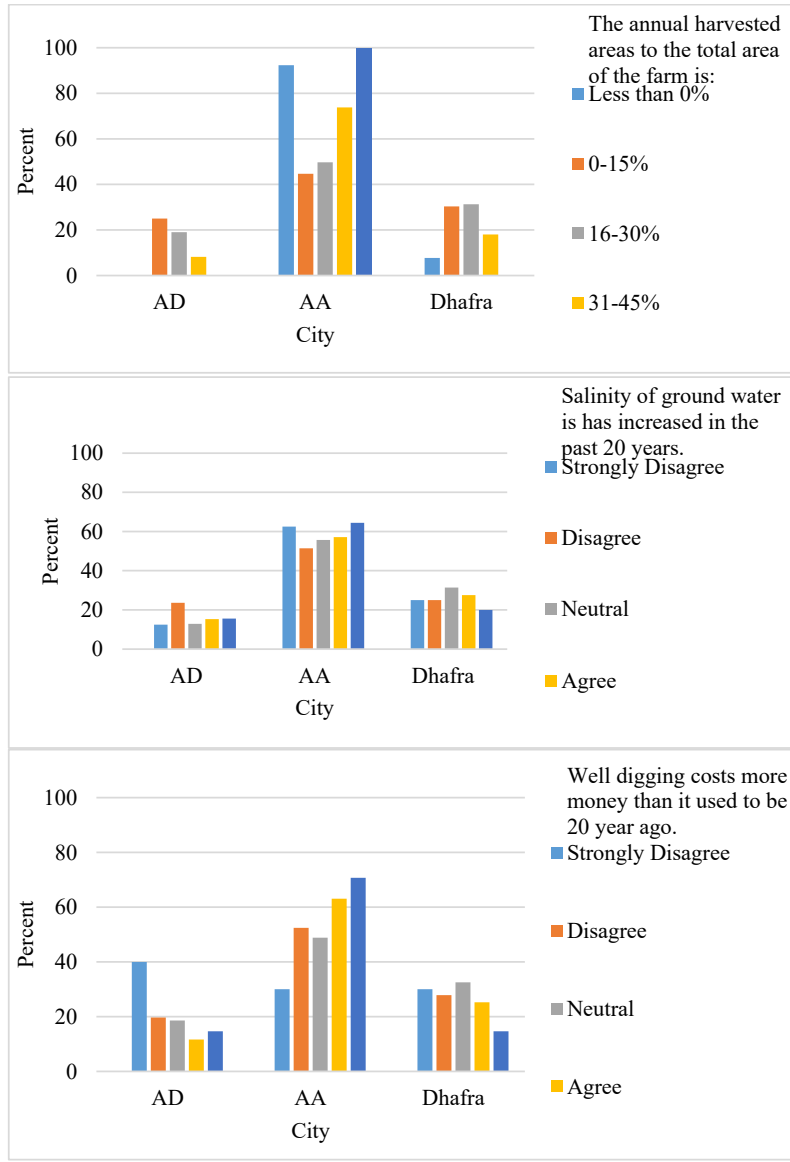


Fig. 4: Survey responses in different cities.

We analyzed the survey questions with each category's average for the survey variables. We excluded gender because there was only one female farmer respondent. The results showed that, for the production category, 39% of the farmers agreed more that production quality and quantity has increased in the past 20 years. In Al-Ain, 46% of the respondents remarked that their production had increased. The farm owners agreed more that their production increasing (47%) in comparison to the workers. In regard to ages, approximately half (50%) of the respondents aged between 20-30 and 51-60 years reported that their production is increasing (Fig. 4).

For the change in production over time category, we found that 41% of the farmers in Al-Ain, 30% in Abu Dhabi, and 33% in AI Dhafrah agreed that there has been a change in production over time. A majority of the UAE nationals (51%) agreed that there has been a change in production over time, with 42% of Arabs and 28% of Asians also agreeing with this statement. (Figs. 5 and 6). The farmers aged between 51-60 years agreed that there has been a change in production over time. Approximately 50% of the farm owners agreed that there has been a change in production, while the majority of the workers responded neutrally on this topic.

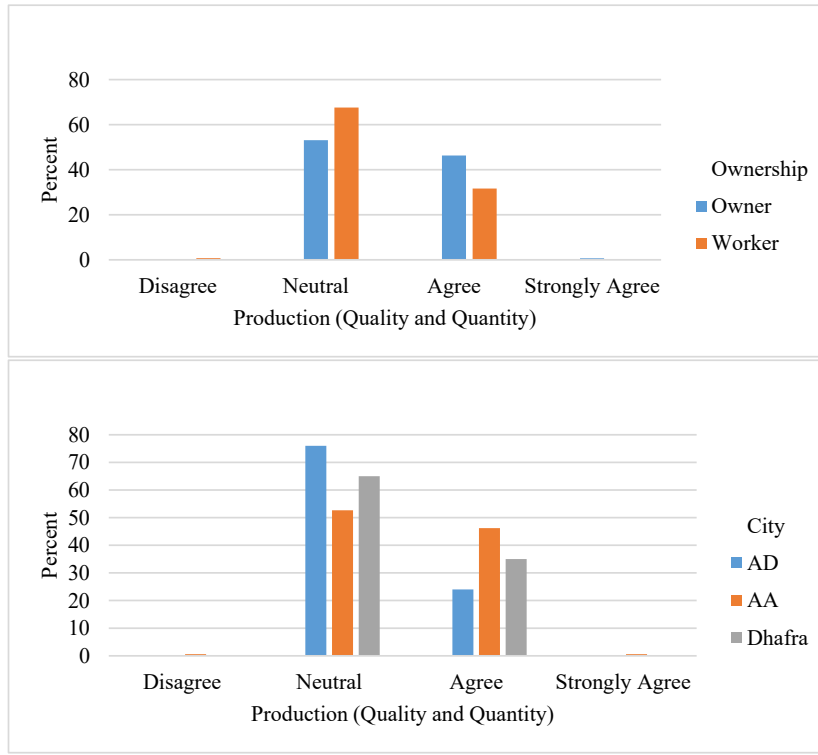


Fig. 5: Survey responses on production quality and quantity questions.

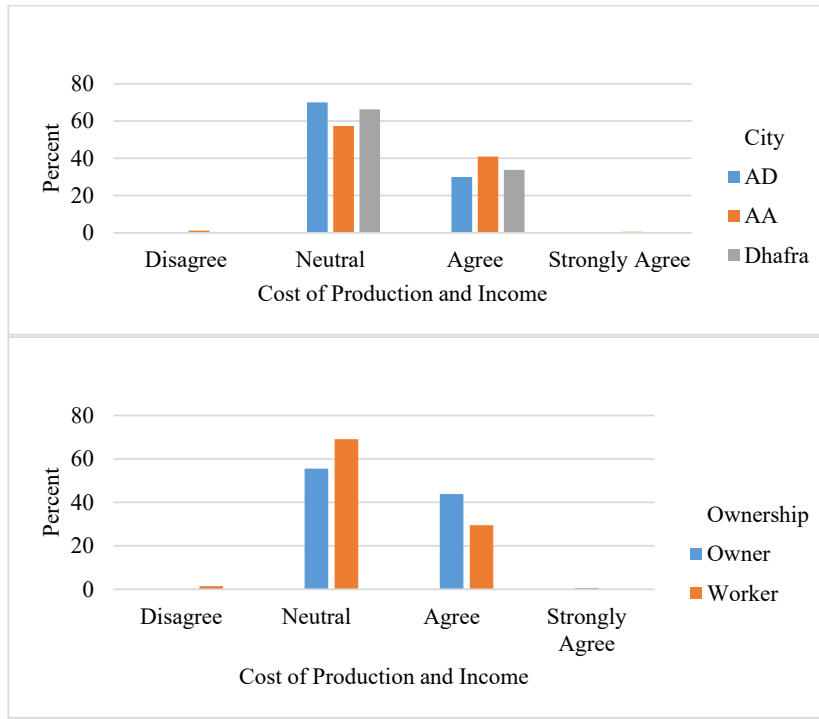


Fig. 6: Responses on the cost of production and income questions.

For the cost of production and income category, both the owners and workers agreed that there has been a change in both production and income. However, more workers disagreed (50%) than workers who agreed (35%) with this statement. In Al-Ain, 40% of farmers agreed that there have been changes in both income and production costs, while only 34% and 30% of the farmers in Al Dhafra and Abu Dhabi agreed, respectively (Fig 7).

Survey responses on wells and groundwater questions are provided in Fig. 8. Farmers aged between 51-60 years agreed more compared to other group ages, that there have been changes in the groundwater level over the past 20 years. Approximately 32% of the workers disagreed that there has been a change in the groundwater quantity, while 18% of the owners agreed. The majority of the respondents were neutral in their responses to this set of questions. The farmers in Abu Dhabi disagreed that there has been a change in the groundwater quantity (26%), and 20% of the AlDhafrah farmers also disagreed. In this case, the percentage of farmers who disagreed was equal to the percentage of farmers who agreed with the groundwater quantity statement (both 18%) in the mentioned cities.

Given that the Likert-type data are ordinal, we used nonparametric techniques to analyze the data. We conducted a Mann-Whitney test to analyze the difference in scoring tendencies between two groups' demographic variables. We also conducted a Kruskal-Wallis test to analyze the difference in the participants' scoring tendencies for the demographic variables of more than two groups. For gender and ownership, we used two variables (Mann Whitney Test), and we used K variables for nationality, city, and age (Kruskul-Wallis Test). The Asymptomatic Significance values are given in Table 3. Our data indicate that there is a significant difference ($P < 0.05$) between nationality and age for question 8 (I plant vegetable crops in my farm). For question 11 (I sell all my produced crops in the market), there is a significant difference between ownership and nationality. There was also a significant difference between ownership and nationality for questions 12, 20, 28 and questions 31-38. For question 22 (the total amount of farm expenses has increased over the last 20 years), there is a significant difference between ownership and nationality and age.

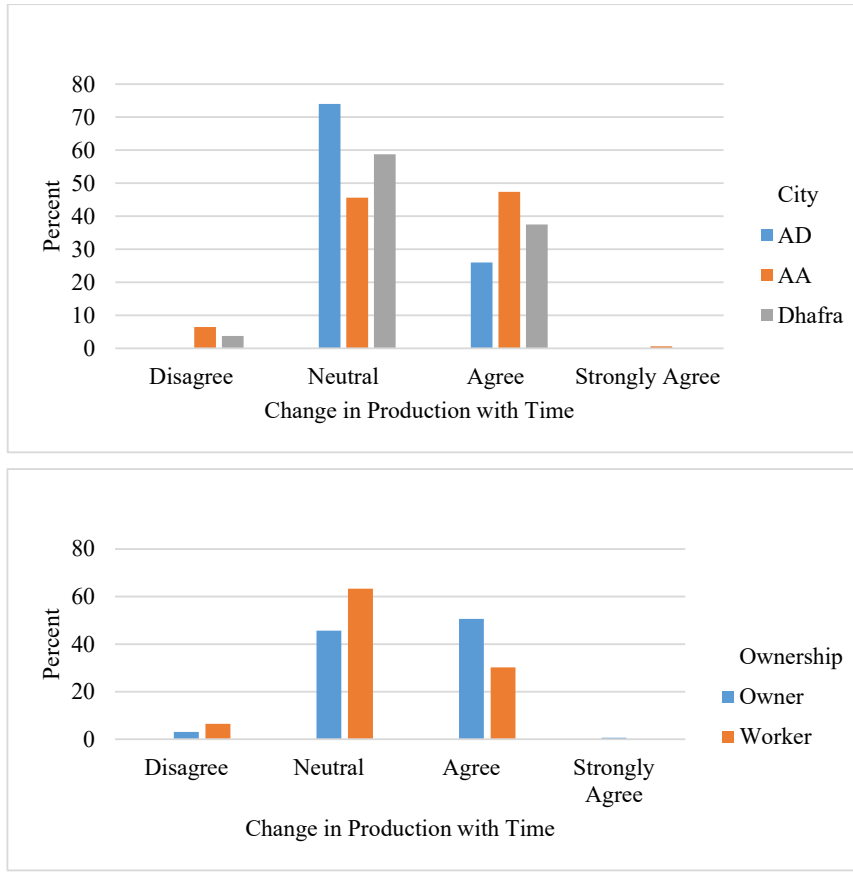


Fig. 7: Survey responses on change in production over time questions.

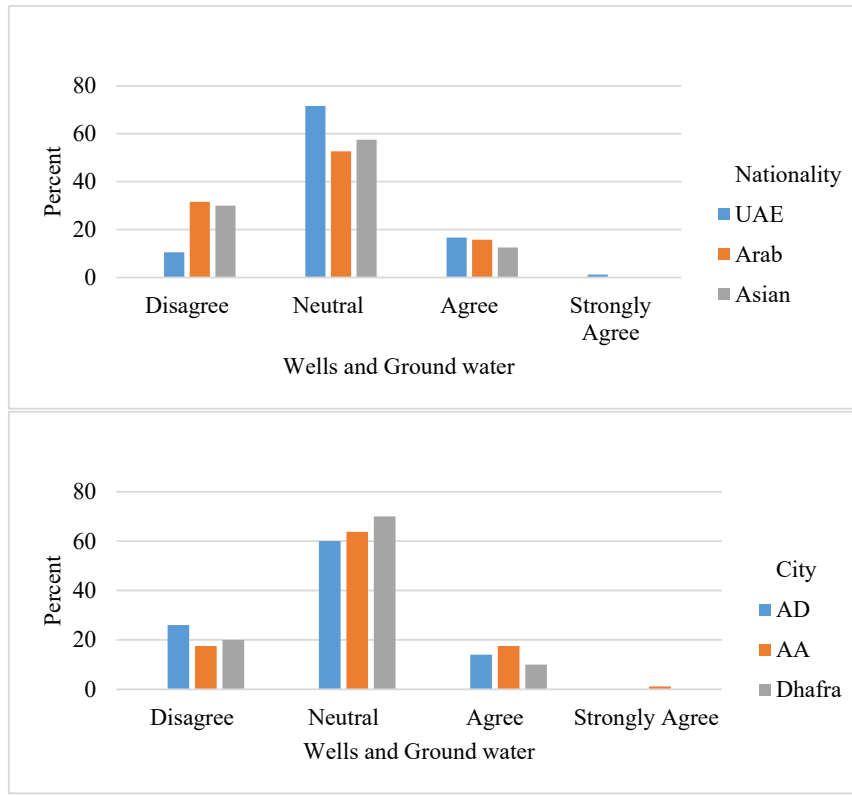


Fig. 8: Survey responses on wells and groundwater questions.

Table 3: Asymptomatic significance values for survey questions.

Question	Gender	Ownership	Nationality	City	Age
1	0.284	0.912	0.542	0.234	0.050
2	0.357	0.006*	0.024*	0.599	0.000*
3	0.479	0.045*	0.091	0.531	0.903
4	0.173	0.165	0.306	0.489	0.015*
5	0.117	0.629	0.636	0.612	0.005*
6	0.415	0.375	0.660	0.066	0.161
7	0.952	0.043*	0.109	0.518	0.209
8	0.988	0.343	0.029*	0.085	0.044*
9	0.085	0.146	0.175	0.00*	0.135
10	0.143	0.034*	0.089	0.00*	0.131
11	0.607	0.012*	0.042*	0.611	0.783
12	0.100	0.001*	0.002*	0.862	0.444
13	0.105	0.298	0.238	0.617	0.347
14	0.571	0.144	0.397	0.359	0.056
15	0.440	0.379	0.414	0.787	0.444
16	0.126	0.671	0.671	0.023*	0.944
17	0.164	0.911	0.451	0.180	0.771
18	0.145	0.056	0.061	0.661	0.974
19	0.653	0.208	0.171	0.594	0.653
20	0.448	0.018*	0.02*	0.369	0.349
21	0.191	0.254	0.183	0.697	0.682
22	0.607	0.005*	0.031*	0.915	0.023*
23	0.471	0.131	0.144	0.434	0.451
24	0.188	0.023*	0.074	0.015*	0.109
25	0.241	0.000*	0.001*	0.554	0.019*
26	0.209	0.708	0.694	0.002*	0.963
27	0.476	0.810	0.954	0.001*	0.033*
28	0.288	0.047*	0.039*	0.000*	0.585
29	0.932	0.000*	0.000*	0.000*	0.206
30	0.179	0.109	0.232	0.083	0.138
31	0.146	0.021*	0.046*	0.328	0.346
32	0.144	0.008*	0.016*	0.256	0.652
33	0.651	0.001*	0.002*	0.007*	0.608
34	0.327	0.000*	0.000*	0.199	0.392
35	1.000	0.001*	0.002*	0.029	0.042*
36	0.302	0.001*	0.001*	0.009*	0.098
37	0.304	0.001*	0.002*	0.001*	0.701
38	0.400	0.000*	0.002*	0.024*	0.320

*Sig. at 0.01 level

DISCUSSION

It is known that climate change will not impact all farms equally (Claessens et al. 2012). While some farms will be greatly impacted by climate change, others will not be impacted at all. Our results indicated that approximately 68% of the respondents in Al-Ain agreed that managing a farm nowadays is much easier and more profitable than it was 20 years ago. In Al Dhafrah, 29% of the respondents agreed with this statement, while the majority of the Abu Dhabi respondents disagreed. These results indicate that the farms in Al-Ain are not influenced by climate change, or, that climate change's influence is more manageable in Al-Ain than it is in Abu Dhabi (where the majority of the farmers were unable to easily manage their farms). Differing governmental policies do not factor in here, as both Al-Ain and Abu Dhabi are governed by the same regulations and codes. Besides, farmers in both Al-Ain and Abu-Dhabi receive similar services and aid amounts from the government.

Instead, climate-related factors may be the drivers of the farms' managing processes. Scanlon et al. (2005) reported that, in the next 50 years, the area of agricultural lands are going to increase by 20%. This supports our results, which indicate that 80% of the Al-Ain farmers strongly agreed that their production has increased over the past 20 years. However, only 28% and 18% of the respondents in AI Dhafrah and Abu Dhabi City agreed, respectively. SCAD (2018) reported that the estimated number of livestock in 2017 was 613,841 in Abu Dhabi, 2,335,268 in Al-Ain, and 595,891 in Al Dhafrah. When questioned about whether the amount of money they spent raising livestock had increased over the past 20 years, the majority of Al-Ain respondents either disagreed or responded neutrally (more than 60%), nearly 70% of respondents in Abu Dhabi disagreed, and 34% of the respondents in Al Dhafrah disagreed. The farmers in the three cities of our study area are managing the livestock properly, and it is clear that they are not facing financial or other possible issues in raising them.

The total value of field crop production was 325,654 AED in Abu Dhabi Emirate, comprised of 74.8% in Al-Ain, 17.3% in Al Dhafrah, and 8.0% in Abu Dhabi City (SCAD 2018). More than 50% of the farm owners (UAE nationals) strongly agreed that their crop-selling income has increased over the past 20 years, while the majority of workers (Arabs and Asians) either disagreed or responded neutrally. The farm owners handle the financial matters related to their farms; the income flows directly to the owners' accounts, while the workers receive their salaries without knowledge of the farm's total profit. Mertz et al. (2009) found that farmers in agricultural countries have been able to develop their strategies for continuously managing the unpredictable climate, pest attacks, and changing policies.

Al-Ain, as the city of an oasis, is known for its agricultural and livestock production, as its people are highly experienced in farming and irrigating techniques and in managing the different cultivation tools. This might explain why the Al-Ain farmers had more positive responses than the respondents in Abu Dhabi and Al Dhafrah. For the *production quality and quantity* category, 50% of the respondents aged between 51-60 years reported that their production is increasing. These respondents likely provided better and more reliable answers, as they have had experience with farming over the last 20 years. For the change in production over time category, we found that 41%, 30%, and 33% of the farmers in Al-Ain, Abu Dhabi, and Al Dhafrah, respectively, noted that production has changed over time. Al-Ain's higher positive percentage for this category is likely also explained by the same reasons listed above. The UAE nationals (51%), Arabs (42%), and Asians (28%) also agreed that there has been a change in production over time. The farmers aged between 51-60 years also agreed that there has been a change in production over time. Approximately half (50%) of the farm owners agreed that production has changed over time, while the majority of workers responded neutrally on this topic. In general, the workers tended to respond neutrally to the questions, which might have been because they were not aware of the issues or because they did not properly understand the questions (due to language differences [for the Asian respondents] or low educational levels [for the Asian or Arab respondents]).

For the cost of production and income category, both owners and workers agreed that income and production costs have changed; however, more workers disagreed (50%) than agreed (35%) with this statement. Approximately 40% of the farmers in Al-Ain, 34% in AI Dhafra, and 30% in Abu Dhabi agreed that income and production costs have changed. The expenses and incomes are both increasing, which has occurred in various businesses due to increases in oil prices, demand for goods, etc. The farm owners thus sell their crops and livestock at higher prices, which could subsequently translate into higher income. Although the total loans given to the farmers in the agricultural sector in Al-Ain was decreased to 166,000 AED, where it was 21,752,000 AED in 2010 and to 147,000 AED in 2017 compared to 4,967,000 AED in Abu Dhabi and AlDhafrah in 2010 (SCAD 2018).

Many farms have been abandoned over the past 20 years due to the new policies and regulations, yet the farmers we surveyed are productive farmers. A study conducted on water consumption rates in the different KSA sectors found that modern irrigation (localized and sprinkler irrigation) covers about 66% of the total irrigated area in the KSA, with the remaining proportion (34%) using surface irrigation (IMF, 2013). Our results showed that almost 60% of the farmers in Al-Ain use sprinklers for their irrigation systems, followed

by the Aflaj and drip systems. The majority of Abu Dhabi farmers use drip irrigation systems (25%), while 34% of the farmers in Al Dhafrah use the Aflaj system.

Many scientists have proposed that climate change might influence the physical characteristics of aquifers (Shah 2009). Our results indicated that farmers aged between 51-60 years agreed more so than did younger farmers, that the groundwater quantities and quality have changed over the past 20 years. This result is important because this age range has the most experience with farming. The majority of the respondents provided neutral responses to this set of questions. It is unclear whether the respondents were not aware of the groundwater's status or if they did not understand the questions due to language differences or other factors. The farmers in Abu Dhabi (26%) and AlDhafrah (20%) disagreed that the groundwater levels had changed.

Alauddin & Sarker (2014) found that the majority of farm households in Bangladesh had observed that both groundwater and surface water were less available during the summers than they used to be. While we did not focus on a specific season, Alauddin and Sarket's study applies to the study region, as the region is dry and hot for most of the year. Groundwater wells are the most important tools that farmers have used to survive droughts. Consequently, well digging tends to peak during drought years, and this is expected to continue and even increase with the amplified hydro-climatic variability (Shah 2009). Approximately 70% of the farmers in Al-Ain strongly agreed that well digging currently costs more money than it used to cost 20 years ago. In Abu Dhabi and Al Dhafrah, 16% and 24% of the farmers agreed that the digging costs had increased, respectively. This cost increase could be due to increases in machinery costs and/or water depths.

CONCLUSION

Of our 301 respondents, 64% of the local farmers agreed that the total farm expenses had increased over the past 20 years. Also, the local farmers strongly agreed that their crop-selling income had also increased over the past 20 years. Approximately 32% of the farmers aged between 51-60 years agreed more compared to the younger groups, that the groundwater levels and quality had changed over the past 20 years. The farmers in Al-Ain agreed that the groundwater's salinity had increased over the past 20 years. Generally, 68% of the Al-Ain respondents reported that managing a farm nowadays is much easier than it was 20 years ago.

In addressing the research questions, it is possible to determine that there has been a change in agricultural and livestock production over the past 20 years and that this change varies (in both amount and nature) across the different regions of Abu Dhabi Emirate. We assumed that climate change has influenced agriculture and livestock production, and we built our survey on this assumption to assess how climate change has impacted farm production and groundwater characteristics. Climate change's negative influence was not indicated in our findings. The responses indicated that both agricultural and livestock production have recently increased. Also, the responses suggest that groundwater characteristics have not been influenced by climate change. We think that our findings open the door for future researchers to further explore the region in regard to climate change's influence on agricultural and livestock production through other factors including groundwater.

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REFERENCES

Al-Maamary, H.M., Kazem, H.A. and Chaichan, M.T. 2017. Climate change: The game changer in the gulf cooperation council region. Renewable and Sustainable Energy Reviews, 76: 555-576.

Al Shidi, H., Sulaiman, H. and Amoatey, P. 2016. Shifting to renewable energy to mitigate carbon emissions: Initiatives by the states of gulf cooperation council. Low Carbon Economy, 7: 123.

Alauddin, M. and Sarker, M.A.R. 2014. Climate change and farm-level adaptation decisions and strategies in drought-prone and groundwater-depleted areas of Bangladesh: an empirical investigation. Ecological Economics, 106: 204-213.

Bruce, J.P., Lee, H. and Haites, E.F. 1996. Climate Change 1995: Economic and Social Dimensions of Climate Change.

Claessens, L., Antle, J., Stoorvogel, J., Valdivia, R., Thornton, P.K. and Herrero, M. 2012. A method for evaluating climate change adaptation strategies for small-scale farmers using survey, experimental and modeled data. Agricultural Systems, 111: 85-95.

Gerber, P., Vellinga, T., Opio, C., Henderson, B. and Steinfeld, H. 2010. Greenhouse gas emissions from the dairy sector: A life cycle assessment. Food and Agriculture Organization of the United Nations, Rome, Italy.

Houghton, J.T., Meiro Filho, L., Callander, B.A., Harris, N., Kattenburg, A. and Maskell, K. 1996. Climate change 1995: The science of climate change: Contribution of working group I to the second assessment report of the Intergovernmental Panel on Climate Change (Volume 2). Cambridge, UK, Cambridge University Press.

Ibrahim, S.B., Ayinde, I.A. and Arowolo, A.O. 2015. Analysis of arable crop farmers awareness to causes and effects of climate change in southwestern Nigeria.

International Journal of Social Economics, 42: 614-628.

IMF 2013. Economic prospects and policy challenges for the GCC Countries.

Technical Report. Report presented at the Annual Meeting of Ministers of Finance and Central Bank Governors, Saudi Arabia.

Kløve, B., Ala-Aho, P., Bertrand, G., Gurdak, J.J., Kupfersberger, H., Kværner, J., Muotka, T., Mykrä, H., Preda, E., Rossi, P. and Uvo, C.B. 2014. Climate change impacts on groundwater and dependent ecosystems. Journal of Hydrology, 518: 250-266.

McGuire, A.D., Sitch, S., Clein, J.S., Dargaville, R., Esser, G., Foley, J., Heimann, M., Joos, F., Kaplan, J., Kicklighter, D.W. and Meier, R.A. 2001. Carbon balance of the terrestrial biosphere in the twentieth century: Analyses of CO_2, climate and land use effects with four process-based ecosystem models. Global Biogeochemical Cycles, 15: 183-206.

Mertz, O., Mbow, C., Reenberg, A. and Diouf, A. 2009. Farmers perceptions of climate change and agricultural adaptation strategies in rural Sahel. Environmental Management, 43: 804-816.

MOE, 2007. Data sheet for the climate of UAE. Technical Report. Ministry of Economics, Metrology Department, Abu Dhabi.

Murad, A.A. 2010. An overview of conventional and non-conventional water resources in arid region: assessment and constrains of the United Arab Emirates (UAE). Journal of Water Resource and Protection, 2: 181.

Olesen, J.E. and Bindi, M. 2002. Consequences of climate change for European agricultural productivity, land use and policy. European Journal of Agronomy,

16: 239-262.

Parry, M., Rosenzweig, C., Iglesias, A., Fischer, G. and Livermore, M. 1999. Climate change and world food security: A new assessment. Global Environmental Change, 9: S51-S67.

Porter, J.R., Xie, L., Howden, M., Iqbal, M.M., Lobell, D., Travasso, M.I., Garrett, K., Lipper, L., McGrath, J., Aggarwal, P. and Hakala, K. 2014. Food Security and Food Production Systems. Methods, 7, pp.1-1.

Rizk, Z., Alsharhan, A. and Shindo, S. 1997. Evaluation of groundwater resources of United Arab Emirates. In: Proceedings of the 3rd Gulf Water Conference, pp. 95-122.

Rosenzweig, C. and Hillel, D. 2000. Soils and global climate change: Challenges and opportunities. Soil Science, 165: 47-56.

Sanderson, M.R. and Curtis, A.L. 2016. Culture, climate change and farm-level groundwater management: An Australian case study. Journal of Hydrology, 536: 284-292.

SCAD 2018. Statistical Yearbook of Abu Dhabi 2018. Technical Report. Statistics Center, Abu Dhabi.

Scanlon, B.R., Reedy, R.C., Stonestrom, D.A., Prudic, D.E. and Dennehy, K.F. 2005. Impact of land use and land cover change on groundwater recharge and quality in the southwestern us. Global Change Biology, 11: 1577-1593.

Shah, T. 2009. Climate change and groundwater: India's opportunities for mitigation and adaptation. Environmental Research Letters, 4: 035005.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., Rosales, M. and de Haan, C. 2006. Livestock's long shadow: Environmental issues and options. Rome, Italy: United Nations Food and Agriculture Organization.

Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, A., Nauels, Y., Xia, V., Bex, V. and Midgley, P. 2013. Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis.

Watson, R.T., Zinyowera, M.C. and Moss, R.H. 1996. Climate Change 1995. Impacts, Adaptations and Mitigation of Climate Change: Scientific-Technical Analyses. Cambridge University Press.

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Water Quality Assessment of River Tungabhadra, India

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ABSTRACT

This study reports the results of an analysis performed on 40.5 km stretch of River Tungabhadra. The results show that there was a significant increase in all the physical and chemical variables of the river towards downstream of Harihara town, particularly in the pre-monsoon season. However, all the variables remain within the standards recommended for drinking water. Based on the Central Pollution Control Board (CPCB) guidelines, the classification of water at various segments of the research focal area is as follows: the 12.08 km stretch from the upstream boundary (Ingalagondi) is designated as class C, based on the Biochemical Oxygen Demand (BOD). However, from 12.08 km to 40.5 km on the stretch of downstream Harihara is designated as Class D, based on the BOD. However, in terms of dissolved oxygen (DO), the river satisfies the requirements for Class C (> 4 mg/L) at all of the locations sampled across all periods.

INTRODUCTION

The increasing populations in both rural and urban areas and rapid industrialization has led to the production of an unmanageable amount of waste. Although the technology for waste management has also evolved considerably, it could not cater to the vast amount of waste being generated. As a result, the rate of deposit of pollutants into the environment does not correlate to the rate of their purification. These inadequacies have led to both short-term and long-term deterioration of the water sources, which are critical to human consumption. In this research, the water quality assessment will be valuable for the control of pollution and the protection of both surface and groundwater. One of the primary factors that cause an outbreak of water-borne diseases in India is the continuous deposition of untreated sewage from towns and villages into water bodies. Biodegradable substances are the core contaminants that affect the dissolved oxygen concentration, and also serves as a major indicator of polluted surface water, estimated data released by the World Health Organization (WHO) indicates that domestic waste is the cause of about 80% of water pollution in developing countries. With respect, the growth rate of the urban areas will be directly proportional to the number of people who lacks safe water and sanitation (Gleick 1993). Several types of research have been conducted to ascertain the quality of water sources. The researchers depend on the evaluation of physiochemical and biological characteristics, as well as the concentration of heavy metals on surface and groundwater (Haribhau 2012, Patil 2012, Manimaran 2012, Kumar 2012, Ranjith et al. 2019 and Ranjan 2012). The core objectives of water quality management in communities is to strike a balance between the interest of the citizens without undermining development potentials while improving and preserving the quality of water. The River Tungabhadra serves as the primary source of drinking water for the people of Davangere district in the northern parts of Karnataka state of India. It is essential to note that besides the increased deposition of domestic sewage in the region, industrial activities are also increasing as well. Given the significant impact that polluted water plays in aggravating human and aquatic health, it is essential to take urgent steps to manage the polluted segment of the river effectively. There has been no prior work that aimed at critically evaluating the water quality of the river stretch that this study focuses on. That is why this research choose the watercourse of River Tungabhadra that stretches through major towns to evaluate the seasonal variations in the quality of water.

MATERIALS AND METHODS

Study Area

The Krishna River (the second largest river of the southern Indian peninsular) has a tributary Tungabhadra River. The recent investigation deals with up to 40.5 km of Tungabhadra River beginning from the Harihara Town upstream, which has a population of above 1.0 lakh. Mudenuru, Rajanahalli, Kumarapatanam, Nalawagalu, Airani and Somalapura villages are located on the stretch of the river after the town of Harihara. These villages have populations varying from 1000 to 30,000. Fig. 1 indicates the stretch of the river chosen for the study. At their location no sewage treatments plants are constructed; therefore, all the waste is disposed without any treating into the river resulting in very serious pollution issues. The MSL (sea level elevation) of the Tungabhadra river is 613 m; having 14.52°N and 75.8°E co-ordinates. This area has a semi-arid climate, with an average to extreme summer and average winter and the erratic rainfall is low. Rain is received in this catchment area in the northeast and southwest monsoon. During May and April, dry weather with severe temperatures prevail-resulting in droughts conditions.

Sampling Stations

There were eight sampling stations selected along the stretch of the river chosen for this study. The sites were chosen based on the point source of waste discharge. The sampling stations were selected at places where the waste is well mixed to ensure that the sample taken represents the water quality of the source. The distance between the sampling stations was optimized to allow for the maximum mixing of the wastewater discharged in the river. This strategy ensures that the samples taken were true representatives of the water quality of various segments of the river.

Sampling Program

Several types of biological and physicochemical variables were chosen as benchmarks for the assessment of the River Tungabhadra water quality in the course of this study. The physical parameters are conductivity, turbidity and temperature. The chemical characteristics are Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), pH, alkalinity and hardness. While the biological variable: Biochemical Oxygen Demand (BOD) and Total Coliform (TC). The water samples for evaluating the physicochemical and biological parameter were taken from all of the sampling stations on the first week of each month for two consecutive years, i.e., 2017-18 and 2018-19. The "dip and grab" sampling method was used to collect the samples at a depth of 15 cm to avoid contamination by surface rubbles and stored at 4 degrees Celsius. At each sampling station, the samples were taken across the width of the river at 1/3, 1/2 and 2/3 of the river width. The samples were analysed for the different parameters mentioned using standard methods (APHA 1998). The temperatures, pH, DO, and conductivity parameters were measured in the field at the time of sample collection with the aid of Portable Star Series Orion (USA) meter. All of the parameters measured were presented as a two-year average for both the pre-monsoon and the post-monsoon seasons.

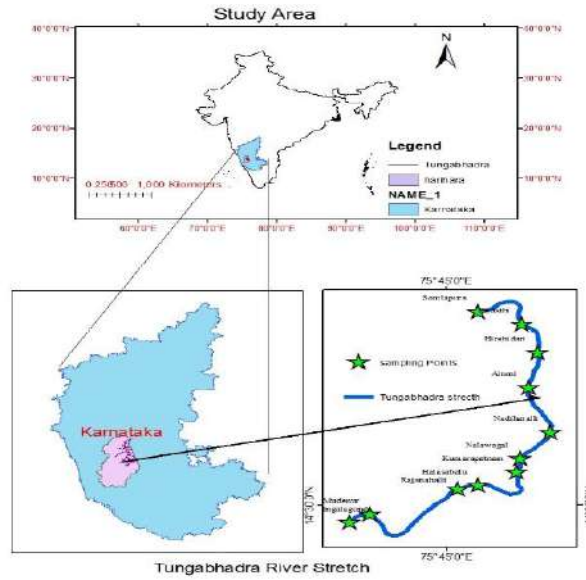


Fig. 1: The stretch of Tungabhadra river selected for the study.

Table 1: Two-year average values during the pre-monsoon season.

S. No.	Parameters	Sampling station (pre-monsoon)							
		SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8
1	Water Temperature	30.05	28.75	28.85	31.65	29.75	29.8	28.15	29.85
2	Turbidity	5.62	16.34	14.45	17.67	14.67	12.75	11.1	8.3
3	conductivity	241.6	301.72	364.62	408.61	396.1	365.4	384.7	450
4	pH	7.24	7.61	7.85	8.2	8.16	7.95	8.1	7.7
5	TA	84.84	141.35	151.6	168.71	162.28	153.1	154.7	151.31
6	CaCO3	86.15	109.65	121.16	155.36	154.1	158.6	160.13	159.8
7	TDS	134.96	176.75	188.1	228.9	231.65	253.1	260.05	271.7
8	Total Coli	1050	4500	4950	4700	4050	4650	3800	2100
9	Faecal coli	450	1250	1650	1750	1255	1400	890	550
10	DO	7.65	8.1	7.93	4.65	5.9	6.85	7.4	8.1
11	BOD	2.9	5.5	6.5	10.95	9.1	7.1	5.04	4.1
12	COD	26.73	40.7	46.1	59.1	79.6	66.3	58.33	61.6

Table 2: Two-year average values during post-monsoon season.

SL.NO	Parameters	Sampling station (post-monsoon)							
		SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8
1	Water Temperature	26.85	27.65	27.85	29.75	28.4	28.3	28.2	28.1
2	Turbidity	4.68	9.63	9.46	12.64	10.7	9.86	10.1	8.75
3	conductivity	221.6	270.7	308.16	371.46	281.8	327.16	316.1	410.73
4	pH	7.32	7.78	7.48	8.41	8.18	7.61	7.76	7.34
5	TA	78.74	127.16	140.05	138.71	143.16	138.6	129.7	148.7
6	CaCO3	71.6	88.7	93.4	110.67	102.7	115.21	118.7	121.6
7	TDS	120.3	133.56	163.4	196.7	197.2	201.1	213.4	234.6
8	Total coli	1100	4750	4850	4900	4600	3250	2800	2200
9	Faecal coli	500	1450	1800	1800	1250	810	700	620
10	DO	8.1	8.8	7.9	5.3	6.1	7.3	7.8	8.4
11	BOD	2.65	4.85	5.4	9.5	8.1	6.4	4.5	3.25
12	COD	20.6	38.6	41.6	50.1	59.85	48.6	46.7	41.25

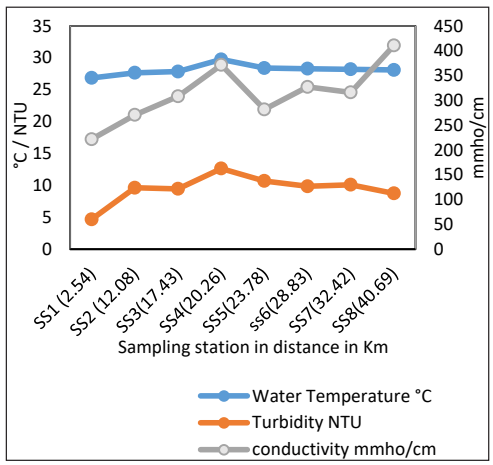


Fig. 2: Variation of temperature, conductivity and turbidity during pre-monsoon season.

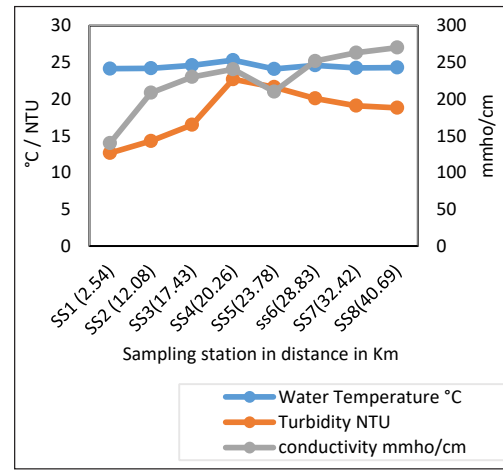


Fig. 3: Variation of temperature, conductivity and turbidity during post-monsoon season.

RESULTS AND DISCUSSION

Tables 1 and 2 attached shows the average values of the various quality parameters at all the eight-sampling station for the post-monsoon and pre-monsoon seasons, respectively. The Figs. 2 and 3 give the graphical representation of the variation of the parameters at all the eight sampling stations during the post-monsoon and pre-monsoon seasons respectively.

Temperature: The temperature help to know the type of biological species present in the aquatic environment and also their level of activity. The variation in the temperature at the different seasons demonstrates the change in the climate of the study environment. At the pre-monsoon season, the temperature of the water varies between 28.15 to 31.65 degrees Celsius for all the eight sampling stations whereas the temperature ranges between 26.85 and 29.75 degree Celsius during the post-monsoon season.

Conductivity: The ability of the water body to carry electric current can be expressed in a numerical value called Conductivity. During the summer season, the conductivity measured at the upstream of Harihara town (SS1) as 241.6 µmhos/cm. and it is found that the conductivity varies from 301.72 to 450.8 µmhos/cm in eight sampling station. However, during the winter season, the values at station-one (SS1) is observed to be 221.6 µmhos/cm, and in rest of the station, it varies between 270.7 and 410.73 µmhos/cm.

There was an abrupt rise in conductivity value from stations 1-2 and the gradual increase from stations 2-8. The changes in the values may be caused due to the discharge of domestic waste into the river from Harihara taluk and neighbouring villages located on the banks of the Tungabhadra river. The study carried out on River Ganga shows that the electrical conductivity increased to an extreme level during the pre-monsoon and reduces during the rainy season (Rao et al. 1990). The research carried out on the Polar River shows similar results (Khare et al. 1986). The peak value for conductivity is noted in the monsoon season at River Ganga which is located at Gazipur (Shukla et al. 1992).

Turbidity: It is expected that the domestic wastewater that finds its way into the river will add a significant amount of both organic and inorganic matter that can increase the turbidity of the river water. The turbidity values are obtained at sampling station 1 during the pre-monsoon and post-monsoon seasons are 5.62 and 4.68, respectively. However, from station 2-8, the turbidity value varies from 8.3 to 17.7 and 8.75 to 12.64 NTU, respectively, indicating that there is a sudden increase in its value, which can be attributed to the domestic wastewater discharge at the downstream of Harihara. As the rainwater carries suspended particles and industries discharge effluents (Unni 1992, 1985), the values of turbidity increases.

pH: It should be noted that a tiny change in pH value also affects the quality of the water significantly. The pH influences other vital factors such as precipitation, trace metal complexation, biological uptake, and their respective reverse pathways. Previous studies have observed alkaline pH values of various riverine systems (Pal et al. 1986). At station 1, the pH value during the pre-monsoon and post-monsoon season was 7.24 and 7.32, respectively. For the other stations 2-8, the pH value varied between 7.6 and 8.2 and 7.3 and 8.4 during the pre-monsoon and post-monsoon season, respectively (Figs. 4 & 5). Results proved that the water samples at all of the stations were slightly alkaline. However, the pH value increased sharply from station 1-2, and from stations 2-8, it continued to increase gradually. Pollution due to the domestic wastewater and industrial waste from Harihara Polyfiber and Grasim industries near Kumarapatanam town and other villages located along the river banks might be responsible for the increase in pH from stations 2 to 5.

Alkalinity: The alkalinity of water is a measure of the capacity of the water to neutralize acids. It is essential to have a slight alkaline environment for many species to thrive. However, when the alkaline content is too high, the taste of a water sample becomes bitter. It is understanding that to reduce the alkaline content of water is the reaction between the alkaline water and cations in the aquatic environment is needed. The precipitations that result from above can cause pipes to clog and foul other water system accessories.

The average values of alkalinity ($CaCO_3$) measurements at Station 1 for the pre-monsoon and post-monsoon seasons were measured as 84.84 and 72.74 mg/L, respectively. However, from station 2-8, the values of $CaCO_3$ vary from 141.35 to 168.71 and 127.16 to 148.7 mg/L for the pre-monsoon and post-monsoon season, respectively.

The results show that the alkalinity levels were high during the pre-monsoon season increasing from station 1 through 8 significantly. The increase was gradual till station 5, then the change became insignificant. The urea-laden wastewater from Harihara and the village on the river banks could be responsible for the increase in alkalinity on the downstream of Harihara.

Earlier researchers reported that the riverine system which receives a considerably high amount of sewage has its alkalinity levels above 50 mg/L (Raina et al. 1984). In the present study, the alkalinity levels at all the stations exceed 60 mg/L across all seasons. The authors found that the alkaline content of the sampled water is within the acceptable levels of concentration (up to 200 mg/L), as per IS: 10500, 1992.

Total hardness: The hardness of water is referred to as

the concentration of various forms of metallic cations in a solution. The levels of hardness that are parallel to the alkalinity are referred to as "carbonate hardness." The excess hardness is termed as "non-carbonate hardness." In practical terms, the levels of hardness of water can be represented as the sum of the calcium and magnesium ions found in the solution. The average hardness levels measured at station 1 during the pre-monsoon and post-monsoon seasons are 86.15 and 71.6 mg/L as $CaCO_3$ respectively. However, from stations 2-8, the values arrived at during the pre-monsoon and post-monsoon seasons varied between 109 to 160, and 88 to 121 mg/L respectively. A close inspection of the results indicates a sharp increase in the values measured from station 1-2, After that, the values increased gradually from stations 2-5. The discharge of domestic waste from Harihara and other settlements by the riverbanks is the likely cause of the increase in the water hardness. Moreover, it was found that the concentration of the hardness increases towards the summer. This change can be as a result of a decrease in the water level as well as a reduction in the velocity of the water currents. The levels of hardness of water still follow the same trend with the change in season. The analysis of the sample tested indicate, the water can be classified as moderately hard (50 to 150 mg/L as $CaCO_3$) during the post-monsoon season. On the other hand, during the pre-monsoon season, the water is moderately hard from station 1-4 only. The other stations contain hard water during the pre-monsoon season. It is essential to note that the water hardness across all seasons are within the acceptable limit for drinking water designated at 300 mg/L (IS: 10500, 1992)

Total dissolved solids: The level of pollution of any water sample is directly proportional to its dissolved solid content. The data computed for the Total Dissolved Solids (TDS) in the pre-monsoon and the post-monsoon seasons for station 1 were 134.96 and 120.3 mg/L respectively. However, from the stations 2 to 8, the TDS values vary from 176.75 to 271 mg/L and 133.54 to 234.6 mg/L during pre-monsoon and post-monsoon seasons, respectively (Figs. 4 & 5).

The higher TDS values occurred during the pre-monsoon season. Throughout all the season, there was a sudden spike in the values observed from station 1-2. Beyond station 2, the TDS value to increase gradually from station 2 through to 8. The increase in the TDS value from stations 2-8 is likely caused by the release of untreated domestic wastewater. Some researchers have observed that a high level of TDS is a pointer to industrial pollutions (Khare & Unni 1986). However, the TDS values measured throughout all the seasons are within the acceptable threshold for drinking water, which is 500 mg/L (IS: 10500, 1992).

Dissolved oxygen: Previous research has established that a groundwater source must have a minimum of 2 mg/L of dissolved oxygen to be able to support higher life forms. For instance, the game fish requires at least 4 mg/L of DO to thrive, and some species may require even more. Besides its ability to support life, DO levels are also crucial in the aquatic environment because the end product of various chemical and biochemical reaction where oxygen is low give rise to unsightly colours and foul taste and odour in water.

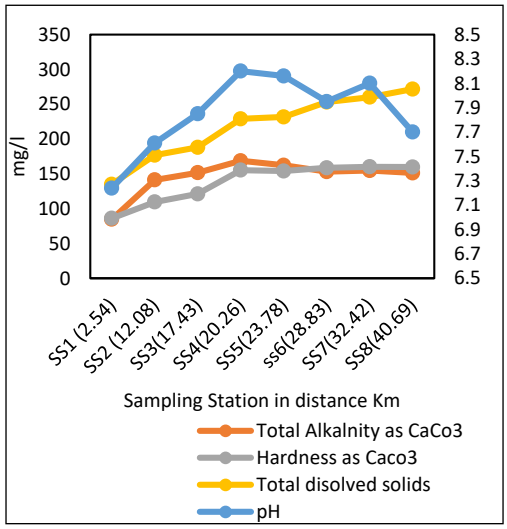


Fig. 4: Variation of pH, TDS, $CaCO_3$ and TA during pre-monsoon season.

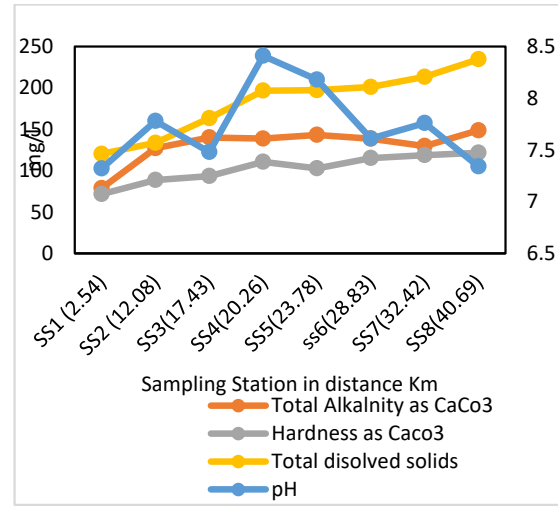


Fig. 5: Variation of pH, TDS, $CaCO_3$ and TA during post-monsoon season.

The present study reveals that the average DO value during the pre-monsoon and post-monsoon seasons are 7.65 and 8.1 mg/L, respectively. On the other hand, the DO values for station 4 is between 4.65 and 5.3 mg/L, respectively. The values measured continued to recover till station 5, then it increased from station 6 to 8 and attained up to 8.1 and 8.4 mg/L during the pre-monsoon and post-monsoon seasons respectively (Figs. 6 & 7).

The differences in the DO values from station 2-6 can be largely attributed to oxidation of dissolved organic matter introduced into the water system through sewages from Rajanahalli to Nadiharahalli, and other villages located on the river banks. The improvement of DO after station 5 is due to atmospheric aeration as there is no further discharge of waste into the water system. As per CPCB, the content of Dissolved Oxygen in the river system aligns with the standards of Class "C" making it drinkable if subjected to conventional treatment and disinfection is done.

The higher atmospheric temperature and increase in the decomposition of organic matter by microbes could be responsible for the reduction of DO during the summer months. The research carried out on Ganga river water quality at Gazipur shows a similar conclusion (Shukla et al. 1992).

Total coliform: When a water body has been contaminated with faecal material, traces of faecal coliform bacteria will be observed in the water body. There is a high likelihood that such water is already contaminated with virulent pathogens such as bacteria and viruses that exist in faecal materials. The occurrence of faecal coliform in natural water can be as a result of contamination through the discharge of domestic sewage, or nonpoint sources of waste from human and animals. The present research shows that the total coliform count for station

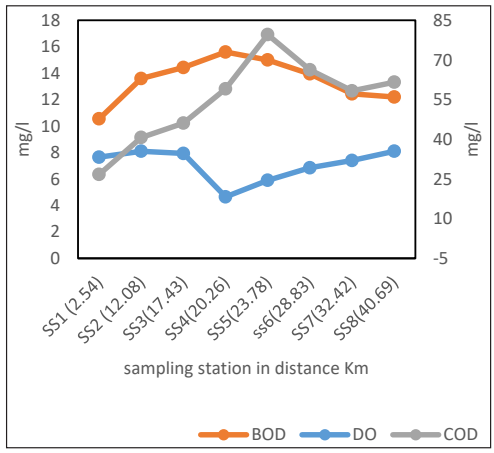


Fig. 6: Variation of BOD, DO and COD during pre-monsoon season.

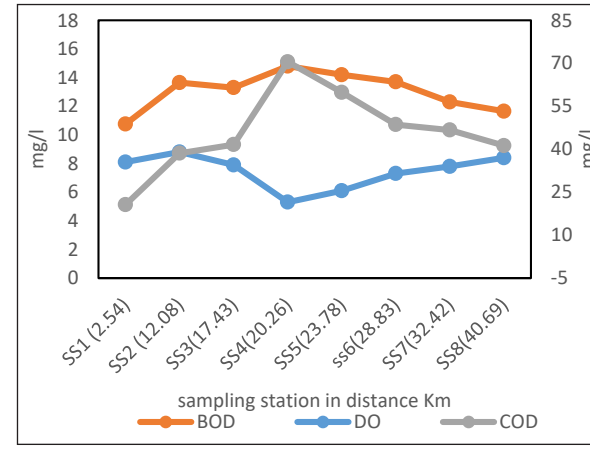


Fig. 7: Variation of BOD, DO and COD during post-monsoon season.

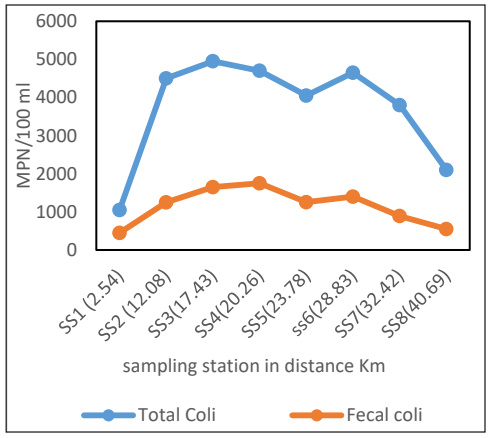


Fig. 8: Variation of Total Coli and Faecal Coli during pre-monsoon season.

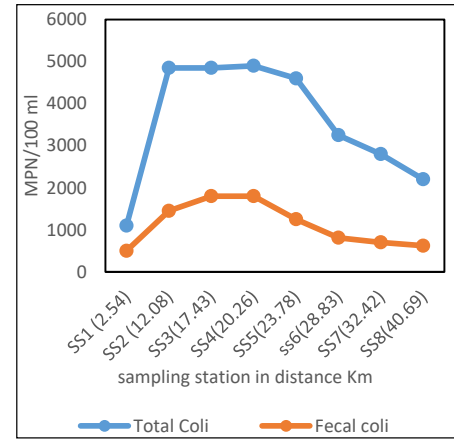


Fig. 9: Variation of Total Coli and Faecal Coli during post-monsoon season.

1 was 1050 and 1100 MPN/100 mL, for the pre-monsoon and post-monsoon seasons respectively (Figs. 8 & 9). For the remaining sampling site (SS2-SS8), the value measured ranges from 550 to 4950 and 2200 to 4850 MPN/100 mL for the pre-monsoon and post-monsoon seasons, respectively. The analysis reveals that the values increased sharply, from station 1 to 2. The same trend continues gradually from stations 3 to 8. Due to the by the discharge of domestic waste at locations upstream of the sampling stations.

CONCLUSION

The present study report on water quality assessment in term of physiochemical and biological parameters across 40.5 km stretch of river Harihara show a sharp increase in values in the readings obtained from upstream of Kumarapatanam to its downstream sampling site. The values observed from stations 3-5 increased gradually. However, all the values are within the acceptable threshold for drinking water (IS: 10500, 1992). The fact that the values measured are increasing while progressing downstream Kumarapatanam shows that there is an urgent need to keep pollution under check by relevant authorities. This intervention parameter within the acceptable limits as industrial activities and the population continues to rise. The BOD value measured at all the monitoring stations exceeds 3 mg/L, except at the upstream Mudenur Village. The highest value for BOD (10.95 mg/L) was obtained. The highest value for the BOD was measured during the pre-monsoon season at 19.9 km upstream of sampling station 1. Taking inference from the various BOD measurements, the only part of the stretch that qualified as Class C is up to 3 km stretch from the upstream boundary. The water obtained from this part of the river is good for drinking, after conventional treatment, and disinfection. However, the results show that the river water derived from 12.8-40.5 km stretch of the area is designated as Class D. As such, the water could be applied for irrigation, industrial cooling, fishery, and control wastewater disposal.

REFERENCES

APHA 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, 20th Ed., Washington DC.

Gleick, P.H. 1993. Water in Crisis: A Guide To The World's Fresh Water Resources. Oxford University Press, New York.

Haribhau, M. G. 2012. Trace metals contamination of surface water samples in and around Akot city in Maharashtra, India, Res. J. Recent Sci., 1(7): 5-9.

Khare, S.K. and Unni, K.S. 1986. Changes in physico-chemical factors and distribution of periphyton in Pollar river Proc. In: K. S. Unni (Ed.), All India Seminar on Water Quality Around Urban Ecosystems.

Kumar, 2012. Physico-chemical and microbiological analysis of underground water in and around Gwalior City, Madhya Pradesh, India, Res. J. Recent Sci., 1(6): 62-65.

Manimaran, D. 2012. Groundwater geochemistry study using GIS in and around Vallanadu Hills, Tamil Nadu, India. Res. J. Recent Sci., 1(7): 52-58.

Patil, A. 2012. Impact of physico-chemical characteristics of Shivaji University lakes on phytoplankton communities, Kolhapur, India. Res. J. Recent Sci., 1(2): 56-60.

Pal, U.C., Bandopadhyay, G. and Santra, S.C. 1986. Importance of phytoplankton study in the assessment of water quality-A case study from Bhagirathi-Hoogly river basin. Proc. All India Seminar on Water Quality around Urban Ecosystem and their Management, pp. 27-29.

Raina, V., Shah, A.R. and Ahmed, S.R. 1984. Pollution studies on river Jhelum-1. An assessment of water quality, Indian J. Environ. Hlth., 26(3): 187-201.

Ranjan, R. 2012. Water quality monitoring of groundwater resources around sugar factory, near East-West Champaran Border, Bihar, India, Res. J. Recent Sci., 2(7): 79-81.

Ranjith, S., Shivapur, A.V., Kumar, P.S.K., Hiremath, C.G. and Dhungana, S. 2019. Water quality model for streams: a review. Journal of Environmental Protection, 10: 1612-1648.

Rao, S.N., Chaubey, R. and Srinivasan, K.V. 1990. Ganga waters quality in Bihar. Indian J. Environ. Hlth., 32: 393-484.

Shukla, 1992. Physico-chemical and Bacteriological properties of the water of river Ganga at Ghazipur, Comp. Physiol. Ecol., 17: 92-96.

Singh, D.K. and Singh, C.P. 1990. Pollution studies on river Subernarekha around industrial belt of Ranchi (Bihar). Ind. J. Environmental Health, 32: 26-83.

Unni, 1992. Preliminary hydro biological studies on river Narmada from Amarkantak to Jabalpur. In : S. R. Mishra and D. N. Saksena (Ed.) Aquatic Ecology.

Unni, K.S. 1985. Comparative limnology of several reservoirs in Central India. Int. Rev. Gesanten. Hydrobiol., 70: 845-856.

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Reduction of Wave Energy Due to Monotypic Coastal Vegetation Using Response Surface Methodology (RSM)

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Cymodocea serrulata
Seagrass meadow
Response surface methodology

ABSTRACT

Information of interactions between waves and aquatic vegetation is becoming increasingly important, in particular, due to the trend of plant-induced wave attenuation towards sustainable coastal management systems. This study aims to understand monotypic vegetation-wave interactions through three-level, four factors, response surface methodology (RSM) using laboratory wave flume under controlled conditions. Cymodocea Serrulata is one of the prevalent monotypic seagrass species found in the Gulf of Mannar, Tamilnadu, South India. It was physically simulated using synthetic plant imitations to create a relationship between wave attenuation (E%) and four direct control factors, i.e., water depth (h), wave period (T), plant density (N) and bed roughness factor (f) using an empiric model. The model developed was tested using the analysis of variance technique (ANOVA) and evaluated for the main and interaction effects of the studied parameters. The findings showed that both individually and in combination, all of the parameters considered were significantly effective on E%. All model-based findings were compared with a new collection of experimental data and validation tests were performed. The comparison of experimental results with model predictions was at a good agreement with a high coefficient of determination (R^2) of 0.98 (with p-value < 0.05).

INTRODUCTION

The rise in sea level and land subsidence along the heavily populated coastline has been recognized as a major threat to coastal regions in many countries around the world. Natural coastal habitats, such as seagrasses, salt marshes and mangrove forests, provide a wide range of ecological services (Larkum et al. 2006, Manca et al. 2012, Specht et al. 2015, Xu et al. 2017) and serve as foreshore vegetation structures (Guannel et al. 2015, Vuik et al. 2018). Such structures potentially mitigate the intensity of tides and waves at locations where they are situated in front of flood control structures. It is well known that vegetated foreshore structures affect local hydrodynamic climate (Koch et al. 2006). The interface of the ocean with dry land reduce wave heights, create mixing layers (Luhar & Nepf 2016) and regulate turbulence due to waves that break in shallow water and attenuate vegetation waves. This natural occurrence has led to the concept of sustainable coastal conservation and protection through the use of natural seagrass wetlands, salt marshes and the mangrove forest network. With a growing population and infrastructure alongside low-lying coastal areas, the potential of coastal vegetation to serve as a bio-shield or non-intrusive buffer to mitigate the combined effects of rising sea levels has recently increased interest (Feagin et al. 2019). In this research, an empirical model based on an experimental design for the transformation of waves over monotypic underwater vegetation (Cymodocea Serrulata species) is constructed within a laboratory flume. A three-level, four-factor, Central Composite Response Surface Methodology (RSM)) was selected for analysis.

THEORETICAL BACKGROUND

For linear waves, Eqn.1 provides a general formula for measuring wave height when translated into energy density (E)

$$E = \frac{1}{8}\rho g H^2 \quad \ldots(1)$$

Where ρ is seawater density, g is the acceleration of gravity, and H is the height of the wave. The reduction in wave energy density was determined by Fonseca and Cahalan (Fonseca & Cahalan 1992) using a percentage reduction in energy density (E%) over a 1 m test segment:

$$E\% = \left[\left\{\frac{E(in)-E(out)}{E(in)}\right\} \times 100\right] \quad \ldots(2)$$

Where E (in) is the energy density entering the 1m test section, and E(out) the energy density leaving the 1 m test section.

MATERIALS AND METHODS

The experiments were performed in the flume of the fluid mechanics laboratory, National Institute of Technology Tiruchirappalli (NITT), Tamilnadu, India. The wave flume measures 12.5 m in length, 0.3 m in width and 0.6 m in depth and is equipped with an electro-hydraulic piston wave generator on one side of the flume. To stop the wave reflection, a 1:7 aspect ratio rubble masonry wave absorber was mounted in the opposite direction of the wave generator. A monotypic artificial plant meadow of one meter in length was placed in the middle portion of the flume. The meadow starts about 4 m away from the wave paddle.

Monotypic conditions on the *Cymodocea serrulata* meadow: Species of Cymodocea serrulata, a coastal seagrass plant with roots, stems and leaves, forming thick vegetative colonies primarily in shallow estuaries (Kuo & den Hartog 2006). It has a smaller leaf structure with whorl-like blades that distinguish it from other seagrass species. India's seagrasses contain 14 populations of about 50 species from seven genera worldwide (Kuo & den Hartog 2006). The Gulf of Mannar and Palk Bay are mainly located in southeast coastal regions as well as island lagoons from Lakshadweep to the Andaman Sea and Nicobar in the East of India's Bay of Bengal.

The physical properties of the selected plants are important for studying wave interactions, leaf bends and the resulting efficiency of wave dampings, such as density and rigidity. In the current research, artificial polypropylene models are chosen on the basis the most similar physical characteristics to real leaves such as the elasticity modulus $E = 0.9$ GPa in the Cymodocee Serrulata meadow (Fig. 1). For a typical natural Serrulata Cymodocea with 4-5 leaves, stems with 1 cm (10-20 cm long and 3-5 mm wide), arranged in opposite directions, each with a length of up to 15 cm can be fitted.

Artificial polypropylene plants of the *Cymodocea serrulata* have a diameter of 0.15 m long leaves with polypropylene streaks (Fig. 1 and 2). Each simulated plant has four leaves attached to a stem of 0.01 m. The mimics were mounted on a standard 1 m and 0.26 m acrylic base frame and connected to a staggered distribution with a width of 43 mm to retain the plant density of 543 stems/sqm. Likewise, a spacing of 21.5 mm was maintained to achieve a plant density of 2163 stems/sqm. Digital HD Video Camera Recorder SONY, Model No: 25 Hz HDR-PJ50V has captured both the experiment and the wave profiles. The captured video images were processed using the MATLAB image processing method and the time series were obtained for the wave profile. The wave height was used to measure wave attenuation before and after the wave.

CENTRAL COMPOSITE DESIGN (CCD)

Experimental design (DOE) was a standard, efficient, statistical approach to research methodology focused on multivariate models and analysis of second order. A standard RSM design called Central Composite Design (CCD) approach for testing the equations of the regression model from the collected data is being used in the current study. The developed regression equations are useful in the analysis of the input parameter interactions affecting the process. The analysis of regression equations draws true and objective conclusions to ensure the experiments are successful. It is also useful to analyze the interactions of the multiple parameters that affect the process.

The RSM technique involves the development of a



Fig. 1: Morphological features used for the synthetic analysis of plant species.



Fig. 2: The plant species imitates the flume under evaluation.

relationship by polynomial expression of the second-order between the variables k. The true link between variables or independent influence factors, x_1, x_2 ... x_k The following expression in Eqn.3 can express x_k and answer Y (dependent variable):

$$Y = f(x_1, x_2, ..., x_k) \quad \ldots(3)$$

Where 'f' is the true response function of an unknown system. The second non-linear polynomial equation to suit the data is given in the form of the following Eqn.4 (Montgomery & Runger 2014):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_{ii}^2 + \varepsilon \quad \ldots(4)$$

Where Y is the predicted response (in the present case, wave attenuation efficiency, $E\%$); X_i and X_j are the independent coded variables; k is the number of factors, b_0 is the model's constant term; b_i, b_{ii}, b_{ij} is the linear, square and interaction effect, and b is the random experimental error respectively. In the present analysis, the Face Centered Central Composite Design (FCCD) approach was used to evaluate and analyze the linear, quadratic, and interaction effects of the variables. Based on the previous analysis four major variables, water depth (h), wave period (T), plant density (N) and bed roughness (f) were selected according to the three levels of each variable with "-1," "0" and "+1" indicating the low, middle and high factor levels respectively as shown in Table 1. The tests, for example, used steel sheet, fine sand, and coarse pebbles to represent the low (-1), medium (0) and high (+1) bed roughness levels. The FCCD method by nature needs 30 experimental runs and a total of 90 experiments were performed at random with 3 replications to eliminate systematic errors. Statistical software (Minitab ® software release 17) has been used for statistical analysis of the experimental data and their response surface graph. Regression analysis was performed on the data obtained to determine the effects of the attribution on the expected response of the selected variables, $E\%$. The simulated seagrass meadow for Cymodocea Serrulata, constructed with plastic as a series of modules on board, was fixed firmly together and onto the floor of the flume. Between the flume walls and the meadow, sufficient gap was created to allow free movement of plant mimics to better represent the actual dispersion of the seagrass meadow in shallow water.

RESULTS

The statistical parameters have been determined using the ANOVA (variance analysis) method. In Eqn.5 the empirical model developed is shown as a coded factor for the $E\%$ prediction:

$$Y = \beta_0 + \beta_1 h + \beta_2 T + \beta_3 N + \beta_4 f + \beta_{1,2} h\,T + \beta_{1,3} h\,N + \beta_{2,3} T\,N + \beta_{2,4} T\,f \ldots + \beta_{1,1}(h)^2 + \beta_{2,2}(T)^2 + \beta_{3,3}(N)^2 + \beta_{4,4}(f)^2 \quad \ldots(5)$$

Where, b_0 is an average of responses and b_0, b_1, b_3, ..., b_{44} are coefficients for regression (Montgomery et al. 2014), which depend on the respective linear, interaction and quadratic terms of variables. The value for each coefficient was determined using the program. The final empirical model was constructed using the following coefficients as defined in Eqn.6:

$$E\% = 28.42 - 24.1h - 9.58T + 6.29\,h * T + 2.43\,h * N - 3.32\,T * N + 19.69\,(h)^2 + 10.24\,(T)^2 - 25.55(f)^2 \ldots(6)$$

ANOVA checks the adequacy and statistical importance of the quadratic response model ($E\%$). The coefficient of multiple determination (R^2) for the quadratic model developed is found to be greater (0.982). The failure to match the F-value of the model was found to be 5.05 (not indicated in the table). This large value may occur because of noise. The expected R^2 value of 0.885 is in good agreement range with the modified R^2 value of 0.965. A new series of experiments were carried out under identical experimental conditions to validate the existing regression model. The collected data were compared with the data generated from the regression model and is graphically illustrated in Fig. 3. Every expected value from the established model is well correlated with its new experimental value.

The high F value (28.42) and the corresponding low p-value (under 0.0001) indicate that the function and the parameters are statistically significant in the development of a regression model. The effect of p-values above 0.05 on the model is statistically negligible. The results show, therefore,

Table 1: Original and coded values of control variables.

Control variables	Unit	Notation	Coded symbol	The original value of coded levels		
				-1	0	+1
Water depth	m	h	b_1	0.10	0.125	0.150
Wave period	s	T	b_2	1.0	2.0	3.0
Plant density	stems/sqm	N	b_3	543	1353	2163
Bed roughness factor	-	f	b_4	0.010	0.017	0.025

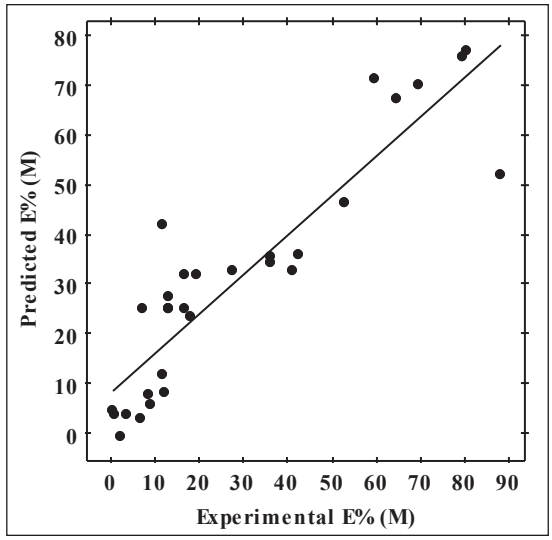


Fig. 3: Comparison between predicted and experimental E%.

that all non-linear terms h and T are relevant; quadratic terms $(T)^2$, $(h)^2$ and $(f)^2$ together with the interaction terms $(h)*(T)$, $(h)*(N)$ and $(T)*(N)$ are significant in response, and $E\%$. The quadratic $(N)^2$ terms and the interaction $(h)*(f)$;$(T)*(f)$ and $(N)*(f)$ terms have a marginal impact within a design range defined.

Impact of parameters: The results from the probability value F-statistics (p less than 0.05) suggested that the Eqn. 6 models could establish a strong correlation between the input and output variables. Parametric variables h, T, N, and f are important for the attenuation of waves caused by vegetation. The graphical representation of the statistically-dependent interaction effects of the two-way factors with their respective regression model ($E\%$) is shown in Fig. 4.

Model parameter interaction results: The interaction effects of all statistically relevant $E\%$ variable on the vegetation meadow are graphically represented in the following sections. The images showing the effect of two-way interaction between the four-wave attenuation parameters are h-T (Fig. 4 (A) and Fig. 5) and h-N (Fig. 4 (B) and Fig. 6) relationship; T-N (Fig. 4 (C) and Fig. 7) inverse relation.

Interaction effect of *h* and *T*: Fig. 5(A) and (B) show the $E\%$ interaction effect of h and T on the vegetation region. In the case of plant stem density N (1353 stems/sqm) and friction factor f (0.0175), $E\%$ is plotted here. At lower h (0.10 m) and T (1.0 s), $E\%$ is the highest (98.32%) and the

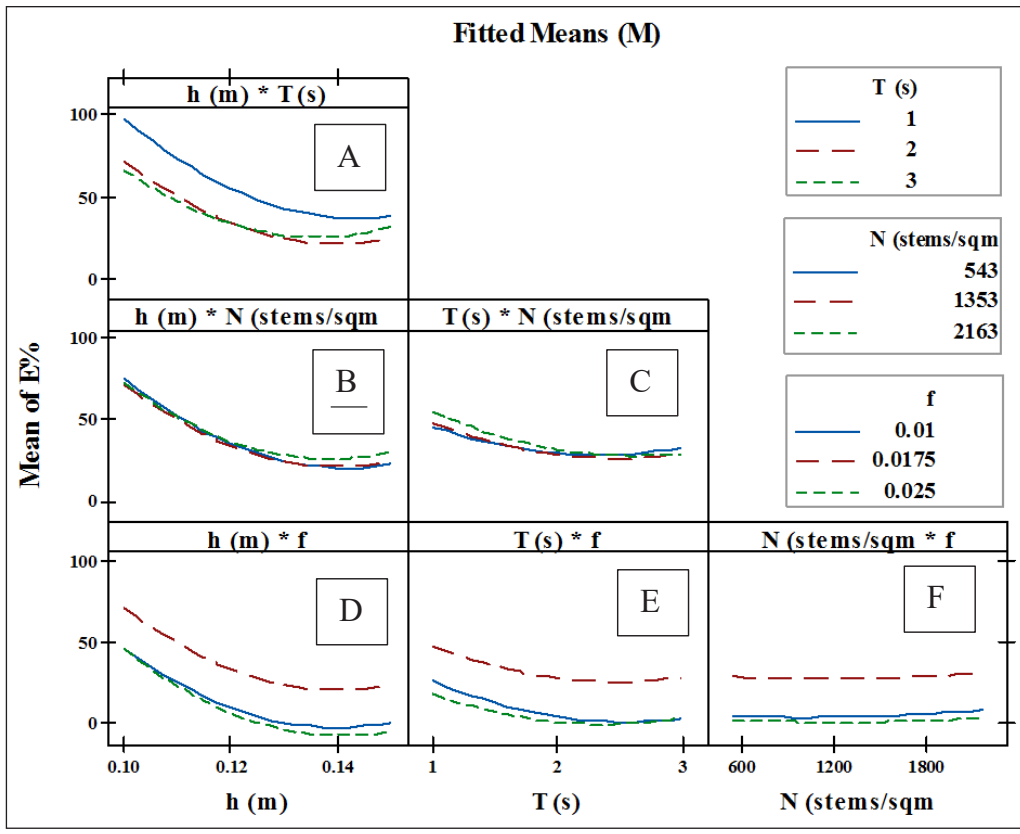


Fig. 4: Two-way interaction effect.

improvement resulted in a decrease in energy loss as the waves passed through the vegetation zone and decreased to as low as (37.53%). With the wave period, the *E%* rises to the small *T*-values point and gradually decreases to the large *T*-values as it travels through the vegetation field. It may be attributed to wider changes in the coefficients of empiric wave decay over short peak wave cycles and almost converging over longer *T*. Also, theoretical coefficients of wave decay are found to be almost independent during variations from peak periods under dips. This is consistent with the study by Huang et al. (2011) that vegetation density and incident wave height are the two variables that often affect wave propagation coefficients.

Interaction effect of *h* and *N*: The regression model developed by Eqn. 6 claimed that the relationship between *h* and *N* had a positive effect on E%, while *N* had a positive effect individually. Fig. 6(A) and (B) display the interaction effect at constant wave period, 2 s and friction factor, 0.0175 as a function of h and *N* at *E%*. The increase from 543 stems/sqm to 2163 stems/sqm in plant density *N* means that the *E%* is around 73.16%. Such findings are consistent with the published results (Bouma et al. 2005), although the published results are not compatible with the effect of *N* on *E%*. As far as the relationship of plant density to wave attenuation is concerned, literature was also contradictory as some studies found that there was no impact from higher plant density (Fonseca & Cahalan 1992) and some other results showed an increase in wave dissipation (Bouma et al. 2005). Nevertheless, the fact that plant morphology (Koch et al. 2006) and mechanical plant morphology such as shoot stiffness (Bouma et al. 2005) have a closer relationship to wave dissipation has been generally acknowledged.

Interaction effect of *T* and *N*: There is also a statistically important interaction effect between *T* and *N* on wave attenuation. Fig. 7(A) and (B) illustrate the interaction effects of *T* and *N* on the vegetation *E%* in the meadow. Indeed, since the cumulative interaction effect is directly proportional to *E%*, an increase of *T* at minimum *N* from 1 s to 3 s results in an increase of *E%*. The surface plot Fig. 7(B) also shows that the shorter wave duration (1 s) results in a higher percentage of wave energy reduction (55%) for maximum plant intensity. At the longest wave period (3 s), however, the highest *N* results in the lowest percentage of wave energy reduction (29.16%). On the contrary, in the shorter wave duration (1 s), the lower *N* results in a higher percentage of wave energy reduction (46%). Möller et al. (1999) stated that, in all wave cycles, salt marshes reduced wave energy to the same degree as flat sand flows at the bottom. However, other studies (Lowe et al. 2007) indicated that attenuation of the vegetation-based wave is primarily dependent on high-frequency wave time. Stratigaki et al. (2011) observed a decrease of 35% in meadow wave height due to a drop in wave frequency. From this analysis, the inverse non-linear effect of the wave cycle on energy variability in the vegetation meadow (Fig. 7A and B) is in good agreement with the published findings.

CONCLUSIONS

The results indicated that all the parameters considered, such as water depth *h*, wave period *T*, plant density *N* and bed roughness factor *f*, have a statistically significant effect on wave energy attenuation under specified conditions. The results also verified that both *h* and *T* have independently negative effects and hence positive nonlinear effects, where-

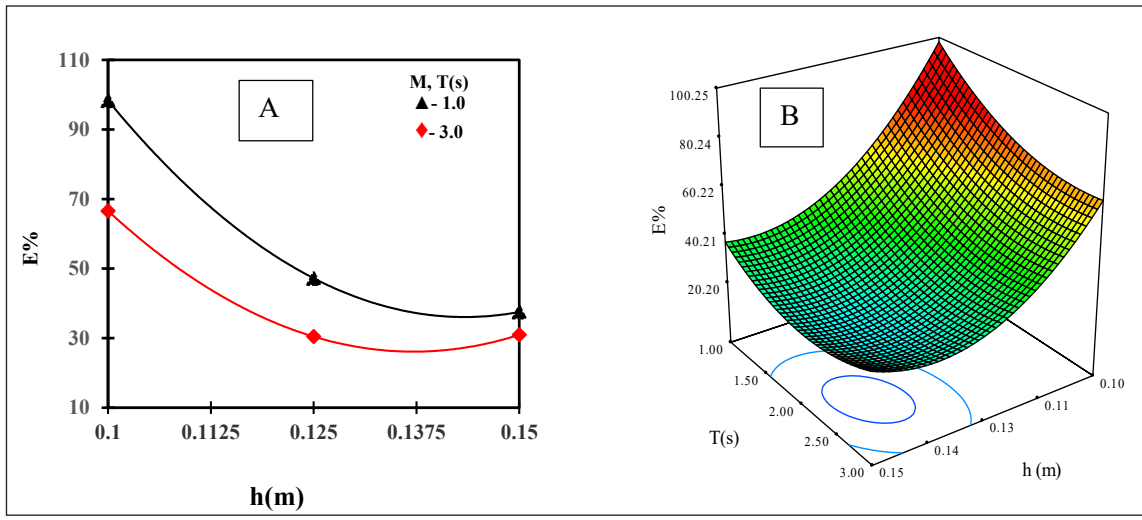


Fig. 5: Interaction effect of *h* and *T* on *E%*, two-way interaction effect (A), surface plot (B).

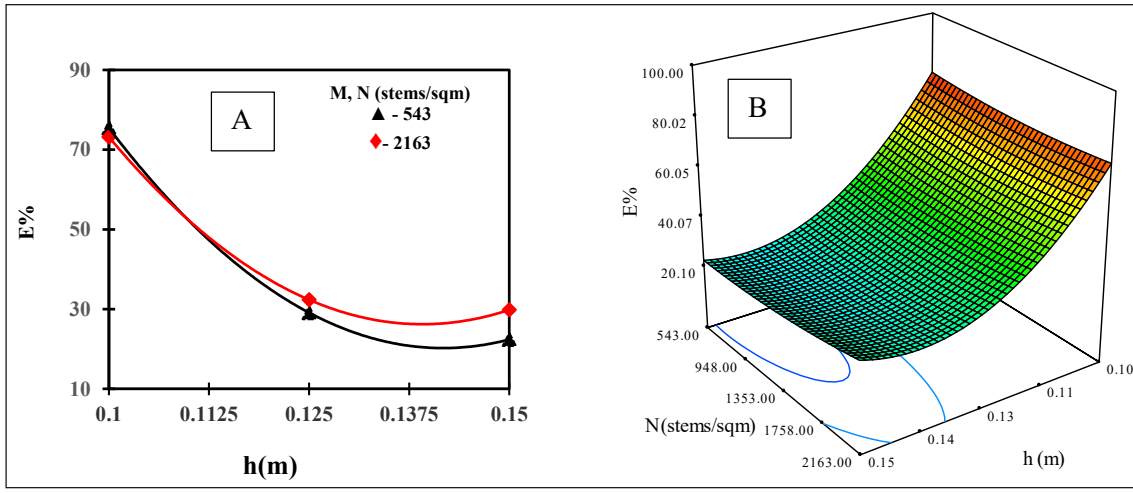


Fig. 6: Interaction effect of *h* and *N* on *E%*, two-way interaction effect (A), surface plot (B).

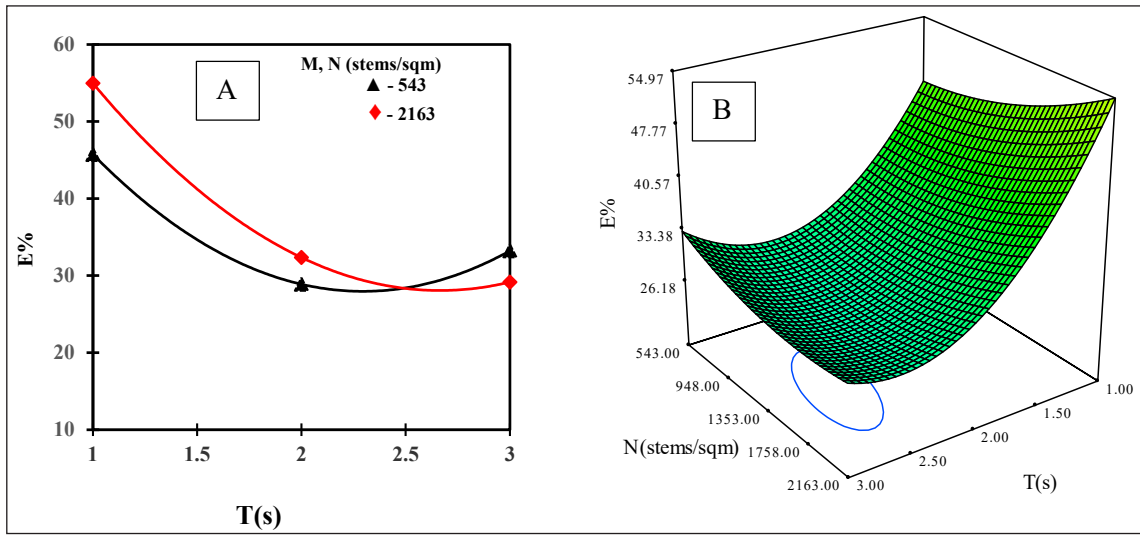


Fig. 7: Interaction effect of *T* and *N* on *E%*, two-way interaction effect (A), surface plot (B).

as *T* and *N* have a negative interaction effect with *f* on *E%*. Water depth contributes most to wave attenuation because it has statistically important bidirectional interactions with two of the other two parameters (*N* and *T*). The RSM technique was found to be extremely effective in predicting the reduction of wave energy by monotypic seagrass meadow as the results of the variance analysis (ANOVA) showed good agreement when comparing the experimental results with the model predictions at a high determination coefficient (R^2) of 0.965 (with p-value < 0.05).

REFERENCES

Bouma, T.J., De Vries, M.B., Low, E., Peralta, G., Tánczos, I.C., Van De Koppel, J. and Herman P.M.J. 2005. Trade-offs related to ecosystem engineering: A case study on stiffness of emerging macrophytes. Ecology, 86 (8): 2187-2199 DOI: 10.1890/04-1588

Feagin, R.A., Furman, M., Salgado, K., Martinez, M.L., Innocenti, R.A., Eubanks, K., Figlus, J., Huff, T.P., Sigren, J. and Silva, R. 2019. Estuarine, coastal and shelf science the role of beach and sand dune vegetation in mediating wave run up erosion. Estuarine, Coastal and Shelf Science, 219 (September 2018): 97-106 DOI: 10.1016/j.ecss.2019.01.018

Fonseca, M.S. and Cahalan, J.A. 1992. A preliminary evaluation of wave attenuation by four species of seagrass. Estuarine, Coastal and Shelf Science, 35(6): 565-576 DOI: 10.1016/S0272-7714(05)80039-3.

Guannel, G., Ruggiero, P., Faries, J., Arkema, K., Pinsky, M., Gelfenbaum, G., Guerry, A. and Kim, C.K. 2015. Integrated modeling framework to quantify the coastal protection services supplied by vegetation. Journal of Geophysical Research: Oceans, 120(1) DOI: 10.1002/2014JC009821.

Huang, Z., Yao, Y., Sim, S.Y. and Yao, Y. 2011. Interaction of solitary waves with emergent , rigid vegetation. Ocean Engineering, 38 (10): 1080-1088 DOI: 10.1016/j.oceaneng.2011.03.003.

Koch, E.W., Sanford, L.P., Chen, S.N., Shafer, D.J. and Smith, J.M. 2006. Waves in seagrass systems: review and technical recommendations (No. ERDC-TR-06-15). (November)

Kuo, J., den Hartog, C. 2006. Taxonomy and biogeography of seagrasses. Seagrasses: Biology, Ecology, and Conservation: 1-23, Availableat: file://localhost/Users/timsherman/Documents/Papers/2006/den Hartog/Taxonomy and Biogeography of Seagrasses 2006 den Hartog.pdf

Larkum, A.W.D., Orth, R.J. and Duarte, C.M. 2006. Seagrasses: biology, ecology and conservation. Seagrasses: Biology, Ecology and Conservation, (May): 1-691 DOI: 10.1007/978-1-4020-2983-7

Lowe, R.J., Falter, J.L., Koseff, J.R., Monismith, S.G. and Atkinson, M.J. 2007. Spectral wave flow attenuation within submerged canopies: Implications for wave energy dissipation. Journal of Geophysical Research: Oceans, 112(5): 1-14 DOI: 10.1029/2006JC003605

Luhar, M. and Nepf, H.M. 2016. Wave-induced dynamics of flexible blades. Journal of Fluids and Structures, 61: 20-41 DOI: 10.1016/j.jfluidstructs.2015.11.007.

Manca, E., Cáceres, I., Alsina, J.M., Stratigaki, V., Townend, I. and Amos, C.L. 2012. Wave energy and wave-induced flow reduction by full-scale model Posidonia oceanica seagrass. Continental Shelf Research, 50-51: 100-116 DOI: 10.1016/j.csr.2012.10.008.

Möller, I., Spencer, T., French, J.R., Leggett, D.J. and Dixon, M. 1999. Wave transformation over saltmarshes: a field and numerical modelling study from North Norfolk, England. Estuarine, Coastal and Shelf Science, 49: 411-426. DOI: 10.1006/ecss.1999.0509

Montgomery, D.C. and Runger, G.C. 2014. Applied Statistics and Probability for Engineers. Eur. J. Eng. Educ., 19(3): 383.

Specht, A., Gordon, I.J., Groves, R.H., Lambers, H. and Phinn, S.R. 2015. Catalysing transdisciplinary synthesis in ecosystem science and management. Science of the Total Environment, 534: 1-3. DOI: 10.1016/j.scitotenv.2015.06.044

Stratigaki, V., Manca, E., Prinos, P., Losada, I.J., Lara, J.L., Sclavo, M., Amos, C.L., Cáceres, I. and Sánchez-Arcilla, A. 2011. Large-scale experiments on wave propagation over Posidonia oceanica. Journal of Hydraulic Research, 49 (SUPPL.1): 31-43. DOI: 10.1080/00221686.2011.583388

Vuik, V., Suh Heo, H.Y., Zhu, Z., Borsje, B.W. and Jonkman, S.N. 2018. Stem breakage of salt marsh vegetation under wave forcing: A field and model study. Estuarine, Coastal and Shelf Science, 200: 41-58 DOI: 10.1016/j.ecss.2017.09.028

Xu, S., Liu, Y., Wang, X. and Zhang, G. 2017. Scale effect on spatial patterns of ecosystem services and associations among them in semi-arid area: A case study in Ningxia Hui Autonomous Region, China. Science of the Total Environment, 598: 297-306 DOI: 10.1016/j.scitotenv.2017.04.009

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Measurement of Radon Concentrations in Mineral Water of Iraqi Local Markets Using RAD7 Technique

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ABSTRACT

The effective technique of RAD7 has been applied to determine the concentrations of radon and annual effective dose of mineral water samples collected from Iraqi local markets. The results show that the level of radon concentrations in mineral water samples ranged between 0.035 and 0.248 Bq/L with an average value of 0.120 Bq/L. In addition to the annual effective dose ranged from 0.129 to 0.905 μSv/y with an average value of 0.440 μSv/y. It was found that the mean value of radon concentration and annual effective dose in all the studied mineral water samples were within the acceptable limits according to the International Commission on Radiological Protection (ICRP) and World Health Organization (WHO).

INTRODUCTION

The water of good quality is free from harmful substances, contaminants and microbes. There are many radioactive materials in different types of waters depending on the type and source of water, seawater contains the highest concentration of potassium-40 (300μc per litre), as for the fountains, contain a high percentage of radium (Canu et al. 2011). Uranium is the most common radioactive element found in the groundwater, with a concentration of 5-10 ppb, which is considered the most dangerous radioactive material (Mohsen & Abojassim 2019, Al-Hamzawi et al. 2014, Al-Hamzawi et al. 2015). Radon, also abundant in groundwater, is a colourless, tasteless and odourless gas with a very short half-life of 3.8 days (Cobb 2013). It is also easy to dissolve in water; however, it does not cause health problems unless it is raised in the form of gas when the water that is dissolved in it is extracted (Castleton et al. 2011). After radon enters the human body through the respiratory system and releases the alpha particles inside the human lung, causing it to damage in the beginning, and then turns into radioactive polonium, which releases the alpha particles then turns into a lead (Kubba 2009). All these disintegrations occur inside the lung, eventually causing lung cancer. Radon can enter the human body through the digestive system while drinking contaminated water. A recent US study suggests that deaths from lung cancer are 14 to 21 thousand cases a year, and the problem is increasing because the gas surrounds us without feeling it (Appleton 2013). Therefore, it is necessary to develop monitoring programs for this dangerous gas to know its concentrations and address the problem of increasing. UNSCEAR organization estimates the radon and its radionuclides produced by its decay, contributions with about three-quarters of the annual effective dose equivalent which are exposed to individuals from terrestrial sources and about half of their doses of all-natural sources combined (UNSCEAR 2000). Method for calculating radon concentrations called (short-term measurements) where the radon concentrations are calculated simultaneously, it is used to observe the level of radon emission for geological sites and earthquake prediction research. In this way, many detectors are employed such as RAD7 detector (Ûrguz et al. 2017). This study aims to develop a database by monitoring the concentrations of radioactive radon gas in most samples of drinking water available in local markets using RAD7 technique as well as determine the annual equivalent dose that reaches the Iraqi people through drinking water.

MATERIALS AND METHODS

Samples Collection

The drinking water was collected in plastic bottles from the markets for several governorates and then was classified according to the trade name and the origin of manufacturing. The total number of water samples was 15 (Table 1). The water samples were labelled with the code of the samples and stored in the refrigerator to the time of analysis.

Table 1: Basic information about mineral water samples.

Sample code	Trade name	Origin
S1	Nawar	Iraq-Najaf
S2	Al-sanam	Iraq-Najaf
S3	Al-taor	Iraq-Najaf
S4	Al-mazaya	Iraq-Najaf
S5	Buratha	Iraq-Najaf
S6	Al-saqee	Iraq-Najaf
S7	Sawa	Iraq-Babil
S8	Al-wahaa	Iraq-Babil
S9	Al-janayin	Iraq-Baghdad
S10	Venezia	Iraq-Baghdad
S11	Muna	Iraq-Kirkuk
S12	Karawan	Iraq-Kirkuk
S13	Life	Iraq-Zakho
S14	Zalal	Iraq-Duhok
S15	Mira	Iraq-Arbil

Experimental Methods

Radon gas concentrations in mineral water samples were measured by using the effective technique of an electronic radon detector RAD7-H_2O (Durridge Co., USA) for one month. This device is customized to determine the levels of radon gas in water at high resolution. The most important feature of this device is the ability to determine the energy of each alpha particle electronically making it possible to tell exactly which isotope. The technique used gives the radon concentrations in the form of a card which represents radon concentrations in the sample (Badhan et al. 2010). A very important factor is the average annual effective dose was determined of water samples received by humans throughout the year. This factor was calculated based on the equation (Kheder et al. 2019):

$$D_w \left(\frac{\mu Sv}{y}\right) = C_w \, C_{RW} \, D_{cw} \quad \ldots(1)$$

Where D_w is annual equivalent dose; C_W is the concentration of radon in water (Bq/L); C_{RW} is consumption amount of water (730 L/y) and D_{C_w} is effective dose coefficient (5 $\times 10^{-9}$ Sv/Bq).

RESULTS

The analytical results obtained from the selected mineral water samples collected from Iraqi markets which represent concentrations of radon and annual effective dose are involved in this study and are shown in Table 2.

From Table 2, the highest value of radon concentrations was 0.248 Bq/L found in sample S13 which belongs to the mineral water, bearing the commercial brand (Life) collected from Zakho city northern of Iraq. While the lowest value of radon concentrations was 0.035 Bq/L found in sample S9 which belongs to the mineral water, bearing the commercial brand (Al-janayin) collected from Baghdad city central of Iraq, with the average value of the radon concentrations was 0.120 Bq/L.

As regards to the annual effective dose, the results ranged between 0.905 to 0.129 µSv/y which were found in sample S13 and S9 respectively, with the average value of the annual effective dose equals to 0.440 µSv/y. Based on these values, the average value of radon concentration is within the permissible limit of radon 0.5 Bq/L as reported elsewhere (Griffiths et al. 2012, Brenner 1994). On the other side, the average value of the annual effective dose is less than the global allowed limit which equals to 1 mSv/y as reported by (WHO 2008, UNSCEAR 1958).

DISCUSSION

According to these results, the concentrations of Rn-222 in mineral water samples collected from different cities of Iraq ranged between 0.248 Bq/L found in sample S13 from Zakho city northern of Iraq and 0.035 Bq/L found in sample S9 from Baghdad city central of Iraq. The reason for the high radon concentrations in mineral water samples from the city of Zakho in northern Iraq with a mountainous nature can be

Table 2: Radon concentrations and annual effective dose in mineral water samples.

Sample code	Radon concentration Bq/L	Annual effective dose µSv/y
S1	0.070	0.258
S2	0.094	0.345
S3	0.212	0.775
S4	0.106	0.388
S5	0.177	0.646
S6	0.141	0.516
S7	0.071	0.259
S8	0.095	0.346
S9	0.035	0.129
S10	0.132	0.484
S11	0.096	0.350
S12	0.089	0.324
S13	0.248	0.905
S14	*BLD	*BLD
S15	0.118	0.430
Mean ± Std Error	0.120 ± 0.058	0.440 ± 0.13

*BLD means below the detection limit

Table 3: Radon concentrations and annual effective dose in water samples for different countries.

Country	Description	Radon concentration Bq/L	Annual effective dose μSv/y	Reference
Iraq	tap water	0.072 – 0.325	1.74	(Al-jnaby 2016)
Iraq	mineral water	0.001 – 0.142	0.096 – 11.402	(Al-Hamidawi 2013)
Iraq	tap water	0.073 – 0.190	0.267 – 0.493	(Tawfiq et al. 2015)
Iran	drinking water	3.79 – 4.17	–	(Keramati et al. 2018)
Syria	drinking water	2.8 – 15.3	–	(Shweikani & Raja 2015)
Jordan	drinking water	0.02 – 0.386	–	(Kullab 2005)
Saudi Arabia	drinking water	1.65 – 3.82	16.3 – 37.5	(El-Araby et al. 2019)
Turkey	tap water	0.0004 – 0.0024	5.87	(Oner et al. 2009)
Iraq	mineral water	0.035 – 0.248	0.129 – 0.905	Present work

attributed to the fact that mountainous areas, in general, have more radioactive materials than low-lying areas as reported elsewhere (Lochard et al. 2009). The average value of radon concentration in selected Iraqi mineral water from different locations of Iraq 0.120 Bq/L is within the permissible limit according to the United States Environmental Protection Agency (US EPA) and International Commission on Radiological Protection (ICRP) 0.5 Bq/L (Griffiths et al. 2012; Brenner 1994). On other hand, the annual effective dose of the mineral water samples varied from 0.905 to 0.129 μSv/y which was found in samples S13 and S9, respectively. The average value of the annual effective in studied samples which equals to 0.440 μSv/y is less than the global allowed limit as reported by United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) and World Health Organization (WHO) 1 mSv/y (WHO 2008, UNSCEAR 1958). These findings indicated that the mineral water samples in Iraqi markets are free from the radiological contaminants and valid for people. Table 3 gives a comparison of the results in the present study with those by the previous researchers in Iraq and different countries. In the present investigation study, the range of radon concentrations and annual effective dose is 0.035 to 0.248 Bq/L and 0.129 to 0.905 μSv/y, respectively. The figures of this study are higher than Turkey and lower than Saudi Arabia, Syria and Iran.

CONCLUSIONS

The results obtained show that the radon concentration levels and annual effective dose of the mineral water samples collected from Iraqi markets were within the acceptable limits. The study indicates that the samples of mineral water are free from radon contamination and suitable for human use.

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REFERENCES

Al-Hamidawi, A.A. 2013. Determining the concentrations of radon and the rate of annual effective dose in some types of drinking water available in the Iraqi markets. Iraqi Journal of Physics, 11(20): 75-80.

Al-Hamzawi, A.A., Jaafar, M.S. and Tawfiq, N.F. 2014. Uranium concentration in blood samples of Southern Iraqi leukaemia patients using CR-39 track detector. Journal of Radioanalytical and Nuclear Chemistry, 299(3): 1267-1272.

Al-Hamzawi, A.A., Jaafar, M.S. and Tawfiq, N.F. 2015. Concentration of uranium in human cancerous tissues of Southern Iraqi patients using fission track analysis. Journal of Radioanalytical and Nuclear Chemistry, 303(3): 1703-1709.

Al-jnaby, M.K.M. 2016. Radon concentration in drinking water sources of the University of Babylon/Iraq. Journal of Karbala University, 14(4): 228-235.

Appleton, J.D. 2013. Radon in air and water. In: Essentials of Medical Geology, pp. 239-277, Springer, Dordrecht.

Badhan, K., Mehra, R. and Sonkawade, R.G. 2010. Measurement of radon concentration in ground water using RAD7 and assessment of average annual dose in the environs of NITJ, Punjab, India. Indian Journal of Pure & Applied Science, 48(1): 508-511.

Brenner, D.J. 1994. Protection against Radon-222 at Home and at Work. ICRP Publication, 65.

Canu, I.G., Laurent, O., Pires, N., Laurier, D. and Dublineau, I. 2011. Health effects of naturally radioactive water ingestion: The need for enhanced studies. Environmental Health Perspectives, 119(12): 1676-1680.

Castleton, J., Elliott, A. and McDonald, G. 2011. Geologic hazards of the Magna Quadrangle. Salt Lake County, Utah: Utah Geological Survey Special Study, 137: 73.

Cobb, A.B. 2013. The Basics of Nonmetals. The Rosen Publishing Group, Inc.

Ćurguz, Z., MirjaniĆ D. and PopoviĆ M. 2017. Comparison of radon concentration measured by short-term (active) and long-term (passive) method. Contemporary Materials, 1(8): 28-32.

El-Araby, E.H., Soliman, H.A. and Abo-Elmagd, M. 2019. Measurement of radon levels in water and the associated health hazards in Jazan, Saudi Arabia. Journal of Radiation Research and Applied Sciences, 12(1): 31-36.

Griffiths, C., Klemick, H., Massey, M., Moore, C., Newbold, S., Simpson, D., Walsh, P. and Wheeler, W. 2012. US Environmental Protection Agency valuation of surface water quality improvements. Review of Environmental Economics and Policy, 6(1): 130-146.

Keramati, H., Ghorbani, R., Fakhri, Y., Khaneghah, A.M., Conti, G.O., Ferrante, M., Ghaderpoori, M., Taghavi, M., Baninameh, Z., Bay, A. and Golaki, M. 2018. Radon 222 in drinking water resources of Iran: A systematic review, meta-analysis and probabilistic risk assessment (Monte Carlo simulation). Food and Chemical Toxicology, 115(1): 460-469.

Kheder, M.H., Ahmad, A.M., Azeez, H.N., Slewa, M.Y., Badr, B.A. and Sleeman, S.Y. 2019. Radon and uranium concentration in ground water of Nineveh plain region in Iraq. In: Journal of Physics: Conference Series, 012033 :(1) 1234. IOP Publishing.

Kubba, S. 2009. LEED Practices, Certification and Accreditation Handbook. Butterworth-Heinemann.

Kullab, M. 2005. Assessment of radon-222 concentrations in buildings, building materials, water and soil in Jordan. Applied Radiation and Isotopes, 62(5): 765-773.

Lochard, J., Bogdevitch, I., Gallego, E., Hedemann-Jensen, P., McEwan, A., Nisbet, A., Oudiz, A., Oudiz, T., Strand, P., Janssens, A. and Lazo, T. 2009. ICRP Publication 111-Application of the Commission's recommendations to the protection of people living in long-term contaminated areas after a nuclear accident or a radiation emergency. Annals of the ICRP, 39(3): 1-4.

Mohsen, A.A.H. and Abojassim, A.A. 2019. Determination of alpha particles levels in blood samples of cancer patients at Karbala Governorate, Iraq. Iranian Journal of Medical Physics, 16(1): 41-47.

Oner, F., Yalim, H.A., Akkurt, A. and Orbay, M. 2009. The measurements of radon concentrations in drinking water and the Yeĝırmak River water in the area of Amasya in Turkey. Radiation Protection Dosimetry, 133(4): 223-226.

Shweikani, R. and Raja, G. 2015. Natural radionuclides monitoring in drinking water of Homs city. Radiation Physics and Chemistry, 106 (1): 333-336.

Tawfiq, N.F., Mansour, H.L. and Karim, M.S. 2015. Measurement of radon gas concentrations in tap water for Baghdad Governorate by using nuclear track detector (CR-39). International Journal of Physics, 3(6): 233-238.

United Nations Scientific Committee on the Effects of Atomic Radiation 2000. Sources and effects of ionizing radiation, ANNEX B, Exposures from natural radiation sources. UNSCEAR 2000 REPORT, New York, 1: 97-99.

United Nations Scientific Committee on the Effects of Atomic Radiation 1958. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation (No. 17). United Nations Publications.

World Health Organization and UNICEF 2008. Progress on drinking water and sanitation: Special focus on sanitation. World Health Organization.

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Assessment of the Surface Water Quality: A Case of Wadi El-Kébir West Watershed, Skikda, North-East Algeria

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ABSTRACT

The considerable increase in the amount of nitrogen in the surface water is a major environmental problem. It has become a great matter of worry because of the multiple environmental effects including eutrophication and health risks. The El-Kebir West watershed is a coastal plain in northeastern Algeria. This study aims to determine the physicochemical quality of waters of the western WadiEl-Kebir and its main tributariesand also to evaluate the spatiotemporal variabilityof its physicochemical quality in particular of mineral nitrogen during wet and dry periods. Four sampling campaigns were realized as follows: January 2015 and February 2016 corresponding to water high season and in March and September 2016 corresponding to the low season. The water samples were obtained from the seven stations located along western Wadi El-Kebir. Temperature, pH,conductivity,dissolved oxygen,SO_4^{2-}, PO_4^{3-}, NO_3^-, $NO_2^-$and NH_4^+ were measured either *in situ* or in the laboratory.A statistical treatment employingthe PCA method (The Principal Components Analysis) was applied for all the obtained results. It has been noticed that the S2 and S3 upstream stations are very rich in ammonium (NH_4^+) at the low water period with average values of 15.22 mg/L and 20.41 mg/L, respectively.This study has shown the influence of seasonal variations and anthropogenic activities on the evolution of physicochemical settings, in general, and on mineral nitrogen in particular. In conclusion,the waters of Wadi El-Kebir were of an average to poor quality.

INTRODUCTION

Pollution affects a large part of our river systems. This is mainly due to the extremely rapid expansion of urban areas that necessarily discharge their treated or untreated wastewaters into river systems, as well as the multiplication of industrial establishments along rivers. But it is also the result of the development of agriculture, which is increasingly using chemical fertilizers, herbicides, insecticides and various pesticides.

The discharge of pollutants can profoundly modify the physicochemical components of receiving aquatic environments as well as the biocenoses inhabiting these environments (Pessonet al.1976). Among the sources of pollution, mineral nitrogen in its various forms (NH_4^+, $NO_2^-$and NO_3^-) represents a direct (toxicity) or indirect (eutrophication) nuisance for the ecological balances of our rivers (Teissier 2001).

The present study takes as field the watershed of WadiKebir West in Ain Charchar, one of the sub-basins of the central Constantine region, located in north-east Algeria. The various economic activities dominated by agriculture and the agricultural processing industry and the continuous expansion of urban areas are sources of pollution of surface and groundwater in this watershed.

This study aims to assess the pollution of the waters of the Wadi El-Kébir-West and its main tributaries, to diagnose the watercourse eutrophication and its receiving environment.

MATERIALS AND METHODS

Study Area

Located in the north-east region of Algeria (Fig. 1), between the Filfila massif in the north and the Constantine mountains in the south, the watershed of the wadi El- Kebir West is part of the greatbasin pouring coasting Constantinois, extends over a total area of about 1130 km^2, and is limited between latitudes 36°30' and 37°00' North and longitudes 7°00' and 7°30' East (Daifallah 2008).This basin has a water potential of about 90 Hm^3.The Wadi El -Kebir West is the main watercourse with a total length of about 43km. Its width varies between 20 and 50 meters and its main tributaries are WadiFendek and Wadi El Aneb.

The climate of the coastal area of the Skikda region is of the humid Mediterranean type. Its annual rainfall reaches

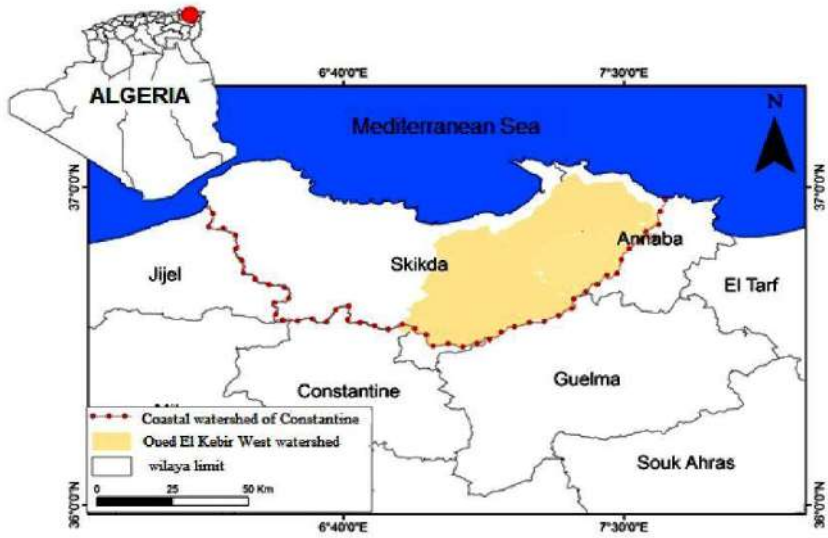


Fig. 1: Geographical location of the Wadi El-Kebir West watershed.

nearly 1300 mm. This area is classified among the most watered regions in northeastern Algeria (Benrabah et al. 2013, Bouchareb et al. 2013).

Water Sampling

Water samples were collected at seven sampling points (stations) selected based on the hydrological importance of the tributaries, the urbanization of the watershed and the ease of access and collection of water samples in the Wadis (Fig. 2).

The geographical coordinates of these different GPS stations are given in Table 1.

The water samples were collected in the middle of the riverbed and introduced intopolyethene bottles previously rinsed with distilled water and the stationwater. They have been then stored at 4°C duringtransporting them to the laboratory of the Soil and Sustainable Development (Badji Mokhtar University Annaba) within the 24 hours of sampling.

Water Analysis

To gradually follow the evolution of water quality in the wadis during both the low and high water periods, seasonal monitoring has been adopted. A total of 4 sampling campaigns were carried out during 2015 and 2016

Table 1: Geographical coordinates of water abstraction stations.

Stations	Coordinates	Station Description
S1 : Wadi Mougger	N 36°42.095' E 007°18.605'	• Upstream of the watershed, • One of the main effluents of the Oued El Kebir watercourse. • Located in an urban area (region from Roknia to Bekkouche Lakhdar). • Presence of thermal sources from which wastewater is discharged into the watercourse.
S2 : Wadi Fendek	N 36° 43.655' E 007°05.008'	• Upstream of the watershed • Azzaba Region
S3 : Wadi Emchekel	N 36° 44.642' E 007°14.374'	• Located in the inner part of the watershed of Oued El Kebir West. • Agricultural and industrial area.
S4 : Wadi El Kébir	N 36°44.468' E 007°26.125'	• Urban and industrial area • Located near the Hadjar Soud Cement Plant.
S5 : Wadi Magroun	N 36°52.439' E 007°25.640'	• Confluence of the Oueds Mougger and Emchekel (Ben Azzouz). • diversified industrial area.
S6 : Wadi Aneb	N 36°52.384' E 007°25.640'	• Downstream of the watershed • Urban and agricultural area. • Length of this Oued greater than 26Km
S7 : Wadi Ennkouche	N 36°58.185' E 007°15.637'	• Located 200 meters from the entry into the sea. • Agricultural area characterized by the existence of marshes.

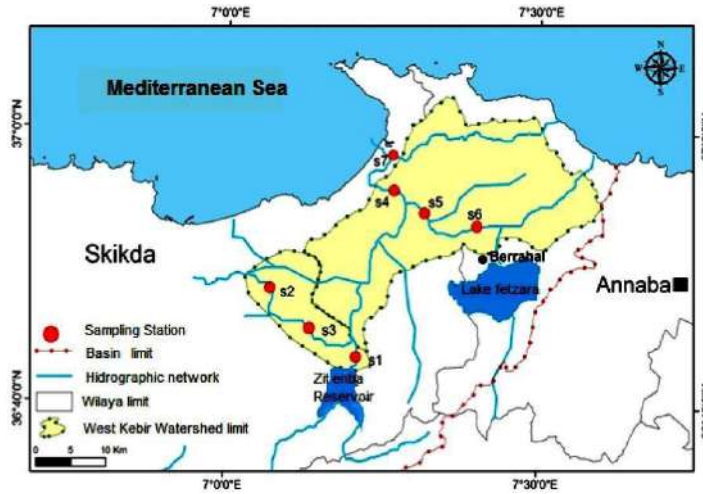


Fig. 2: Location of water sampling stations in the Wadi El-Kebirwest watershed.

(two during the wet season and two during the dry season), i.e. January 2015-February 2016 and May 2016-September 2016.

For each sample collection, *in situ* measurements of temperature, pH, electrical conductivity and dissolved oxygen were performed to determine certain characteristics of the sampling environment. Thesewere measured using a multi-parameter (HANNA instruments HI 9828).

The chemical parameters were determined byusing the standardized methods of Rodier (1978) using a spectrophotometer. Nitrate ions (NO_3^-) were determined by the sodium salicylate method and nitrite ions (NO_2^-) by the Zambelli reagent method. The determination of ammonium ions (NH_4^+) was carried out by the Nessler reagent method. The orthophosphate (PO_4^{3-})dosing was analysed by the ammonium molybdate spectrophotometric method.The sulphate ion (SO_4^{2-})was determined by gravimetry after precipitation in the form of barium sulphate.

We have treated the data of the physicochemical analysis by the statistical method called Principal Component Analysis (ACP) using XLSTAT 2016 software.

RESULTS AND DISCUSSION

Physicochemical Characteristics

Water temperature is an important factor in the aquatic environment because it regulates almost all physical, chemical and biological reactions (Nouayti et al.2015).

The water temperature values of Wadi El -Kebir West vary according to the seasonal rhythm, with a maximum value of 23.5°C (Station S2) in the dry period (4th campaign) and a minimum value of 11.5°C (Station S4) in wet period (1st campaign) (Fig. 3a). The temperature of surface waters (rivers, lakes and reservoirs) varies greatly according to the season and can go from 2°C in winter to 30°C in summer (Potelon & Zysman 1998).

Electrical conductivity indicates the ability of an aqueous solution to conduct electrical current. It represents the total content of the mineral salts present dissolved in an aqueous solution (Ayad 2016). The minimum value (198µS/cm) was observed at station S6 during the wet season (2nd season). The highest conductivity value (3024 µS/cm) measured at station S7 (downstream of the wadi) in the dry period (3rd campaign) indicates high mineralization probably due to urban wastewater discharges and agricultural leaching.

The temporal variation in the electrical conductivity of the waters of Wadi El-Kebir shows a decrease during the high water period (Fig. 3b). Abundant rainwater during this period would be the main factor in reducing the electrical conductivity of the water (Doubi et al. 2013) in Wadi El-Kebir West.

The pH of the water measures the concentration of H^+ions found in the water. It summarizes the stability of the balance established between the different forms of carbonic acid and it is linked to the buffer system developed by carbonates and bicarbonates (Makhoukh et al. 2011).The observed values do not show any significant variations and tend to be basic with a minimum of 7.37 at station S4 in the 1st campaign and a maximum of 8.85 at stations S3 and S5 (3rd campaign) (Fig. 3c).

The increase in pH during the high water period is thought to be related to the dissolution of carbonate from limestone rocks and the addition of humic acids during soil leaching (Bou Saab 2017).

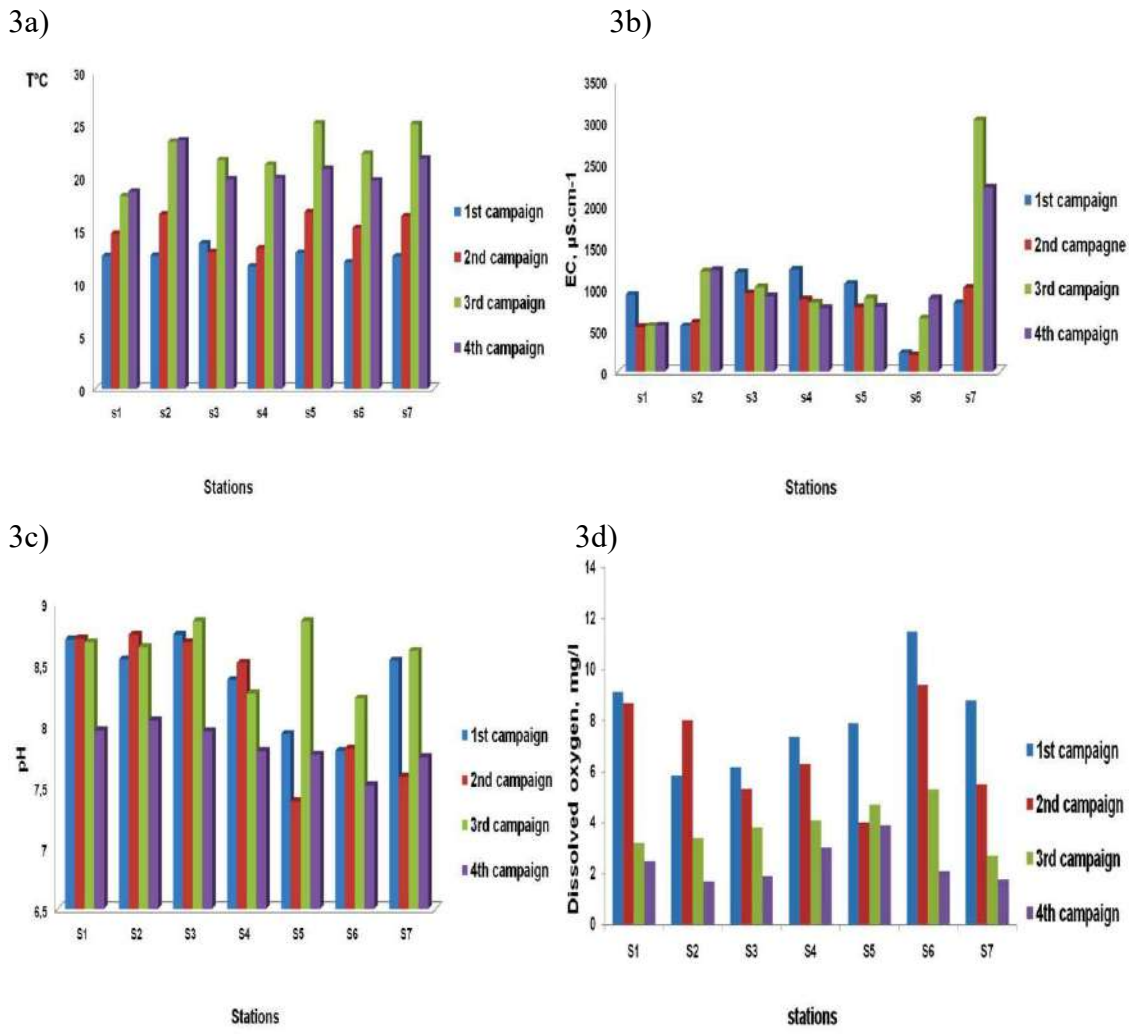


Fig. 3:Spatial and temporal variation of (a) temperature, (b) electrical conductivity, (c) pHand (d) dissolved oxygen in the waters of the Wadi El-Kebir West.

The quantification of the concentration of dissolved oxygen in water (DO) in a hydro-system is a fairly important factor because it is involved in the majority of chemical and biological processes in these aquatic areas. Its value provides us with information on the degree of pollution and therefore on the degree of self-purification of a watercourse (Makhoukh et al. 2011).

In our study, the seasonal evolution of dissolved oxygen shows higher concentrations in wet periods than in dry periods. Indeed, the reported levels (Fig. 3d) range from 1.8 mg/L (S7, 4th campaign) to 11.5 mg/L (S6, 1st campaign). The concentration of dissolved oxygen in natural waters can be influenced by several factors; the increase in this parameter in the waters of Wadi El-Kebir would be related to rainwater loaded with atmospheric oxygen and strong flow rates promoting air diffusion in the waters of rivers (Makhoukh et al. 2011).

The low dissolved oxygen levels recorded in the dry period (S2, S3 and S7) are probably related to the high organic loads of urban discharges (Reggam et al. 2015).This situation is favourable to the self-purification of the wadi and the oxidation of organic and ammoniacal nitrogen to nitrate ion.

Nitrate is naturally present and soluble in soil, entering soil and groundwater and discharging into watercourses. It generally comes from the decomposition of organic matter by bacterial oxidation of nitrites and is thus the ultimate product of nitrification. However, it is also synthetically provided by fertilizers and is one of the factors contributing to the degradation of water quality (Abboudi et al.2014).

The histogram of nitrate ion concentrations in the waters of Wadi El- Kebir West (Fig. 4a) shows a slight variation in these concentrations, which oscillate between the minimum value of 0.2 mg/L registered at station S6 (1st campaign) and the maximum value found at station S1 (4th campaign) with 14.21 mg/L. These results do not exceed the accepted standard for surface water of 50 mg/L (ABH 2010).

Nitrite, also called nitrous nitrogen, it is an intermediate product between nitrate and ammonium ion in nitrification and denitrification processes. This form of mineral nitrogen, the least oxygenated and most unstable, is an ecotoxic precursor in aquatic environments and a human health risk. Nitrites are spread in soil, water and plants but in relatively small quantities (Aissaoui 2012).

The highest concentration (1.04 mg/L) was recorded at station S5 in the 3rd campaign (dry season) (Fig. 4b). The presence of nitrites in significant quantities in the waters of the watercourse would indicate contamination resulting from the discharge of wastewater and an oxygen deficit in the environment and/or a reduction in nitrates by organic matter (Benrabah et al. 2013).

The found values do not exceed the accepted standard for surface water 1 mg/L(ABH 2010), except at station 5 (3rd campaign), but they indicate pollution of the waters of Wadi El-Kebir.

Ammoniacal nitrogen is a good indicator of the pollution of watercourses by urban effluents. It is one of the links in the complex nitrogen cycle in its primitive state. It is a water-soluble gas. In surface waters, it comes from nitrogenous organic matter and gaseous exchanges between water and the atmosphere(Makhoukh et al. 2011)

Analysis of the ammonium profile (Fig. 4c) shows that the levels are between the minimum value of 0.44 mg/L recorded at station S2 (1st campaign) and the maximum value found at station S3 with 30.98 mg/L (4th campaign). Ammonium values found in wet weather are lower than those found in dry weather. The effect of dilution by rainwater is the good oxygenation of the waters of the wadi leading to the oxidation of nitrogen so that these values are consistent with the values of dissolved oxygen and the values of nitrate ion concentrations in the winter period (Debieche 2002). In natural waters, the detection of ammonium in large quantities ($\geq$5 mg/L) is a pollution criterion (Dussart 1966, Bengherbia et al. 2014)

Phosphate (PO_4^{3-})is the result of the degradation by bacteria of organic phosphorus contained mainly in wastewater. They can also be introduced in significant quantities into watercourses by runoff from agricultural fields enriched with phosphate fertilizers. They are also involved in the eutrophication of watercourses.

The analytical results illustrated by (Fig. 4d) show that the concentrations of orthophosphate ions in the waters of Wadi El-Kebir West vary between 0 mg/L (S6) in the 1st campaign and 1.92 mg/L (S4) in the 3rd campaign. This variation has a relatively increasing trend in dry periods. The high concentrations of orthophosphates upstream of the Wadi El-Kebir are linked to urban discharges from neighbouring urban areas and wastewater from the Hammams situated in this area.

Surface waters contain sulphate ion in variable quantities; concentrations are generally between 2.2 mg/L and 58 mg/L (Meybeck et al. 1996, Abboudi et al. 2014). In the analyzed waters of Wadi El-Kebir West, they are variable and oscillate between 0 mg/L (S5 and S6) in the first campaign and 87.5 mg/L (S7) in the 4th campaign (Fig. 4e).

Statistical Analysis

The Principal Component Analysis (ACP) is a tool for analysis of data which makes it possible to explain the structure of the correlations by using linear combinations of the original data. The ACP aims to present, in a graphical form, the maximum of contained information in data table based on the principle of double projection on the factorial axes(Ramdani&Laifa 2017). This method is widely used to interpret hydrochemical data(N'diaye et al. 2013).

It allowed us to quickly extract an interesting amount of information from a set of dimensional data using two simple graphs; the correlation circle and the observation graph. The data cover all 7 water sampling stations in Wadi El-Kebir West in two periods: wet season (high water) and dry season (low water).

Nine variables were treated; pH, temperature, electrical conductivity (EC), dissolved oxygen, sulfate ion, nitrate ion, nitrite ion, ammonium ion and orthophosphate ions.

Period of High Waters

The major part of the information is explained by the factor axes F1 and F2. These axes show the correlations between the studied variables that are related to the wet season.The analysis of the factorial plan F1 and F2 shows that 76.28% of the variance is expressed (Fig. 5).

The factorial plan F1 is expressed at 47.94%; this axis is mainly represented by SO_4^{2-}, PO_4^{3-}, NH_4^+, NO_2^- as well as pH and EC. It reflects both mineralization and organic pollution of water.Axis F2 is expressed at 28.79% and represented by temperature, NO_3^- and OD in the negative direction. It reflects a significant presence of oxidized nitrogenous forms (NO_3^-).

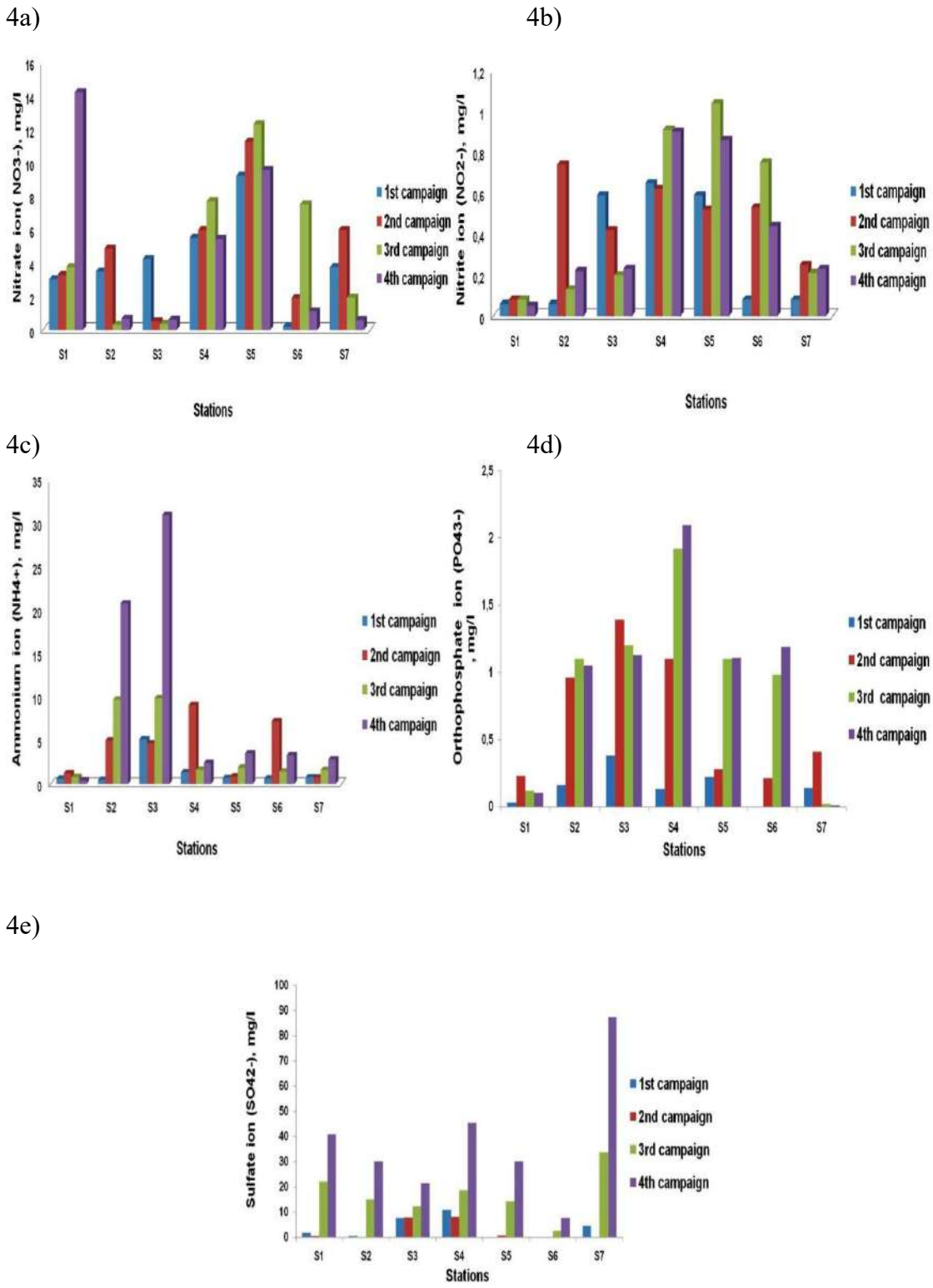


Fig. 4: Spatial and temporal variation of the concentration of (a) nitrate ion, (b) nitrite ion, (c) ammonium, (d) orthophosphates and(e)sulfate in the waters of Wadi El-Kebir West.

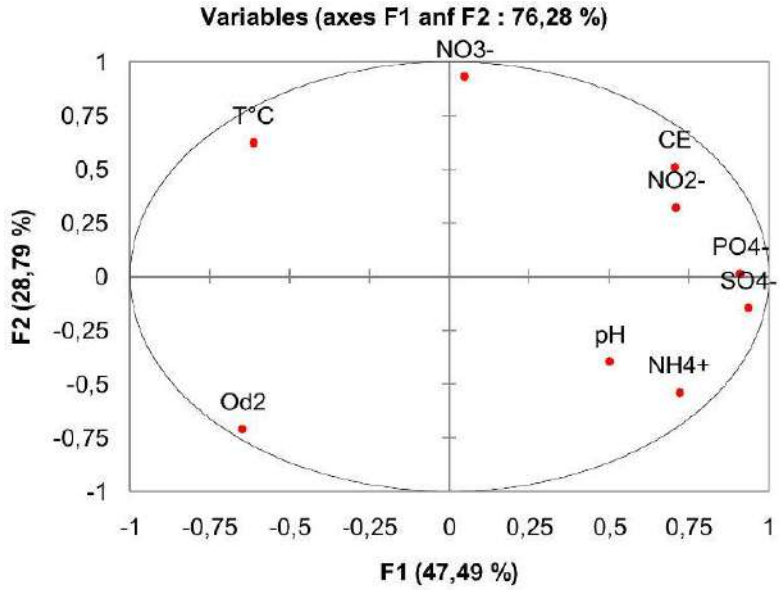


Fig. 5: Projection of the physicochemical parameters of the water on plan F1 and F2 (High water period).

Period of Low Waters

The factorial plan F1 and F2 shows that 65.79% of the total information is expressed (Fig. 6). Axis F1 represents 34.16% of the information. It is expressed towards its positive pole by OD, NO_3^-, NO_2^-, PO_4^{3-} and in the negative sense by temperature and EC. These variables describe both eutrophication and water mineralization. Axis F2 is expressed at 22.64% and represented by NH_4^+, pH and SO_4^{2-}, reflecting an organic matter load.

With regard to the representation of stations, the ACP results show that the different stations are positioned (on axes F1 and F2) according to the degree of pollution of their waters.

Period of High Waters

The most polluted study stations S3 and S4 are located on the positive side of the F1 axis. They are characterized by

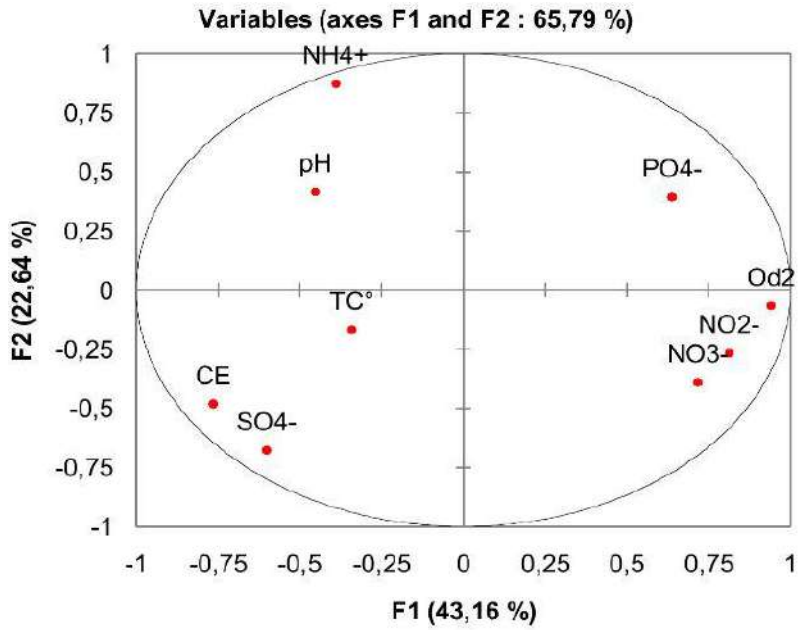


Fig. 6:Projection of the physicochemical parameters of the wadi waters on plan F1 and F2 (Low water period).

high concentrations of SO_4^{2-}, PO_4^{3-}, NH_4^+, NO_2^-, high pH and EC values, and low concentrations of OD. Overall, they are the most loaded with organic material from domestic waste, agricultural waste and cement from the Hadjar Soude factory. Stations S1 and S6 are located on the negative side.

Stations S2, S5, S7 are positioned on the positive side of the F2 component characterized by a significant presence of NO_3^- (Fig. 7).

Period of Low Waters

Stations S4, S5 and S6 are located on the positive side of axis F1 and contain large amounts of nutrients (NO_3^-, NO_2^- and PO_4^{3-}) from discharges from agricultural activities and urban wastewater. Station S7, located downstream of Wadi El-Kebir, is characterized by high electrical conductivity. Stations S2 and S3 upstream of the wadi are presented on the positive part of the factorial axis F2. They are the richest in NH_4^+ ion (Fig. 8).

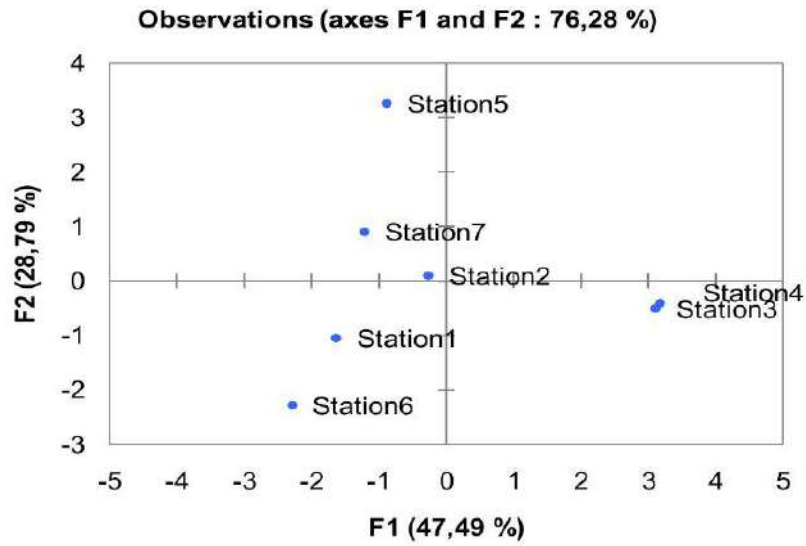


Fig. 7: Representation of the water sampling stations on the factorial plan F1 and F2 (high water period).

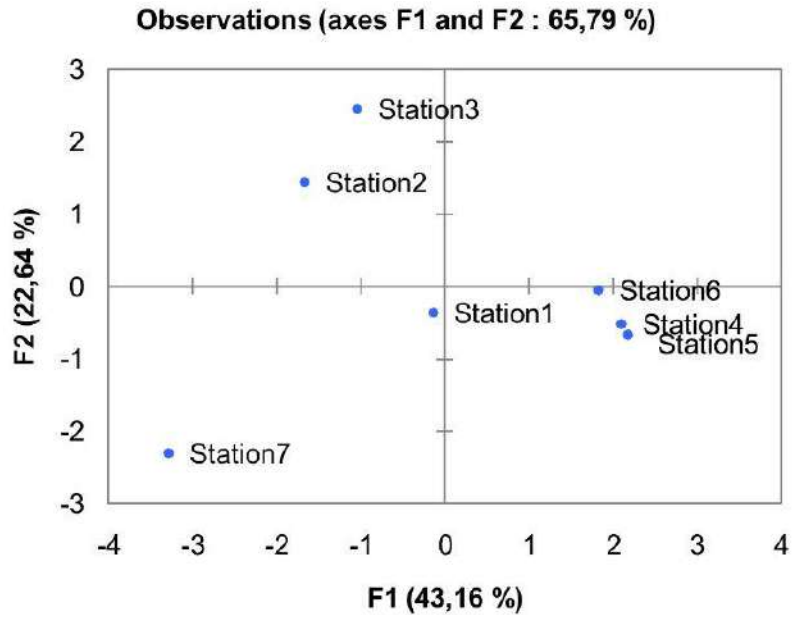


Fig. 8: Representation of the wadi water sampling stations on the plan factorial F1 and F2 (low water period).

CONCLUSIONS

In the light of the results of physicochemical analyses carried out on the waters of Wadi El-Kebir West, a degradation of water quality has been observed.The study of the spatial and temporal evolution of the measured parameters shows a significant difference between the sampling stations and between high water and low water periods. However, there is a degradation of quality in dry weather which results in high values of some parameters. This can be explained by the low flows of the wadis during this period, which corresponds to a phase of concentration and accumulation of degradation products of the materials contained in the waters of the wadi.

The statistical treatment of physicochemical data by analysing the main components allowed us to differentiate a zonality of water quality in the sequence of the wadi under study.The sampling stations located upstream and in the middle part (Azzaba, Ain Charchar and Ben Azouz plains) are the most affected by pollution linked to anthropogenic activities, in particular, the infiltration of wastewater (urban and industrial) and the use of chemical fertilizers in agriculture. Strong mineralization was detected downstream of the wadi (station 7) during the dry period.

Overall, the water quality of Wadi El-Kébir West is between the middle to poor classes. As a result, the control and preservation of this important watercourse become essential because its degradation constitutes an environmental and health risk. The most appropriate solution to this problem would be to set up wastewater treatment plants.

REFERENCES

Abboudi, A.,Tabyaoui, H. And El Hamichi,F.2014.Study of the physico-chemicalquality and metallic contamination of surface waters of the Guigou watershed, Morocco.European Scientific Journal. 23:1857-7431.

Agence De Bassin Hydrographique De Constantine «ABH» (Constantine River Basin Agency).2010. Surface Water Quality in the Constantine Coastal and Highlands Basins 2004-2007. The Agency's Workbooks. Ministry of Water Resources.

Aissaoui, A. 2012. Evaluation of the level of contamination of the Grouz hammam dam water in the Oued Athmania region (Mila wilaya) by agricultural activities.. Magister's thesis. Mouloud Mammeri Tizi-Ouzou University, 133p.

Ayad, W. and Kahoul,M. 2016. Assessment of the physicochemical and bacteriological quality of well water in the region of El-Harrouch (N.E. -Algeria).J. Mater. Environ. Sci.,7: 1288-1297.

Bengherbia, A., Hamaidi, F., Zahraoui, R., Hamaidi, M S. andMegateli, S. 2014. Impact of wastewater discharges on the physicochemical and bacteriological quality of the Beni Aza wadi (Blida, Algeria). Lebanese Science Journal,15: 39-51.

Benrabah, S., Bousnoubra, H., Kherici, N. and Cote, M. 2013. Characterization of water quality in the Kebir Quest oued (North East Algeria). Revue des Sciences et de la Technologie; Synthese, 26: 30-39.

Bouchareb, N. 2013.Nitrogen, phosphorus and silicon transfers and geochemistry from the Kebir-Rhumel, West Kebir and Saf-saf basins to the coast.MSc Thesis. Badji Mokhtar Annaba University.

Bou Saab, H., Nassif, N., ElSamrani, A G., Daoud, R., Medawar, S. and Ouaïni, N. 2017.Monitoring the bacteriological quality of surface waters (Nahr Ibrahim River, Lebanon). Revue Science de l'eau, 20: 341-352.

Daifallah, T. 2008.Water Resources And Integrated Management in the Oued El Kebir West Watershed (North East Algeria).Magister's thesis. Badji Mokhtar Annaba University.

Debieche, T. H. 2002.Evolution of water quality (salinity, nitrogen and heavy metals) under the effect of saline, agricultural and industrial pollution. Application to the low plain of the Seybouse-North-East Algeria.M.Sc. thesis. Badji Mokhtar Annaba University.

Doubi, M., Dermaj, A., Ait Haddou, B., Chebabe, D., Erramli, H., Hajaji, N. and Etsrhiri, A. 2013.Contribution to the study to the physico-chemical study of Oued Moulouya and an influent in the Outat El Haj region. Larhys Journal, 16:91-104.

Dussart, B.(ed.)1966.Limnologie. L'étudeDes EauxContinentales. Gauthier-Villars ed.Paris, p. 677.

Makhoukh, M., Sbaa, M., Berrahou, A. and Clooster, M. Van 2011. Contribution to the physico-chemical study of the surface waters of the Moulouya Oued (Eastern Morocco).Larhyss Journal, 09:149-169.

Meybeck, M., Friedrich, G., Thomas, R. and Chapman, D. (ed.)1996.Water Quality Assessments: A Guide to the Use of Biota, Sediments and Water in Environment Monitoring.Chapman E & FN Spon, London.

N'diaye a,D., OuldSid'Ahmed, M., OuldKankou, A., Sarr, D. and Lo, B. 2011. Contribution of the main components analysis to the assessment of the colour of Nouakchott city effluents.Larhyss Journal,09:139-147.

Nouayti, N., Khattach, D. and HilalI, M. 2015. Assessment ofphysico-chemical quality of groundwaterof the Jurassic aquifers inhigh basin of Ziz (Central High Atlas, Morocco). J. Mater. Environ. Sci., 6(4): 1068-1081.

Pesson, P., Leynaud, G., Riviere, J., Cabridence, R., Bovard, P., G. Tuffry, G., Vivier, P., Laurent, P., Angely, N., Descy, J P., Wattez, J R. andVerneaux, J. (ed.)1976.La Pollution Des EauxContinentales. Incidence Sur Les BiocénosesAquatiques. Ed. Gauthier-Villars. Paris, 285p.

Potelon, J. L. and Zysman, K. (ed.)1998. Le Guide DesAnalyses de l'eauPotable. Edition La Lettre du CadreTerritorial, Voiron, France.

Ramdani, H. and Laifa, A. 2017. Physicochemical quality of Wadi Bous namoussasurface waters (Northeast of Algeria). Journal of Water and Land Development,35:183-191.

Reggam, A., Bouchelaghem, H. and Houhamdi, M. 2015. Physicochemical quality of the waters of the Oued Seybouse (North-East Algeria): Characterization and analysis in main components. Journal. Mater. Environ. Sci., 6(5):1417-1425.

Rodier, J. (ed.) 1978.L'analyse de l'eau: EauxNaturelles, EauxRésiduaires, Eau De Mer; Chimie, Physico-Chimie, Bactériologie, Biologie. Dunod Tech, Paris, p. 1135.

Teissier, S. 2001. Assessment of nitrogen transformations in rivers. Methodological developments of the measurement of fluxes at the sediment-water column interface and applications (sediments, epilithic biofilms in the river Garonne). University Toulouse III,pp. 212.

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Removal of Azo Dyes Reactive Black from Water by Zero-Valent Iron: The Efficiency and Mechanism

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ABSTRACT

The removal kinetics and mechanism of active black removal by zero-valent iron were investigated. The experimental results showed that the rate of reactive black removal by zero-valent iron increased with the decreasing of pH and initial dye concentration, and increased with the increasing of temperature and ZVI dosage. SO_4^{2-} promoted the removal rate of reactive black. Ca^{2+} had an inhibitory effect on the removal of reactive black in the early stage by zero-valent iron and promoted it in the later stage, while Mg^{2+}, CO_3^{2-}, ClO_4^-, NO_3^-, PO_4^{3-} and HCO_3^- all inhibited the removal rate of reactive black by zero-valent iron. The activation energy was 26.38 KJ mol^{-1} by using the Arrhenius formula, indicating that this reaction was easy to occur. The degradation process was further analysed by UV-Vis, SEM and XRD, and the main reaction product was Fe_2O_3.

INTRODUCTION

Reactive black is a large-volume, typical and widely used double-activated azo dye (Wu et al. 2017). It is used for dyeing and printing cotton, hemp, silk and nylon. Reactive black is soluble in water and is blue and black (Qiu 2010). In addition to the azo bond, the reactive black also contains a group such as a benzene ring, a naphthalene ring or a sulfonic acid group (Zhang et al. 2015). Although reactive black itself is less toxic, studies have shown that its hydrolysate toxicity is significantly increased (Zhang et al. 2004). Besides, the dyed waste liquid will have a large amount of reactive black entering the water environment endangering human health. At present, dye wastewater treatment methods mainly include adsorption method, electrochemical method, advanced oxidation method, biological method, etc. However, they all have certain limitations, such as easy to cause secondary pollution, high cost, low efficiency and harsh operating conditions (Feng et al. 2014).

As a kind of water treatment agent with wide source, simple operation and low price, zero-valent iron (ZVI) has been widely used in groundwater remediation, industrial wastewater treatment and drinking water treatment with groundwater contaminated by heavy metals (Bao et al. 2017). The standard oxidation electrode of ZVI has a low potential (E=-0.44 V) and can reduce most of the contaminants. The mechanism of ZVI to remove pollutants is mainly the use of iron reduction, micro-electrolysis, and coagulation adsorption (Fu 2010). At the end of the 20th century, scholars had studied the removal of chlorinated organic wastewater from water by zero-valent iron. With the gradual serious pollution of groundwater arsenic at home and abroad, zero-valent iron was widely used to repair the pollution of groundwater arsenic (Ludwig et al. 2009). A large number of scholars have studied the use of zero-valent iron to remove refractory nitrate wastewater (Rodríguez-Maroto et al. 2008), and zero-valent iron is also used to remove azo dyes. Epolito et al. (2008) studied the effects of different operating conditions on decolorizing reactive blue 4 of zero-valent iron. Khan et al. (2016) using ultrasonic waves to promote the surface of zero-valent iron to treat printing and dyeing wastewater. Guan et al. (2015) summarized the possible reactions of zero-valent iron in water as follows:

$$Fe^0+2H^+ \rightarrow Fe^{2+}+H \quad \ldots(1)$$

$$2Fe^0+O_2+2H_2O \rightarrow 2Fe^{2+}+4OH \quad \ldots(2)$$

$$Fe^{2+}+2H_2O \rightarrow Fe(OH)_2\downarrow+2H^+ \quad \ldots(3)$$

$$6Fe^{2+}+O_2+6H_2O \rightarrow 2Fe_3O_4\downarrow+12H^+ \quad \ldots(4)$$

$$4Fe^{2+}+O_2+10H_2O \rightarrow 4Fe(OH)_3\downarrow+8H^+ \quad \ldots(5)$$

$$4Fe^{2+}+O_2+6H_2O \rightarrow 4FeOOH\downarrow+8H^+ \quad \ldots(6)$$

$$Fe^0+O_2+2H^+ \rightarrow Fe^{2+}+H_2O_2 \quad \ldots(7)$$

$$Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+\cdot OH+OH^- \quad \ldots(8)$$

However, there were still few studies investigated the mechanism of the removal of reactive black by zero-valent iron. Hence, this study selected reactive black as the target pollutant and looked for the best operating conditions for zero-valent iron removal of reactive black, the influence of common coexisting ions in the water on the removal process were studied, and the degradation mechanism was also studied.

MATERIALS AND METHODS

Materials

The ZVI used in the experiment was purchased from Shanghai Haotian Co., Ltd. Reactive black, nitric acid and other reagents were analytical grade and purchased from Sinopharm Co., Ltd. The maximum absorption wavelength of reactive black is 587 nm and the dye structure is given in Table 1.

Batch Experiments and Chemical Analysis

This experiment used a mechanical stirrer to stir, the rotation speed was 400 r/min, so that the ZVI was evenly dispersed in the water. The temperature was kept constant by using a cryostat during the experiment. 0.01 M acetic acid-sodium acetate buffer was used in the experiment to keep the pH value constant during the reaction. In the experiment, a certain amount of ZVI was weighed and placed in a jar containing 500 mL of dye solution. After a certain period of reaction, used a plastic syringe to take 5 mL of the water sample, passed the 0.45 μm filter and directly measured the spectrophotometric value at 587 nm using a spectrophotometer. The solid sample after the reaction was subjected to suction filtration through a vacuum suction filter, freeze-dried, and stored for use, and subjected to solid-state characterization by a scanning electron microscope (SEM, Zeiss EVO LS-185) and an X-ray diffractometer (XRD, D/Max-2550 PC).

RESULTS AND DISCUSSION

Effect of ZVI Dosage on the Removal of Reactive Black by ZVI

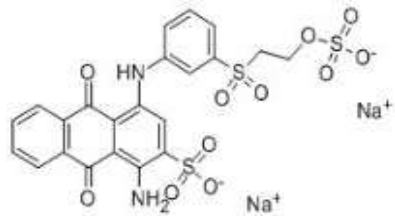
The study investigated the effect of different ZVI dosages on the removal of reactive black by ZVI, as shown in Fig. 1(A). When the ZVI dosage was 0.1 $g.L^{-1}$, the removal rate of the reactive black by ZVI was only 40 % and reactive black cannot be degraded after 30 min. When the ZVI dosage was 0.3 $g.L^{-1}$, the removal rate was reached 60 % at 15 min, and the reactive black removal rate reached 80 % at 30 min. When the ZVI dosage was 0.5 $g.L^{-1}$, the reactive black removal rate reached 100 % at 15 min. The reason was that the reduction reaction of ZVI occurs on the surface of iron powder particles (Cao et al. 1999). Therefore, the more iron powder was added and the larger the surface area of the iron powder provided by the unit reaction volume, that made the removal rate faster.

Effect of Different pH on the Removal of Reactive Black by ZVI

The effect of different pH on the removal of reactive black by ZVI was studied, as shown in Fig. 1(B). ZVI completely removed the reactive black in 15 min when pH = 4. When pH = 5, the removal rate of reactive black by ZVI was about 60% at 15 min and the removal rate can reach 80% at 30 min. When pH = 6, the removal rate of reactive black by ZVI was about 27% at 60 min, the degradation rate was slower and the reaction was incomplete. When pH = 7, ZVI hardly removed the reactive black. The reason was that, under alkaline conditions, a passivation film was formed on the surface of the ZVI and preventing further occurrence of the removal reaction. Under acidic conditions, it was beneficial to increase the corrosion rate of ZVI, promoted the reduction reaction and improved the effect of degrading reactive black. This is consistent with the conclusions of Bai & Wang (2008). The reactive black removal by ZVI was more pH dependent.

Effect of Reaction Temperature on the Removal of Reactive Black by ZVI

Table 1: Molecular structure of the reactive black.

Name	Dye structure	Maximum absorption wavelength
Reactive black		587 nm

The experiment investigated the effects of different temperatures on the removal of reactive black by ZVI, as shown in Fig. 1(C). When the temperature was 298 K, the ZVI removed about 80 % of the reactive black at 60 min. When the temperature was 278 K, the degradation rate of reactive black was poor. However, the reactive black was completely degraded by ZVI for about 60 minutes at a temperature of 318 K. This may be due to the increase in temperature, which accelerated the corrosion of ZVI surface and thereby increasing the degradation rate. This is consistent with the conclusions of Mielczarski et al. (2005). The experimental data showed that the increase of temperature was beneficial to the degradation of reactive black by ZVI, indicating that the degradation reaction of reactive black removal by ZVI was endothermic.

In general, the reaction temperature plays a key role in the chemical reaction. In the course of the reaction, the concentration of the remaining solution in the study according to Shu et al. (2007) and Fan et al. (2009) can be expressed by Eq. (9).

$$C_t = C_{ultimate} = (C_0 - C_{ultimate}) \times \alpha \times \exp(-Kt) \quad \ldots(9)$$

Where, C_0 is the initial concentration of the solution, C_t is the solution concentration at the reaction time t, Cultimate is the final residual concentration of the solution, αis the coefficient of variation of each experiment, and K is the empirical rate constant (min^{-1}). Using the experimental data for nonlinear regression, the estimated constant of *K* can be obtained. Using the reaction rate constant K at different temperatures obtained by Eq. (9), the activation energy of the ZVI removal reactive black process can be obtained according to the Arrhenius equation.

$$lnK = -\frac{Ea}{RT} + lnA \quad \ldots(10)$$

The relationship between ln*K* and 1/T is shown in Fig. 2. Ln*K* had a good linear relationship with 1/T. By calculation, the activation energy of the ZVI to remove the reactive black reaction Ea=26.38 KJ mol^{-1}. This value

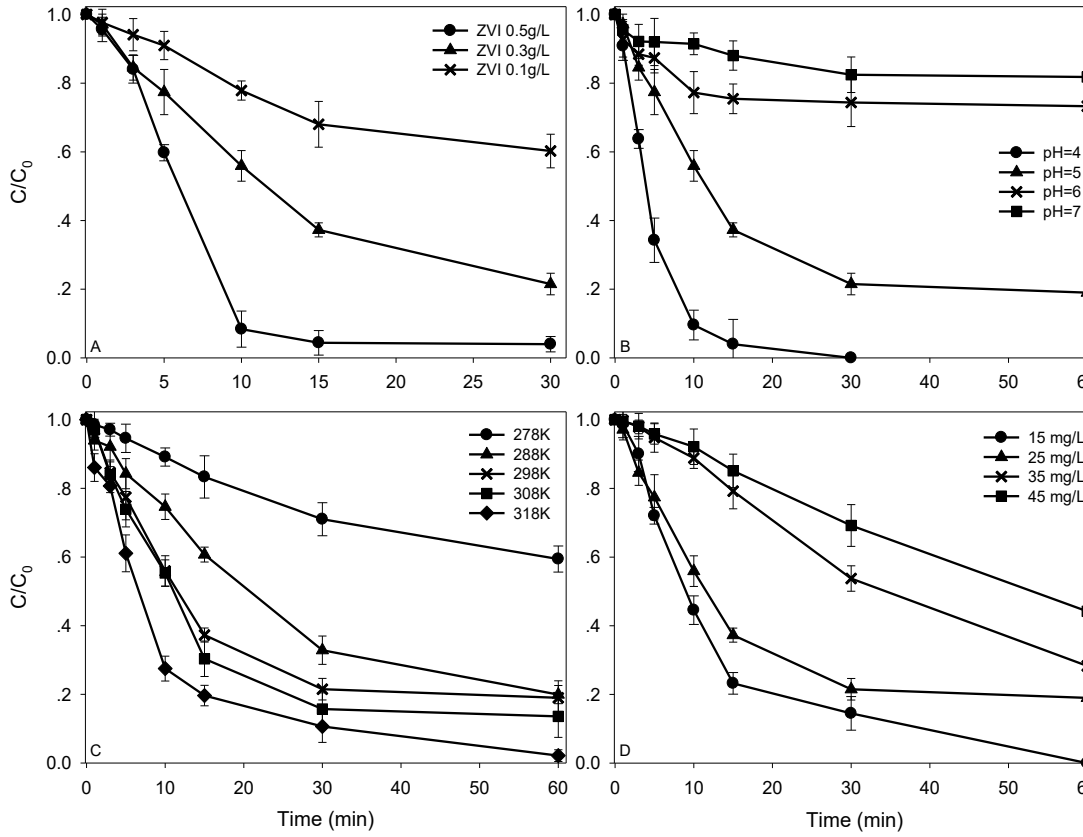


Fig. 1: Effect of different operating conditions on the removal of reactive black by ZVI.
[A:T=298K, 0.01M HAc-NaAc buffer pH=5, reactive black 25 $mg.L^{-1}$; B: T=298K, ZVI 0.3 $g.L^{-1}$, reactive black 25 $mg.L^{-1}$, 0.01M HAc-NaAc buffer pH=4 and pH=5, 0.01M MES buffer pH=6, 0.01M TRIS buffer pH=7; C:ZVI 0.3 $g.L^{-1}$, 0.01M HAc-NaAc buffer pH=5, reactive black 25 $mg.L^{-1}$; D: T=298K, 0.01M HAc-NaAc buffer pH=5, ZVI 0.3 $g.L^{-1}$] by combination of nano zero-valent iron and a EGSB Reactor. Environ. Sci. Technol., 39(05): 80-84.

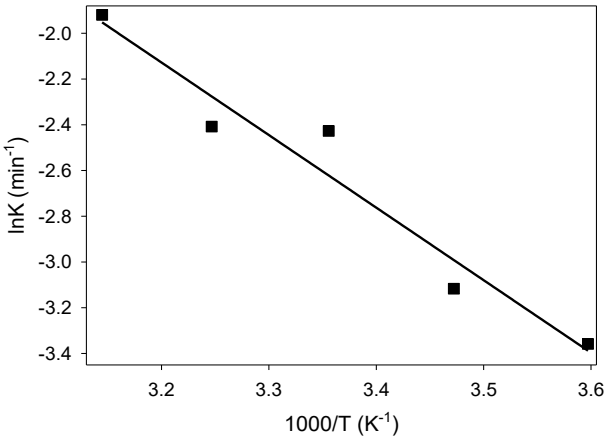


Fig. 2: Arrhenius plot for reactive black removal by ZVI. [0.01M HAc-NaAc buffer pH=5, reactive black 25 mg.L^{-1}, ZVI 0.3 g.L^{-1}]

was close to the ZVI removal Se(IV) activation energy Ea (32.86 KJ mol^{-1}) (Liang et al. 2015). The activation energy Ea of a general chemical reaction is between 60 and 250 KJ mol^{-1} (Fan et al. 2009). The value of the activation energy was small indicating that this reaction was easy to occur and the temperature dependence on practical applications was small.

Effect of Reactive Black Concentration on the Removal of Reactive Black by ZVI

The experiment investigated the effect of different dye concentrations on the removal of reactive black by ZVI, as shown in Fig. 1(D). When the initial concentration of the solution was 15 mg.L^{-1}, the removal rate of reactive black was about 76 % and the ZVI almost completely removed the reactive black at 60 min. As the concentration of reactive black increased, the removal rate of ZVI decreased. This is consistent with Guo et al. (2010). Zhong et al. (2016) had also found that in the case of a certain concentration of pollutants, with the same dosage and as the initial concentration of the solution increases, the removal rate of pollutants in the same reaction time would decrease.

The Effect of Coexisting Ions on the Removal of Reactive Black by ZVI

A large amount of dissolved inorganic anions and metal cations usually present in the dye wastewater, and they cannot be completely removed even after the pretreatment of adsorption or filtration. Therefore, the effect of coexisting ions on the reaction was investigated. Two cations (Ca^{2+}, Mg^{2+}) and six anions (CO_3^{2-}, SO_4^{2-}, ClO_4^-, HCO_3^-, PO_4^{3-}, NO_3^-) were selected to investigate the effect of coexisting ions on reactive black removal by ZVI. The results were shown in Fig. 3.

From Fig. 3 A, it could be seen that SO_4^{2-} promoted the removal of reactive black by ZVI. SO_4^{2-} promoted the removal of reactive black by ZVI and promoted the effect gradually on the reaction after 15 minutes. At 15 min, the removal rate of reactive black by ZVI with the non-ion group was about 62 %, while the removal rate of SO_4^{2-} group was about 77 %. As the reaction progressed, the promotion of SO_4^{2-} decreased gradually within 15 to 30 minutes and the promotion of SO_4^{2-} gradually stabilized after 30 minutes. Also, the removal rate of the reactive black by the ZVI with the non-ion group was about 62 %, and the removal rate with

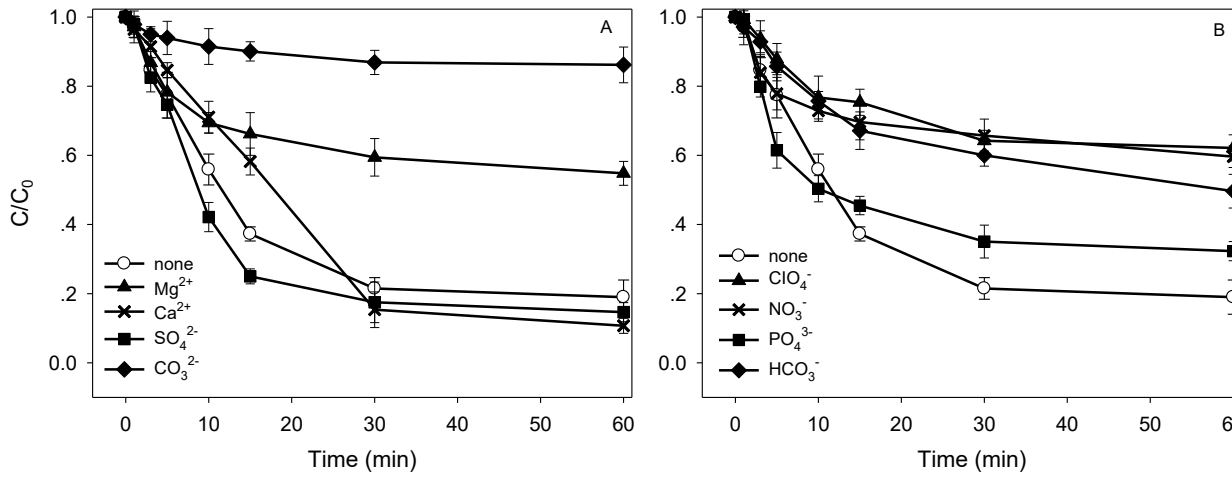


Fig. 3: Effect of coexisting ions on the removal of reactive black by ZVI. [T=298K, 0.01M HAc-NaAc buffer pH=5, reactive black 25 mg.L^{-1}, ion concentration 10 mmol.L^{-1}, ZVI 0.3 g.L^{-1}]

the Ca^{2+} group was about 41 % after 15 min. As the reaction progressed, it was observed that the degradation rates with the non-ion group and the Ca^{2+} group were almost the same at about 27 min. After 27 min, the Ca^{2+} group gradually increased the promoting effect of ZVI removal of reactive black and the growth rate of the promoting effect decreased gradually after 30 min. At 60 min, the removal rate with Ca^{2+} group was about 88 %, while the removal rate with non-ion group was about 80 %. In general, the Ca^{2+} group had an inhibitory effect on the removal of the reactive black by ZVI and promoted it later.

The degradation rates of Mg^{2+}, CO_3^{2-}, ClO_4^-, NO_3^-, PO_4^{3-} and HCO_3^- were lower than those with the non-ion group, as shown in Fig. 3 A and B. Among them, PO_4^{3-} group promoted ZVI removal of reactive black in the first of 12 minutes. However, after 12 min PO_4^{3-} gradually hindered the degradation of reactive black by ZVI. At 30 min, the degradation rate of the PO_4^{3-} group gradually stabilized by about 63 % lower than that of the non-ion group by 78 %. Besides, CO_3^{2-} had an obvious inhibitory effect on the reaction. The removal rate of ZVI with the non-ion group was about 62 % at 15 min, compared with about 8 % with CO_3^{2-} group. The removal rate of CO_3^{2-} group increased slowly and reached stability after 30 min. Meantime, Mg^{2+} group also had an inhibitory effect on the reaction. In the first 10 minutes, the removal rate of Mg^{2+} group increased rapidly and reached 29 % at 10 min. After 10 minutes, the removal rate of Mg^{2+} group increased slowly and reached 43% at 60 min, while the removal rate of the non-ion group was about 80 %. Furthermore, the HCO_3^-, NO_3^-, ClO_4^- groups all hindered the degradation of reactive black by ZVI. It was indicated that these six ions had inhibitory effects on the removal of reactive black by ZVI to the extent of $CO_3^{2-} > ClO_4^- > NO_3^- > HCO_3^- > Mg^{2+} > PO_4^{3-}$.

Mechanism Analysis

UV-Vis scanning analysis: To investigate the process of degrading reactive black with ZVI, the types of intermediate products were qualitatively analysed and the water samples at a different time in the reaction process were studied. UV-Vis scan with a scanning range of 200 nm~600 nm. The decolourization of dye was seen from the UV spectrum, and the degradation process of dye was analysed. As shown in Fig. 4, there are four absorption peaks at 230, 310, 390, 510 nm. Among them, 510 nm in the visible region is the characteristic absorption peak of the azo double bond. The absorption peak of 390 nm represents the cis structure absorption peak of dye. 230 nm, 310 nm are aromatic functional groups, which represent the characteristic absorption peaks of naphthalene ring structure and benzene ring structure of reactive black molecules, respectively.

With the progress of degradation reaction, the characteristic peak at 510 nm in visible light region weakens rapidly. When the reaction reached 30 min, it can be seen that the characteristic absorption peak of azo double bond at 510 nm had disappeared, indicating that the azo double bond in dye molecule had been destroyed at this time (Zhang et al. 2015). It can be calculated from Fig. 4 that the removal rate of reactive black by ZVI was about 90 % when the reaction was 30 min, which also showed that the azo bond in dye molecule was easy to be destroyed (Zhang et al. 2013). At the same time, it can be observed during the experiment that the original blue and black colour of the dye had disappeared, and the solution presented a faint fuchsia colour. The absorption peak at 390 nm at 30 min also basically disappeared, which indicated that the conjugated system of dye and the structure of dye are destroyed at the same time. In addition, with the progress of the reaction, it can be observed from the diagram that the absorption peak of the naphthalene ring structure of 230 nm was gradually weakened. However, the absorption peak of benzene ring structure at 310 nm increased gradually, which indicated that the new benzene ring was produced by the degradation and ring-opening of naphthalene ring (Yang et al. 2012). After the reaction, there was still an absorption peak in the structure of the benzene ring at 310 nm, which indicated that the benzene ring had not been completely degraded.

Characterization of solid products: To find out the variation of corrosion products after the reaction, scanning electron microscope (SEM) and x-ray diffraction (XRD) were used to analyze the samples after ZVI removal of reactive black for 120 min. As can be seen from Fig. 5, it can be seen that the ZVI particles are smooth and irregular in the 0 min of reaction, and the distribution is uniform. After the reaction

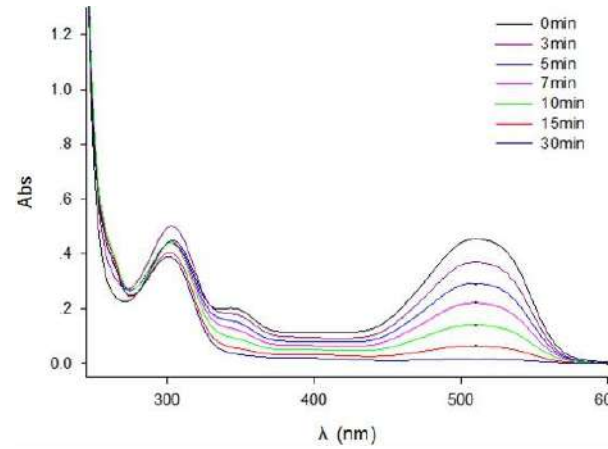


Fig. 4: UV-Vis scanning spectra of reactive black removed by ZVI at different reaction time.
[T = 298 K, 0.01 M HAc-NaAc buffer pH = 5, reactive black 25 $mg.L^{-1}$, ZVI 0.3 $g.L^{-1}$]

of 120 min, the shape of ZVI particles did not change much. However, the surface of the particles became rough, which was caused by the corrosion of the particle surface and the formation of iron oxide or iron hydroxide during the reaction process (Zhao et al. 2018).

To further understand the product change before and after the removal of reactive black by ZVI, the material after the reaction was characterized by x-ray diffraction, as shown in Fig. 6. Among them, 2θ was 44.7, 65.04, 82.34 was the characteristic diffraction peak of ZVI (PDF 06-0696) and the characteristic diffraction peak may be that the incomplete reaction residual of ZVI was more. The weak characteristic diffraction peak of Fe_2O_3 (PDF 21-920) was observed when 2θ was 38.44, which indicated that part of the material was oxidized after the reaction. It was consistent with the conclusion of SEM analysis.

CONCLUSIONS

Removal of reactive black by ZVI had a great degradation effect. The addition of ZVI, pH value of the solution, initial concentration of reactive black, temperature and common coexisting ions in the water had different effects on the removal of reactive black by ZVI. Among them, the lower the pH, the more acidic conditions helped to increase the corrosion rate of ZVI. It promoted a reduction reaction and can improve the effect of degradation reactive black. The

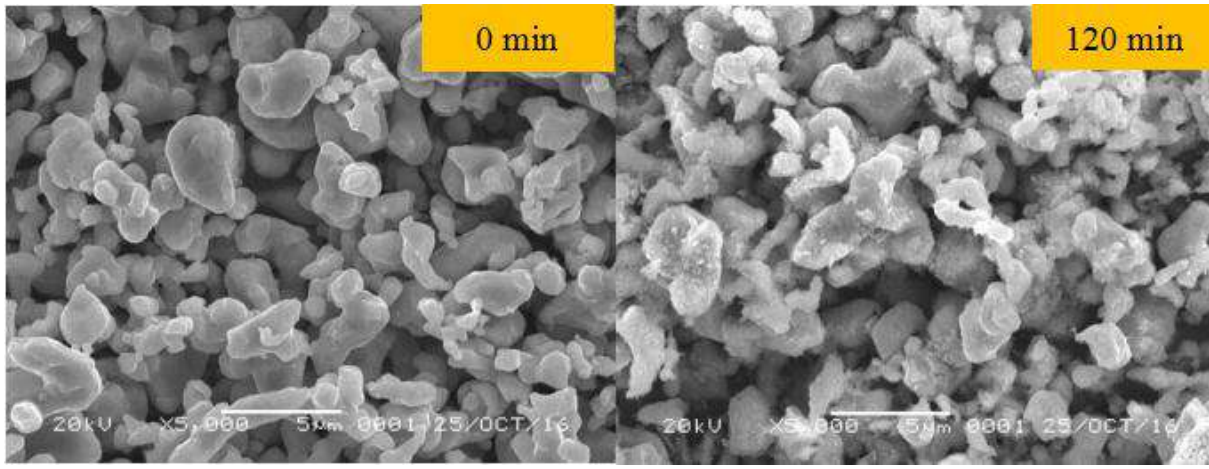


Fig. 5: SEM images of reactive black removal by ZVI at different time.
[T = 298K, 0.01M HAc-NaAc buffer pH = 5, reactive black 25 mg.L^{-1}, ZVI 0.3 g.L^{-1}]

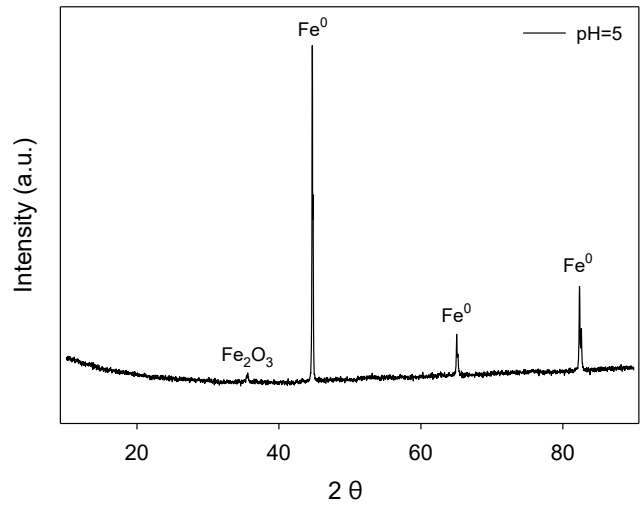


Fig. 6: XRD pattern of activity black removed by ZVI at pH = 5.
[T = 298 K, 0.01M HAc-NaAc buffer pH = 5, reactive black 25 mg.L^{-1}, ZVI 0.3 g.L^{-1}]

existence of SO_4^{2-} can promote the removal of reactive black by ZVI and the existence of Ca^{2+} can inhibit the removal of reactive black by ZVI at the early stage and promote at the later stage. However, the existence of Mg^{2+}, CO_3^{2-}, ClO_4^-, NO_3^-, PO_4^{3-} and HCO_3^- can inhibit the removal of reactive black. The degradation process was analysed by UV-vis, SEM, XRD. The main reaction product was Fe_2O_3.

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REFERENCES

Bai, S.Y. and Wang, M.Y. 2008. Review on contaminated water remediation by nanoscale zero-valent iron (in Chinese). Water Purif. Technol., 27(1):35-40.

Bao, Q.Q., Li, J.X. and Guan, X.H. 2017. Improving the reactivity of zerovalent iron toward various azo dyes by pre-magnetization. Environ. Chem., 36(07):1467-1473.

Cao, J.S., Wei, L.P., Huang, Q.G., Wang, L.S. and Han, S.K. 1999. Reducing degradation of azo dye by zero-valent iron in aqueous solution. Chemosphere, 38(3): 565-571.

Epolito, W.J., Yang, H., Bottomley, L.A. and Pavlostathis, S.G. 2008. Kinetics of zero-valent iron reductive transformation of the anthraquinone dye Reactive Blue 4. J. Hazard. Mater., 160(2-3): 594-600.

Fan, J., Guo, Y.H., Wang, J.J. and Fan, M.H. 2009. Rapid decolorization of azo dye methyl orange in aqueous solution by nanoscale zerovalent iron particles. J. Hazard. Mater., 166(2-3): 904-910.

Feng, P., Zhou, Q.L., Guan, X.H. and Zhou, G.M. 2014. Preliminary study on the effect of zero valent iron enhanced by weak magnetic field on the azo dyes decoloration in the water. Sichuan Environ., 33(4): 1-6.

Fu, F.L. 2010. Newest research progress in the application of zero-valent iron to the treatment of wastewater. Ind. Water Treat., 30(6): 1-4.

Guan, X.H., Sun, Y.K., Qin, H.J., Li, J.X., Irene, M.C.L., He, D. and Dong, H.R. 2015. The limitations of applying zero-valent iron technology in contaminants sequestration and the corresponding countermeasures: The development in zero-valent iron technology in the last two decades (1994-2014). Water Res., 75: 224-248.

Guo, Y., Xiao, L.P., Deng, Z.Y. and Tang, W.Q. 2010. Laboratory study on the decolorization of reactive brilliant blue X-BR treated by an integrated Fe^0–anaerobic biological system. China Environ. Sci., 30(4): 510-515.

Khan, A., Prabhu, S.M., Park, J., Lee, W. and Lee, G. 2016. Azo dye decolorization by ZVI under circum-neutral pH conditions and the characterization of ZVI corrosion products. J. Ind. Eng. Chem., 47: 86-93.

Liang, L.P., Jiang, X., Yang, W.J., Huang, Y.Y., Guan, X.H. and Li, L. 2015. Kinetics of selenite reduction by zero-valent iron. Desalin. Water Treat., 53(9): 2540-2548.

Ludwig, R.D., Smyth, D.J.A., Blowes, D.W., Spink, L.E. and Weisener, C.J. 2009. Treatment of arsenic, heavy metals, and acidity using a mixed ZVI-compost PRB. Environ. Sci. Technol., 43(6): 1970-1976.

Mielczarski, J.A., Atenas, G.M. and Mielczarski, E. 2005. Role of iron surface oxidation layers in decomposition of azo-dye water pollutants in weak acidic solutions. Appl. Catal., B., 56(4): 289-303.

Qiu, L.J. 2010. Study on adsorption and degradation of Reactive Black KN-B by three kinds of sludge. Chin. J. Environ. Eng., 4(1): 96-100.

Rodríguez-Maroto, J.M., García-Herruzo, F., García-Rubio, A., Gómez-Lahoz, C. and Vereda-Alonso, C. 2008. Kinetics of the chemical reduction of nitrate by zero-valent iron. Chemosphere, 74(6): 804-809.

Shu, H.Y., Chang, M.C., Yu, H.H. and Chen, W.H. 2007. Reduction of an azo dye Acid Black 24 solution using synthesized nanoscale zerovalent iron particles. J. Colloid Interface Sci., 314(1): 89-97.

Wu, Q.Y., Xie, M.Y., Yang, Y., Zhu, R., Han, X.R. and Zou, H. 2017. Study of hydrogen peroxide accumulation for degradation of Reactive Black 5 in the zero-valent aluminum/acid/oxygen system. Environ. Sci. Technol., 40(06): 49-53.

Yang, B., Xie, H. and Zhou, P. 2012. Indirect anodic oxidation applied for the treatment of simulated wastewater containing reactive dyes. Chin. J. Environ. Eng., 5(05): 1091-1095.

Zhang, F.F., Liang, X.M., Zhang, Q., Chen, J.P., Ayfer, Y. and Antonius, K. 2004. Investigation of hydrolysis products of Reactive Black 5 by high performance liquid chromatography/mass spectrometry. Chin. J. Anal. Chem., 32(8): 1019-1022.

Zhang, N., Zou, H., Wu, Q.Y. and Zhu, R. 2015. Enhanced the degradation of Reactive Black 5 by zero valent-aluminum / acid system. Environ. Chem., 34(07): 1343-1349.

Zhang, Y.P., Li, Y.B., Li, F., Li, J. and Li, J. 2013. Degradation efficacy and mechanism of reactive black KBR with ferrate solution. Ind. Water Treat., 33(1): 58-61.

Zhao, Y., Zhu, F. and Ren, W.T. 2018. Kinetics of nitrate removal in groundwater using green synthesized nanoscale zero valent iron-nickel. Environ. Eng., 36(7): 71-76.

Zhong, X.H., Yu, Y., Liu, S.Y., Zhao, J.J., Liu, J., Xia, D.F. and Pan, F. 2016. Study on advanced treatment of printing and dyeing wastewater by combination of nano zero-valent iron and a EGSB Reactor. Environ. Sci. Technol., 39(05): 80-84.

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Acute Toxicity and Haematological Studies of Textile Based Industrial Effluent of Pali City on a Freshwater Fish *Clarias batrachus* (L.)

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ABSTRACT

The acute toxicity bioassay of textile industrial effluent (TIE) was carried out by the probit analysis method. The 12, 24, 48, 72 and 96 h LC50 values were obtained as 56.23 %, 28.84 %, 22.38% and 16.59% respectively. LC50 values were significantly decreased with increase in effluent concentration. The safe value was found to be 32.88 %. The experimental fish *C.batrachus* exhibited stressful behaviour which increased with toxicant concentration. Due to acute toxicity of textile based industrial effluent, experimental fish *C. batrachus* expressed high secretion of mucus, uncoordinated and tailfin movement, surfacing, loss of buoyancy, escaping tendency, hyperactivity, and discolouration of the skin. Mortalities were observed in all treatments except control. The haematological analysis was also carried out in experimental fish *C.batrachus* exposed to various periods in textile-dyeing effluents. Haematological data were evaluated for parameters such as Hb, RBCs, WBCs, PCV, MCH, and MCHC of the test species. The alterations of these parameters have been discussed. So it can be concluded that the TIE is toxic to fishes and aquatic organisms.

INTRODUCTION

Water quality of aquatic habitat is altered due to anthropogenic activity (Makwana 2020a). Besides this, the textile industryand its effluents are one of the main sources of severe pollution problems worldwide. The important pollutants in textile wastewater are mainly organics, dye and toxicants. The wastewater contains a different variety of chemicals from the various stages of operations which include printing, scouring, bleaching and dyeing (Deepali & Gangwar 2010). Berardi et al. (2019) have described the different classes of dyes and pigments used in textile processes like dyeing and printing.

The printing and dyeing textile industries cover a major portion of the industrial section in Pali city. The textile effluents discharged through these industries are toxic (Satish et al. 2008). Improperly treated wastewater from the textile and dyeing industries of Pali is affecting groundwater quality and its surrounding areas due to the discharge of wastewater.

Textile effluents discharged in Bandi River, Pali city are highly toxic and alter the quality of river water and severely affect aquatic organisms (Makwana 2020b). It is observed in the study that the pH of river water is more than the permissible limit while most of the parameters like BOD and total hardness are much higher. TDS, chloride and sulphate concentrations are more in the outlet sample than the inlet sample. The main pollutants in the wastewater released from each step in the wet process of textiles as predicted by Holkar et al. (2016). According to Yaseen & Scholz (2016), the textile dye effluents are dark in colour and have high pH, suspended solids, chemical oxygen demand and biochemical oxygen demand.

Taiwo et al. (2018) suggested that the study of textile effluent samples from an industry placed at Oba Akran Road (Ikeja) reported some form of pre-treatment is being embarked upon within the premises before releasing into the environment. Textile wastewater can alter the quality of water and create several hazardous and health problems like allergies, dermatitis, skin problems and cancers in humans (Sarvajith et al. 2018).Due to the widespread use of textile dyes, these toxic contaminants can be present in the environment and store biologically throughout the food chain in aquatic organisms (Hossain et al. 2018).

Bioassay is a test to estimate the relative strength of a chemical by comparing its effects on animals with that of standard preparations (Rand et al. 1985). Several studies have been carried out on the effect of different industrial effluents on fish (Maruthi& Rao 2001). Relative toxicity of bleaching, dyeing and printing textile mill effluents to the freshwater fish *Oreochromis mossambicus*have been studied by Haniffa et al. (1991).

Effects of dairy effluent on the behaviour of *Cyprinus carpio were studied* (Amutha et al.1999). Nagarajan & Ramesh (2001) noticed the impact of Sago effluent on the freshwater fish *Catla catla.* Nagarajan & Shasikumar (2002) observed similar findings on the fish *Labeo rohita* exposed to sago industry effluent. Shanthi (2003) has reported the effects of the tannery effluent on the freshwater fish *Catla-catla.* Nagarajan & Boopathi Raja (2004) have noticed the toxic impact of dyeing effluent to the fish *Catlacatla.* Similar observations have been made by Nagarajan & Suresh (2005) in the fish *Cirrhinus mrigala* in sago effluent. Nagarajan &Aruna(2006) have studied the impact of distillery effluent on the fish *Labeo rohita.*

Many bioassays have been carried out to monitor and assess the toxicity of wastewater from domestic and various industries (Hader 2018). Acute toxicity bioassay of pretilachlor (herbicide) by probit analysis method in the fish *Clarias batrachus* was investigated by Soni et al. (2018). Arya et al. (2018) evaluated the 96 hr LC50 value of lead acetate for the fish *Oreochromis mossambicus*. The acute toxicity test was performed according to the standard methods of APHA and the values were calculated by probit analysis.

Haematological parameters were evaluated for the fish *Oreochromis mossambicus* exposedto the textile effluent by Amte et al. (2013). Srivastav & Dayalanand (2015) studied haematological parameters of a freshwater catfish *Heteropneustes fossilis* exposed to dye. Haematological parameters are very important in determining the health and physiological status of fishes (Clauss et al. 2008, Adeyemo et al. 2009).

The present investigation was designed to study the acute toxicity of textile effluent to the fish *Clarias batrachus* and effect of the effluent on its behavioural response.

MATERIALS AND METHODS

Collection and Acclimatization of the Experimental Fish

Clarias batrachus fish were collected locally and transported to the laboratory in large aerated polythene bags containing water from the collection site. Healthy fish were placed in a large glass aquarium. Before the experiment, the tanks were washed and fish were treated with 0.1% of $KMnO_4$ solution to protect from any dermal infection.

The fish were acclimatized in laboratory conditions for 2 weeks before they were used in the bioassay tests. Water was changed every 48 hours to remove faecal matter and waste metabolite of fish and enhance oxygen content in the water. During acclimatization, the fish were fed with the protein diet. The fish were starved for 24 hours before the experiment. The method employed in this experiment is based on the recommended method for the test of acute toxicity of pollutants to fish described by APHA (2000).

Acute Toxicity Bioassay and Statistical Analysis of Data

Six graded concentrations of textile effluents were used as 0% (control), 10%, 30%, 50% and 70%. Control did not contain the textile effluent. Ten juvenile fish were used and estimated for mortality rate after 24, 48, 72 and 96 hours. Dead fish were removed immediately to prevent water pollution. The 24, 48, 72 and 96hr lethal median concentration (LC50) was determined as a probit analysis using the probit mortality versus the log concentration. The LC50 value was extrapolated from probit 5 to the log concentration. The antilog values provide the LC50 in %. Percentage mortality probit values were taken from Finney's Table (Finney 1971)



Fig. 1: Flow of textile effluents into Bandi River near Pali industrial area.

Mortality data were recorded after 24, 48, 72 and 96 h to get LC50 of respective intervals. The toxicity of textile effluent on morphological and behavioural parameters was recorded. Control and textile effluent treated group of fish were consistently monitored in intervals to get the frequency. The data were recorded as suggested by Gupta & Dua (2010).

Haematological Parameters

The haematological indices of mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated using the total red blood cell count (RBC), haemoglobin concentration (Hb), and haematocrit (HCT) as given below.

Mean corpuscular volume (MCV) = HCT/RBC

Mean corpuscular haemoglobin (MCH) = [Hb × 10]/RBC

Mean corpuscular haemoglobin concentration (MCHC) = [Hb × 10]/HCT × 100

RESULTS AND DISCUSSION

Lethal Toxicity Test

The LC50 values with 95% confidence limits of different concentrations of the textile effluent were determined and represented in Table 6. Significant differences in ($p < 0.05$) in LC50 values were observed for different times of exposures. As the exposure time increases the median lethal concentration (LC50) decreases.

Flow of textile effluent directly into bandi river near Pali industrial area is shown in Fig1. Exposure to *C. batrachus* with different concentrations of textile industrial effluent (TIE) showed a varying degree of mortality with a wide range of concentrations (Table 1). The percentage of mortality was significantly increased with the increase in the concentration of the toxicant as well as the duration of the experiment that is given in Tables 1-5 and Figs. 1-5.

Table 1: Mortality rate of *Clarias batrachus* exposed to different concentrations of the effluent.

Mortality Percentage									
Concentration % (v/v)	No. of fish	24 hrs		48 hrs		72 hrs		96 hrs	
		M	M %	M	M %	M	M %	M	M%
Control	10	0	0	0	0	0	0	0	0
10 %	10	0	0	1	10	2	20	3	30
30 %	10	2	20	3	30	5	50	7	70
50 %	10	3	30	5	50	7	70	9	90
70 %	10	6	60	10	100	-	-	-	-

Table 2: Log concentrations and probit values when exposed to textile effluent after 24 h.

S. No.	Conc. % (v/v) 24 hrs	Log Conc.	No. of Fish	24 hrs	
				Mortality %	Probit
1	Control	0	10	0	0
2	10 %	1.0000	10	0	0
3	30 %	1.4771	10	20	4.1
4	50 %	1.6989	10	30	4.48
5	70 %	1.8450	10	60	5.25

Table 3: Log concentrations and probit values when the fish exposed to textile effluent after 48 h.

S. No.	Conc. % (v/v) 48 hrs	Log Conc.	No. of Fish	48 hrs	
				Mortality %	Probit
1	Control	0	10	0	0
2	10 %	1.0000	10	10	3.72
3	30 %	1.4771	10	30	4.48
4	50 %	1.6989	10	50	5
5	70 %	1.8450	10	100	7.37

Table 4: Log concentrations and probit values when the fish exposed to textile effluent after 72 h.

S. No.	Conc. % (v/v) 72 hrs	Log Conc.	No. of Fish	72 hrs	
				Mortality %	Probit
1	Control	0	10	0	0
2	10 %	1.0000	10	20	4.1
3	30 %	1.4771	10	50	5
4	50 %	1.6989	10	70	5.52
5	70 %	1.8450	10	100	7.37

Table 5: Log concentrations and probit values when the fish exposed to textile effluent after 96 h.

S. No.	Conc. % (v/v) 96 hrs	Log Conc.	No. of Fish	96 hrs	
				Mortality %	Probit
1	Control	0	10	0	0
2	10 %	1.0000	10	30	4.48
3	30 %	1.4771	10	70	5.52
4	50 %	1.6989	10	90	6.28
5	70 %	1.8450	10	100	7.37

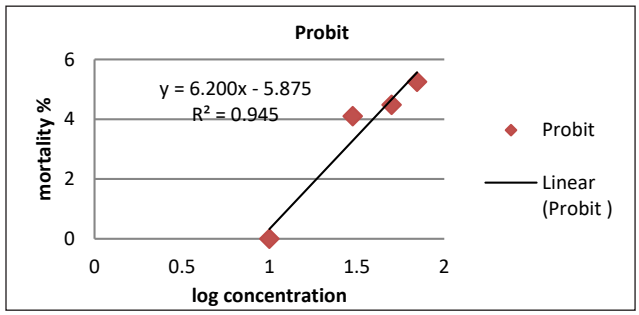


Fig. 2: Linear regression curve of log concentration and mortality response of C.batrachus after 24 hrs. exposure to textile effluent.

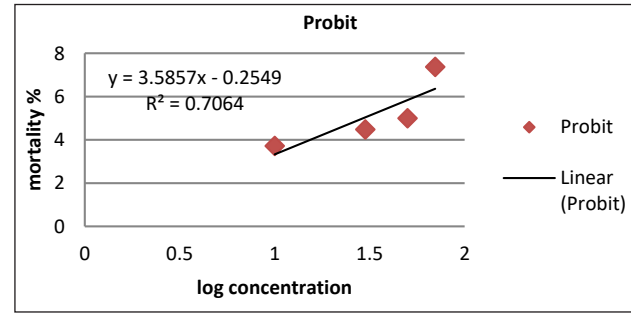


Fig. 3: Linear regression curve of log concentration and mortality response of *C.batrachus* after 48 hrs exposure to textile effluent.

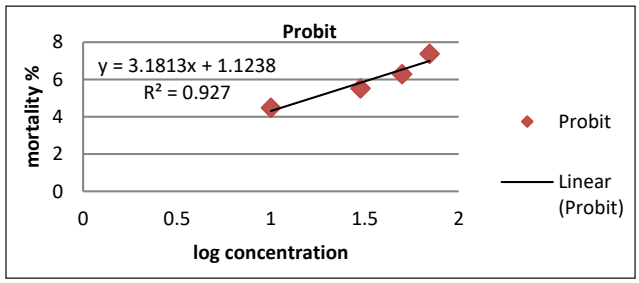


Fig. 4: Linear regression curve of log concentration and mortality response of *C.batrachus* after 72 hrs exposure to textile effluent.

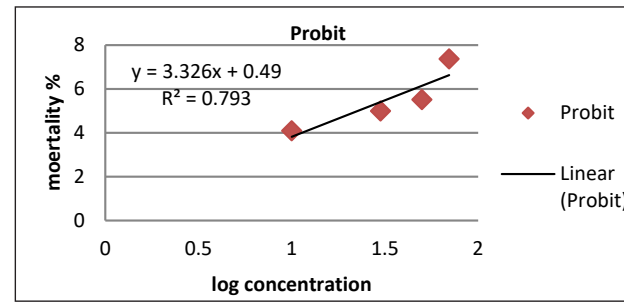


Fig. 5: Linear regression curve of log concentration and mortality response of C.batrachus after 96 hrs exposure to textile effluent.

Table 6: Lethal concentrations of textile effluent at various exposure times for *C. batrachus.*

S.No.	Time period	LC50 %	Regression line /slope	Coefficients	95% Confidence Interval		Safe concentration
					Lower Bound	Upper Bound	
1.	24 h	56.23	y = 6.200x - 5.875	6.1992 ±1.050	1.67725	10.72132	
2.	48h	28.84	y = 3.585x - 0.254	3.5853±1.6343	-3.4468	10.6176	32.88 %
3.	72 h	22.38	y = 3.181x + 1.123	3.3263±1.2012	-1.8422	8.4950	
4.	96h	16.59	y = 3.326x + 0.49	3.1810±0.6311	0.4654	5.8965	

Lethal concentration values in rows with different letters significantly differ at $p < 0.05$.

No mortality was found in the control group of fish. In the experimental group of fish, results were noted as 10% to 70% mortality during the experiment at 24, 48, 72 and 96 hours (Table 6). The LC50 values were found to be 56.23%, 28.84%, 22.38%, 16.59% at 24, 48, 72 and 96 hours respectively as summarised in Table 6. From the above results, it was observed that the toxic effect of effluent is very high to *Clarias batrachus and* it was considered to be highly toxic wastewater to the fishes.

Safe concentration for toxic textile effluent to fish *Clarias batrachus* was calculated by the method described by Hart et al. (1945). Safe concentration was found to be 32.88 % (Table 6). The lethal toxicity test reported that fish in the control tank survived whereas mortalities were recorded in all treatment concentrations. Graphs were plotted between log concentration (mortality %) and probit value at different internal exposure 24, 48, 72 and 96 hrs respectively (Figs.1, 2, 3 and 4).Mortality increased with increasing concentration of the toxicant in water. A similar observation of different fish species exposed to different levels of toxicants is reported by other workers (Agbon et al. 2002, Omoregie et al. 2009, Umar et al. 2010).

The variation in acute toxicity may be due to alteration in water quality and test species. Variability in acute toxicity even in a single species and single toxicant depending on the size, age, experimental species and factors (Farah et al. 2004). The existing physiological parameters of water were reported by Eaton & Gilbert (2008). It is evident from the results that the high concentration of textile effluent with chemical dye, toxic elements and heavy metals has a direct effect on the LC50 values of the respective fish.

Sreelekshmy et al. (2016) studied acute toxicity of industrial effluent on catfish and reported that mills effluents are the main pollutants in the aquatic environment and affect the aquatic organisms especially fishes. Mortality rate increases with increasing toxicity level of textile effluent. LC 50 values were declined by increasing concentration of textile effluent and time exposure.

Behavioural and Morphological Alterations

The behavioural responses of the test fish to the toxicant at different concentrations at the period of 96 h (Table 7) indicated high secretion of mucus,uncoordinated movement, restlessness, tailfin movement, loss of balance surfacing, loss of buoyancy, escaping tendency, hyperactivity and discolouration of the skin. Mortalities were observed in all the treatments except control. The fish exhibits stressful behaviour which increased with the toxicant concentration. Similar behaviour of *C. gariepinus*was observed by Fafioye et al. (2001) and of *C. punctatus*by Rahman et al. (2002) with the effluent.

Behaviour is an organism-level effect defined as the action, reaction or functioning of a system under a set of specific circumstances. We rationalize that a greater understanding of behavioural responses in effect to chemical stress may increase. Therefore, in the current scenario, there is a need for developing newer and effective methods to study behavioural responses. Behavioural changes in a fish form an efficient index to measure any alterations in the environmental conditions (Sharma 2019).

Behavioural changes are one of the most sensitive indicators of potential toxic effects in fishes (Banaue et al. 2011). Based on the results obtained and data shown in the Table 7, it can be concluded that the TIE is toxic to the aquatic environment and fishes. Due to toxicity, morphology and behaviour of *C.batrachus* are altered and it exhibits hyperactivity with high effluent concentration.

Table 7: Behaviour of Clariasbatrachusto textile effluent during acute toxicity test.

Behavioural and morphological response	0 % Concentration (v/v)	10% Concentration (v/v)	30% Concentration (v/v)	50% Concentration (v/v)	70% Concentration (v/v)
Mucus secretion	None	Mild	Moderate	Strong	Very strong
Uncoordinated movement	None	Mild	Strong	Very strong	Very strong
Restlessness	None	Mild	Moderate	Strong	Very Strong
Tailfin movement	None	Mild	Moderate	Strong	Very Strong
Loss of balance	None	None	Moderate	Strong	Very strong
Surfacing	None	None	Moderate	Maximum	Maximum
Loss of buoyancy	None	None	Mild	Moderate	Maximum
Escaping tendency	None	None	Mild	Maximum	Maximum
Hyperactivity	None	None	Mild	Moderate	Maximum
Discolouration of skin	None	None	Mild	Moderate	Maximum

Haematological Parameters

Haematological analysis can provide valuable knowledge for monitoring the health and condition of both wild and culture fish.Haematological tests and analysis for serum constituents have shown useful information in the detection and diagnosis of metabolic disturbance and diseases in fishes (Jamalzadeh et al. 2009).

Bhanu et al. (2013) studied that due to the influence of toxic mill effluent the amount of RBC and Hb have been decreased in the blood of fish Bat the amount of WBC has been increased as an immunological defence to survive against the toxic substance in the effluent. Haematological parameters of the blood of fish such as Hb%, total WBC count, Differential count of WBC, and total RBC count after exposure of *T. mossambica* to 100% untreated and treated textile effluent for a period of 15 days showed different morphological changes. The morphological change of the blood cells was more prominent in the untreated sample than that of treated blood samples due to the high toxicity of untreated effluent (Deepika & Noorjahan 2018).

Haematological changes in the blood due to Textile effluent in the freshwater fish, *C.batrachus* on different exposure periods is shown in Table 8.

Different haematological parameters were evaluated. RBC, haemoglobin (Hb), packed cell volume (PCV), mean corpuscles haemoglobin concentration (MCHC) and mean corpuscles haemoglobin (MCH) values exhibited significantly decreased (Table 8 and Figs. 6-11, 13) whereas total white blood corpuscles (WBC) and glucose level were gradually increased (Table 8 and Figs. 12 and 14) in treated fish when compared with controls (Table 8). The present study clearly indicates the toxic effects of these chemicals on haematological parameters of fish that are seen in Figs. 6-14.

CONCLUSION

The present study was carried out to evaluate the acute toxicity and behavioural responses in *Clarias batrachus* (Linnaeus) exposed to textile industrial effluent.The results clearly indicate that exposure of fishes to industrial based textile effluent resulted in increased mortality with increasing concentration of the effluent. In this study it is observed that textile effluent caused many behavioural and morphological alterations, which may result in severe physiological problems, ultimately leading to the death of fish.Most of the haematological parameters like RBC, Hb, PCV, MCV,

Table 8: Haematological changes in the blood due to Textile effluent in the freshwater fish, *C.batrachus* on different exposure periods.

S.No.	Blood parameter	Control	24hr	48hrs	72hrs	96hrs
1.	RBC (1×10^6 mm^3)	2.70 ± 0.03	2.65 ± 0.08	2.46 ± 0.14	2.35 ± 0.06	2.24 ± 0.05
2.	WBC (1×10^3 mm^3)	5.90 ± 0.12	6.35 ± 0.08	6.58 ± 0.16	6.85 ± 0.05	7.10 ± 0.16
3.	Haemoglobin (gm/dl)	7.65 ± 0.03	6.90 ± 0.05	6.25 ± 0.08	5.90 ± 0.12	4.65 ± 0.04
4.	Haematocrit % (PCV)	24.2 ± 0.14	22.10 ±0.14	20.15 ± 0.14	18.6 ± 0.14	15.8 ± 0.14
5.	MCV(fl)	89.62 ± 1.15	83.58 ± 1.35	81.91 ± 0.12	79.14 ± 0.08	70.53 ± 1.35
6.	MCH(pg)	28.33 ± 1.06	26.03 ± 1.04	25.40 ± 0.07	25.10 ± 0.14	20.25 ± 1.10
7.	MCHC(g/dl)	31.61 ± 0.09	31.15 ± 1.11	31.01 ± 0.06	31.71 ± 0.09	29.43 ± 1.15
8.	Protein g/dl	7.90 ± 1.12	6.85 ± 1.10	6.43 ± 0.54	5.78 ± 0.84	4.70 ± 0.08
9.	Glucose mg/mL	80.5 ± 3.14	82.7 ± 2.54	84.80 ± 3.10	85.35 ± 1.24	90.20 ± 2.44

Values are mean ± SE of 6 values; p ≤0.001 Highly significant; p ≤0.01 Significant; p ≤0.05 Almost significant

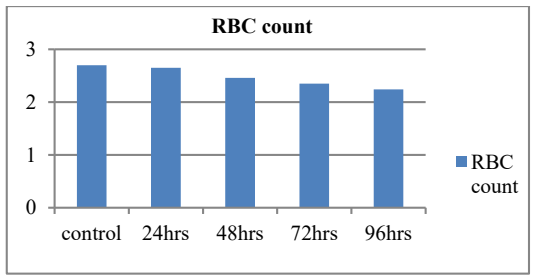


Fig. 6: Percentage changes in RBC of *C.batrachus* exposed to various concentrations of textile effluent.

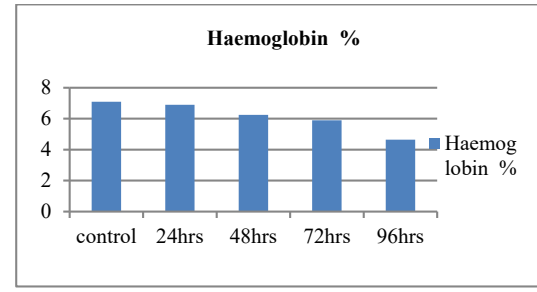


Fig. 7: Percentage changes in Hb % of *C.batrachus* exposed to various concentrations of textile effluent.

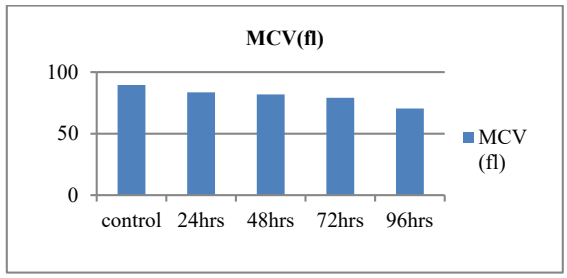


Fig. 8: Percentage changes in MCV of *C.batrachus* exposed to various concentrations of textile effluent.

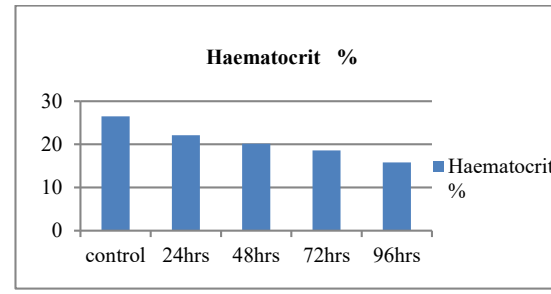


Fig. 9: Percentage changes in Haematocrit % of *C.batrachus* exposed to various concentrations of textile effluent.

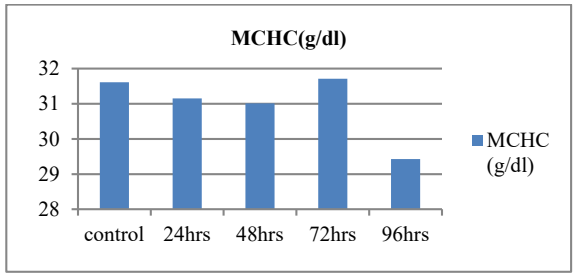


Fig. 10: Percentage changes in MCHC (g/dl) of *C.batrachus* exposed to various concentrations of textile effluent.

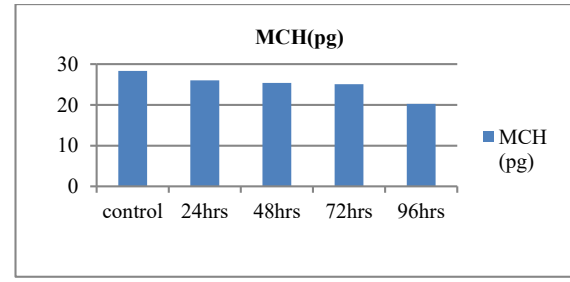


Fig. 11: Percentage changes in MCH(pg) of *C.batrachus* exposed to various concentrations of textile effluent.

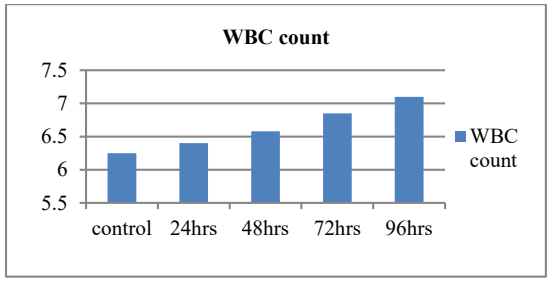


Fig. 12: Percentage changes in WBC of *C.batrachus* exposed to various concentrations of textile effluent.

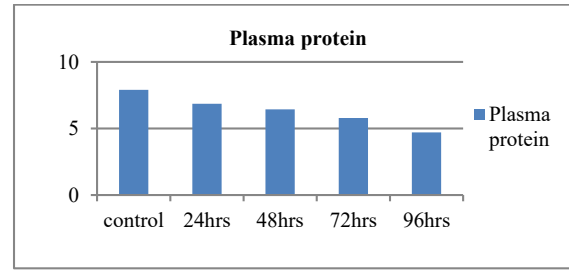


Fig. 13: Percentage changes in Plasma Protein (g/dl) of *C.batrachus* exposed to various concentrations of textile effluent.

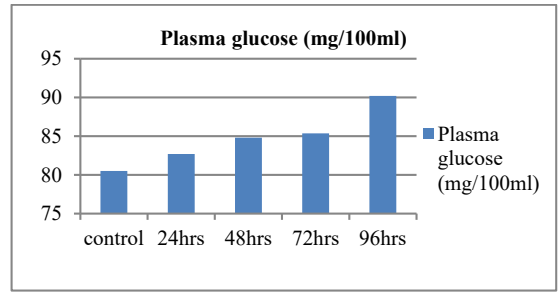


Fig. 14: Percentage changes in Plasma glucose level of *C.batrachus* exposed to various concentrations of textile effluent.

MCH, MCHCand protein level were found to be increased while WBC and glucose level were decreased when fish was exposed to textile effluents in different time.In the present investigation, it can be concluded that industrial-based textile effluent of different textile industries of Pali city is highly toxic to freshwater fish *C.batrachus*. This study observed that CETP (common effluent treatment plants) of Pali city is not properly working and many textile mills discharging

untreated effluents into Bandi River directly. It shows that textile effluent is highly polluted and treatment is necessary prior to discharge of wastewater in Bandi River of Pali city.

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REFERENCES

Adeyemo, O.K., Naigaga, I. and Alli, R.A. 2009. Effect of handling and transportation on haematology of african catfish (*Clarias gariepinus*). J Fish Sci. Com., 3: 333.

Agbon, A.O., Omoniyi, I.T. and Teko, A.A. 2002. Acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on *Oreochromis niloticus* and haematological changes resulting from sub lethal exposure. Journal of Aquatic Sciences, 17(1): 5-8.

Amte, G.K. and Mhaskar, T.V. 2013. Impact of textile-dyeing industry effluent on some haematological parameters of freshwater fish *Oreochromis mossambicus*. Nature Environment and Pollution Technology, 1)12): 93-98.

Amutha, P. and Mahalingam, S. 1999. Effect of dairy effluent on body composition of *Cyprinus carpio* (Cyprinidae). J. Ecotoxicol. Environ. Monit., 9(1): 3-8.

APHA 2000. Standard Method for the Examination of Water and Wastewater. American Public Health Association, 19th edition, American Public Health Association, Washington, DC.

Arya, P.D., Joseph, M.L. and Mathew,A.2018. Determination of LC50 of lead acetate for a fish *Oreochromis mossambicus* (Peters). International Journal of Fisheries and Aquatic Studies, 6(6): 180-182.

Banaue, M., Sureda, A., Mirvaghefi, A.R. and Ahmadi, K. 2011. Effects of diazinon on biochemical parameters of blood in rainbow trout (Oncorhynchus mykiss). Pestic. Biochem. Physiol., 99 (1): 1e6.

Berardi, M., Hsissou, R., Khudhair, M., Mohammed Assouag, M., Cherkaoui, O., Bachiri, A.E. and Harfi, A. E. 2019. Textile finishing dyes and their impact on aquatic environs.Heliyon, 5: e02711.

Bhanu, A. Paratha and Deepak, M. 2013. The Influence of Textile Mill Effluent on Haematological Changes of *Labeo rohita.* International Journal of Innovative Research in Science, Engineering, and Technology, 8003-8001 :(12)2.

Clauss,T.M., Dove, A.D. and Arnold, J.E. 2008. Hematologic disorders of fish. Vet. Clin. North Am. Exot. Anim. Pract., 11: 445-462.

Deepali and Gangwar, K.K. 2010. Metals concentration in textile and tannery effluents associated soils and groundwater, New York Science Journal, 3(4): 82-88.

Deepika, T. and Noorjahan, C. M. 2018. Impact of untreated and treated textile effluent on haematological parameters of freshwater fish, *Tilapia mossambica.* International Journal of Advanced Scientific Research and Management, 3 (6): 24-28.

Eaton, D.L. and Gilbert, S.G. 2008. Principles of toxicology. Casarett&Doull's Toxicology: The Basic Science of Poisons, Eighth ed. McGraw-Hill Medical, New York, pp. 11e43.

Fafioye, O.O., Adeogun, O.A., Olayinka, E.A. and Ayoade, A.A. 2001. Effect of Sub-lethal concentrations of lead on growth of *Clarias gariepinus*. Nigerian Experimental Biology, 5(1): 61-68.

Farah, M.A., Ateeq, B., Ali, M.N., Sabir, R. and Ahmad, W. 2004. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere, 55(2): 257e265.

Finney, DJ. 1971. Probit Analysis. Cambridge University Press, London.

Gupta, N. and Dua, A. 2010. Mercury induced behavioral alterations in *Channa punctatus.* Pollution Research, 29(4):721-723.

Hader, D.P. 2018. Ecotoxicological monitoring of wastewater. In: Häder, D.P. andErzinger, G.S. (eds) Bioassays, Advanced Methods and Applications. Elsevier Press, Cambridge, pp. 369-386.

Haniffa, M.A. and Arul, Selvan, S. 1991. Haematological and biochemical effects of textile mill effluent on the freshwater fish, *Oreochromis mossambicus.* Uttar Pradesh J. Zool., 11(1): 661-662.

Holkar, C.R., Jadhav, A.J., Pinjari,D.V., Mahamuni, N.M. and Pandit, A.B. 2016. A critical review of textile wastewater treatments: Possible approaches. J. Environ.Manag., 182: 351e366.

Hart, W.B., Doudoroff, P. and Greenbank, J. 1945. The evaluation of the toxicity of industrial wastes, chemical and other substances to freshwater fishes. Atlant. Refinding Co. (Phill).

Hossain, L., KantiSarker S. and Samad Khan,M. 2018. Evaluation of present and future wastewater impacts of textile dyeing industries in Bangladesh. Environ. Develop., 26: 23-33.

Jamalzadeh, H.R., Keyvan, A., Ghomi, M.R and Gherardi, F. 2009. Comparison of blood indices in healthy and fungal infected Caspian Salmon (*Salmo trutta caspius*). African Journal of Biotechnology, 8(2): 319- 322.

Makwana, S. 2020a.Impact assessment of anthropological activities on water quality of historical Gulab Sagar pond, Jodhpur, Rajasthan (India). International Journal for Research in Applied Science & Engineering Technology, 8(10): 178-185

Makwana, S. 2020b. Effect of textile industrial effluents on water quality of Bandi River (Pali) Rajasthan, India. International Journal for Research in Applied Science & Engineering Technology, 8(4): 21-28

Maruthi, Y. A. and Rao, S. R. 2001. Effect of sugar mill effluent on organic reserves of fish. Poll. Res., 20(2): 167-171.

Nagarajan, K. and Aruna, Devi, P. S. 2006. Histological changes in Indian freshwater major carp *Labeo rohita* due to distillery effluent. Nature Environ. Polln. Tech., 5: 1 -6.

Nagarajan, K. and Boopathy, Raja, A. 2004. Studies on the effects of dyeing effluent on certain physiological aspects of the freshwater fish *Cath catla.* J. Exp. Indian, 1(1): 101-104.

Nagarajan, K. and Ramesh, F. 2001. Influence of Sago effluent on certain physiological and histological aspects of the freshwater fish *Catla catla.* Proc. ENVIRO 2001. Nat Conf. Cont. Ind. Poll Environ.Degrad., 470-475.

Nagarajan, K. and Shasikumar, R. 2002. Effect of sago effluent on selected physiological aspects of the freshwater fish *Labeo rohita.* J.Ecotoxicol. Environ. Monit., 12(3):233-238.

Nagarajan, K. and Suresh, K. 2005. Impact of treated sago effluent on the levels of acid phosphatase, alkaline phosphatase, and lactate dehydrogenase in *Cirrhinus mrigala.* J.Indust.Polln. Cont., 21(1): 115 -120.

Omoregie, E., Malachy, N.O., Ajino, I., Romanus, I. and Kazimierz, W. 2009. Effect of Single super-phosphate fertilizer on survival and respiratory dynamics of *Oreochromis niloticus*. Acta Ichthyological Et Piscatoria, 39(2): 103-110.

Rahman, M.Z., Hossain, Z., Mollagh, M.F.A. and Ahmed, G.U. 2002. Effect of Diazinon 60EC on Testudineus (*C. punctatus* and *B. gonionotus*). The ICLARM Quarterly, 25(2): 8-12.

Rand, G. M. and Petrocelli, S. R. (Eds.)1985. *In:* Fundamentals of Aquatic Toxicology Methods and Applications. *Hemisphere Publishing Corporation,* Washington, U.S.A., pp. 1 -28.

Sarvajith, M., Reddy, G., Kiran Kumar and Nancharaiah, Y.V. 2018. Textile dye decolorization and ammonium removal over nitrite in aerobic

granular sludge sequencing batch reactors. J. Hazard Mater., 342: 536-543.

Shanthi, M. 2003. Effects Of Tannery Effluent On Certain Physiological And Histological Aspects Of The Freshwater Fish *Catlacatla.* M. Phil. Dissertation. Bharathiar University.

Satish and Sharma,K. C. 2008. Waste Water Management with Reference of Textile and Dyes Industries of Pali District. M. Phil. Thesis (Environmental Management), M.D.S. University, Ajmer.

Sharma, M. 2019. Behavioural responses in effect to chemical stress in fish: A review. International Journal of Fisheries and Aquatic Studies, 7(1): 01-05.

Soni, Rakesh and Verma, S.K. 2018. Acute toxicity and behavioural responses in *Clarias batrachus* (Linnaeus) exposed to herbicide pretilachlor. Heliyon, 4: e01090.

Sreelekshmy, S.G., Miranda, M.T.P. and Rajesh, B.R. 2016. Acute toxicity of industrial effluent on the marine catfish *Arius nanga* (Hamilton, 1822).International Journal of Fisheries and Aquatic Studies, 4(3): 215-219.

Srivastav, A.K. and and Roy, D. 2015. Effects of malachite green (Triarylmethane dye) and Pyceze (Bronopol) on the hematological parameters of a freshwater catfish *Heteropneustes fossilis* (Bloch). International Journal of Fisheries and Aquatic Studies, 2(6): 119-122.

Taiwo, O., Durotoye, Aderonke A., Adeyemi, David, O. Omole and OlumuyiwaOnakunle, Amimul Ahsan2018. Impact assessment of wastewater discharge from textile industry in Lagos, Nigeria. Cogent Engineering, 5: 1, DOI: 10.1080/23311916.2018.1531687.

Umar, M., Stephen, S.H., Abdullahi, M. and Garba, 2010. M. Dichlorvos concentrations in locally formulated pesticides (Ota-pia pia) utilized in Northeastern Nigeria. Scientific Research and Essay, 5(1): 049-054.

Yaseen, D.A. and Scholz, M. 2019.Textile dye wastewater characteristics and constituents of synthetic effluents: A critical review. *Int. J. Environ. Sci. Technol.,* **16:** 1193-1226 https://doi.org/10.1007/s13762-018-2130-z.

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Hammer, D.A. (ed.) 1989. Constructed Wetlands for Wastewater Treatment-Municipal, Industrial and Agricultural. Lewis Publishers Inc., pp. 831.

Haynes, R. J. 1986. Surface mining and wetland reclamation. In: Harper, J. and Plass, B. (eds.) New Horizons for Mined Land Reclamation. Proceedings of a National Meeting of the American Society for Surface Reclamation, Princeton, W.V.

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