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Assessing Phytoremediation Potential of *Aloe barbadensis*, *Chrysopogon zizanioides* and *Ocimum tenuiflorum* for Sustainable Removal of Heavy Metals from Contaminated Soil

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ABSTRACT

India's fast industrialization and population expansion have resulted in heavy metal accumulation from many operations, which has caused massive waste generation and poisoning of soils. Therefore, it is necessary to design reclamation to improve th T.Ne soil. Phytoremediation presents itself as a viable, economical, and environmentally sustainable solution to this problem. This study was carried out by using plants namely, aloe-vera (Aloe-Barbadensis), tulsi (Ocimum Tenuiflorium), and vetiver (Chrysopogon Zizanoides) plants which were planted in a simulated soil of Cd, Zn and Pb, for 4 weeks. The sample of plant and soil were taken in 9 different pots, (15 cm diameter and 25 cm height) among 9 potted soils one will be tested as a controlled sample. An aqueous solution of lead, cadmium and zinc were added separately to the dry soil samples. The moisture level of the soil was maintained to near field water capacity (35.6%) and equilibrated for two weeks. The saplings of vetiver grass, aloe vera and tulsi were selected and pruned (the shoots were originally 20 cm high and the roots 8 cm long), and then transplanted into the pots. The AAS test was conducted after 4 weeks of growing in simulated soil. Tulsi demonstrated the highest efficacy in reducing Zn concentrations from 300 mg/kg to 188.3 mg/kg, followed by vetiver (179.3 mg/kg) and Aloe vera (158.3 mg/kg). Similarly, for Pb, tulsi exhibited the most substantial reduction (from 600 mg/kg to 188.3 mg/kg), followed by vetiver (164.3 mg/kg) and Aloe vera (179.6 mg/kg). Regarding Cd, tulsi reduced concentrations from 80 mg/kg to 18.62 mg/kg, while vetiver achieved a 17.62 mg/kg reduction. The result highlights Tulsi's superior remediation potential, attributed to its efficient heavy metal uptake and translocation mechanisms. Thus, using these plants in the phytoremediation process, the heavy metals are extracted more economically than other plants. This technique highlights the innate ability of hyper-accumulator plant species, which flourish in situations high in heavy metals, to extract contaminants from contaminated soil.

INTRODUCTION

Heavy metal pollution of soil has become a global environmental concern due to increased industrialization and intensification of agricultural practices. Industrialization has emerged as a prominent catalyst for heavy metal pollution, primarily due to its involvement in the extraction, processing, and utilization of metals for manufacturing and energy generation purposes (Mohammed et al. 2011, Kumari et al. 2021). Similarly, contemporary agricultural methodologies that rely extensively on the application of chemical fertilizers, pesticides, and herbicides have inadvertently contributed to the accumulation of heavy metals in soil (Rashid et al. 2023). Although these chemical fertilizers have enhanced agricultural productivity, increased crop yields, and mitigated hunger, have also unintentionally played a role in the deterioration of soil quality and the accumulation of heavy metals (Bakshi et al. 2018). For instance, using phosphate fertilizers is associated with elevated levels of cadmium, resulting in the accumulation of cadmium in soil, thereby leading to contamination (Lin et al. 2022). Thus, the presence of pollutants resulting from industrial activities, agricultural practices, and urbanization has gradually infiltrated the fundamental structure of our terrestrial environment, posing significant obstacles to ecological integrity, agricultural sustainability, and human health (Zhang & Wang 2020).

There are a variety of repercussions that can arise because of heavy metal contamination in soil. Firstly, contaminated soil poses a significant threat to agricultural productivity, hampering soil fertility, nutrient cycling, and microbial diversity, ultimately leading to reduced crop yields and compromised quality (Ali et al. 2019). The uptake of heavy metals by plants can also lead to their introduction into the food chain, which eventually puts human health at risk through consuming contaminated crops (Wuana et al. 2011). As a result of prolonged exposure of humans to heavy metals, a wide variety of health problems have been identified, including damage to organs, neurological disorders, and effects that are carcinogenic diseases. Furthermore, heavy metal contamination in soil is a significant environmental threat that has the potential to disrupt ecosystems and biodiversity (Priya et al. 2023, Saxena et al. 2023). Soil-dwelling organisms, essential for the cycling of nutrients and the health of the soil, are especially susceptible to the toxic effects of heavy metals, which can result in population declines and ecological imbalances (Rashid et al. 2023). Furthermore, heavy metals have the potential to leach into groundwater, which can contaminate sources of drinking water and perpetuate a cycle of environmental degradation. Therefore, removing heavy metals from soil is necessary to mitigate these adverse effects and safeguard environmental and human well-being. The removal of heavy metals from soil can be accomplished through various approaches, each of which has its own benefits and drawbacks. One of the most common methods is physical remediation, which entails removing contaminated soil through excavation, dredging, or washing (Azhar et al. 2022). The physical remediation process, despite its efficacy, can be expensive and disruptive to the surrounding environment (Kumar et al. 2021). Heavy metals are dissolved and extracted from soil particles using chemical remediation techniques. Some examples of these techniques include washing soil with chelating agents or acids (Kirpichtchikova et al. 2006). On the other hand, these methods frequently produce hazardous waste and may pose unintended environmental consequences (Khalid et al. 2017).

Thus, among all the available technologies for removing heavy metals from polluted sites, phytoremediation is regarded as the most efficient, cost-effective, environmentally favorable, and preferred method for cleaning up contaminated areas (Singh et al. 2023). It is a more sustainable and environmentally friendly alternative, utilizing biological processes to degrade, sequester, or transform heavy metals in soil (Pang et al. 2023). Phytoremediation, in particular, harnesses the natural abilities of certain plant species to uptake, accumulate, and detoxify heavy metals from the soil. Phytoremediation plants refer to the utilization of living plants to reduce, degrade, or eliminate toxic toxins from soil. Using green plants to decontaminate soil is a progressive and sustainable method that minimizes the need for hefty machinery or additional contaminants by a significant margin (Li et al. 2022). Lucerne, sunflower, corn, date palms, certain mustards, and even willow and poplar trees can be used to remediate contaminated soil in an inexpensive, environmentally friendly, and sustainable manner (Rungwa et al. 2013, Haller et al. 2023). While a wide array of plant species has been employed in phytoremediation projects, recent attention has turned to the unique potential of aromatic plants.

Aromatic plants are cultivated to produce essential oils in addition to their use in culinary applications. Aromatic plant essential oils are utilized in producing soaps, detergents, insect repellents, cosmetics, scents, and even in food preparation. In contrast to grains, legumes, and vegetables, these plants cannot be digested by people or animals and are, therefore, not directly consumed by them. The essential oil derived from aromatic plants does not provide any risks associated with the buildup of heavy metals in plant biomass (Zheljazkov et al. 2006). As a direct outcome of aromatic plant phytoremediation, heavy metals are prevented from entering the food chain (Gupta et al. 2013). Wild animals tend to avoid damaging or eating aromatic crops because of the distinctive odor they give off. There is an abundance of aromatic plant resources that are capable of being utilized on a massive basis. It is a novel approach to the phytoremediation of heavy metal-contaminated sites to utilize these plants in the remediation process (van der Ent & Rylott 2024, Lancheros et al. 2024, Araujo et al. 2024). Aromatic plants that are both ecologically sustainable and practically useful include vetiver (*Vetiveria zizanioides*), lemon grass (*Cymbopogon f lexuosus*), citronella (*Cymbopogon winterianus*), geranium mint (Mentha sp.), and tulsi (*Ocimum basilicum*) (Pandey et al. 2015). Perennial and resistant to the effects of stress are two characteristics shared by several aromatic grasses, including lemon grass, palmarosa, citronella, and vetiver. The plant can be picked for the hydrodistillation of its essential oil in successive years after it has been grown. These goods have a high value but require minimal effort from the buyer.

Thus, heavy metal pollution of soil, driven by industrialization and intensive agricultural practices involving chemical fertilizers and pesticides, has become a global environmental concern due to its detrimental impacts on soil fertility, agricultural productivity, ecological balance, and human health. Traditional remediation methods, while effective, often have significant drawbacks, making phytoremediation a more sustainable and environmentally friendly alternative. When it comes to phytoremediation strategies, the use of aromatic plants presents a new alternative. This is because aromatic plants can absorb heavy metals without it entering the food chain.

Phytoremediation is a green technology that uses the remarkable abilities of plants to clean up contaminated soil. These "nature's vacuum cleaners" absorb pollutants through their roots, transport them to leaves, and either store or transform them into less harmful substances. The process begins with the roots of these plants absorbing pollutants such as heavy metals, pesticides, and hydrocarbons from the soil. Once absorbed, these contaminants are transported through the plant's vascular system to the shoots and leaves and sometimes stored in the roots. Inside the plant, several processes can occur: pollutants can be sequestered in the vacuoles of cells, reducing their bioavailability; they can be transformed into less toxic compounds through metabolic processes such as phytodegradation or phytotransformation; or volatilized through phytovolatilization, where contaminants are converted to gaseous forms and released into the atmosphere at low concentrations (Barroso et al. 2023). Additionally, some plants excrete root exudates that can enhance the microbial degradation of pollutants in the rhizosphere, the zone of soil influenced by root secretions and associated soil microorganisms (Rathore & Kaur 2023). This multifaceted approach not only removes contaminants from the soil but also improves soil structure and fertility, promoting a healthier ecosystem.

A safe, economically viable, and environmentally friendly method for phytoremediation using aromatic plants like tulsi, vetiver, and aloe vera, which are abundantly available, is discussed. The aforementioned plants were assessed for their efficiency in the contaminated soil with cadmium, lead, and zinc. The contamination of the soil was done in relation to the permissible concentration of heavy metals in the soil. The remediation of these elements is considered very important due to their relatively wider spread when compared to other heavy metals and their negative impacts on the ecosystem and human health. Finally, the plants were evaluated for the effects of phytoremediation on the quality of the soil, which included changes in the concentrations of heavy metals and pH levels as shown in Fig.1.

MATERIALS AND METHODS

Red soil: The red soil sample was meticulously collected from the campus of Aarupadai Veedu Institute of Technology, situated in Vinayaka Nagar, Old Mahabalipuram Road, Paiyanoor, Kancheepuram District, Chennai, Tamil Nadu, India. The geographical coordinates of this esteemed institution are approximately 13.0847° N latitude and 80.1924° E longitude. The collected soil was tested and confirmed that it was free from other minerals and heavy metals. The red soil was characterized by its distinctive red hue due to its iron oxide content. They can offer several benefits for plant health. With its excellent drainage properties, red soil prevents waterlogging and facilitates healthy root growth by ensuring adequate aeration (Gray et al. 2006). Rich in nutrients like iron provides essential elements for plant growth and development (de Souza Costa et al. 2021). Furthermore, its ability to absorb and retain heat promotes root establishment and encourages robust growth, particularly in cooler climates. After characterization, the collected soil was contaminated with zinc, lead, and cadmium. Aqueous solutions containing each metal were individually added to the dry soil samples for experimental studies. Subsequently, phytoremediation studies are conducted on the contaminated soil.

Grow pots and conditions: Nine flower pots were used for this investigation. These pots were categorized into three groups, each representing soil contaminated with zinc (Zn), cadmium (Cd), and lead (Pb), respectively. Within each contamination group, three different plant species were planted separately: Vetiver (*Chrysopogon zizanioides*), Aloe Vera (*Aloe barbadensis* Miller), and Tulsi (*Ocimum tenuiflorum*). The contaminated soil was then carefully placed into the flower pot. The soil moisture level was carefully maintained at near-field water capacity (35.6%) and allowed to equilibrate for two weeks. Saplings of the selected plant species, each pruned to a uniform height of 20 cm with roots measuring 8 cm in length, were transplanted into the pots. These plant species were chosen based on their resilience and ability to thrive under varying soil conditions. The pots were watered daily to 60% of the field water capacity throughout the experiment to ensure optimal growth conditions.

Metal powder: Heavy metals, including zinc, lead, and cadmium, were procured from Labogens Fine Chem, Maharashtra, to serve as crucial elements for the deliberate soil contamination in our experimental setup. These specific metals were selected due to their known environmental significance and prevalence in soil pollution scenarios (Kumari & Mishra 2021). By introducing zinc, lead, and cadmium into our experimental setup, we aimed to replicate real-world conditions of soil contamination, facilitating comprehensive investigations into phytoremediation strategies and their effectiveness in mitigating heavy metal pollution.

Zinc (Zn): The deteriorating environmental conditions attributed to the presence of zinc in the soil prompted our investigation into mitigating its effects through phytoremediation studies. Zinc contamination in soil arises from various sources, including industrial activities such as mining and manufacturing, mining operations alongside other metals like lead, agricultural practices utilizing zinc-

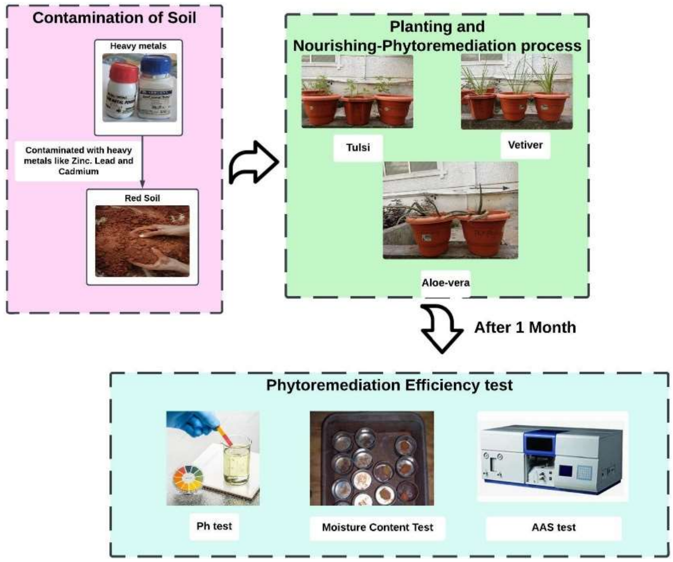

Fig.1: Materials and experimental procedure utilized.

based fertilizers and pesticides, and urban runoff from vehicular traffic and industrial areas (Zhao et al. 2012). The effects of zinc contamination on soil quality encompass soil acidification, reduced fertility, and alterations in soil structure, which impact nutrient availability and hinder plant growth (Korzeniowska et al. 2023). Environmental repercussions of zinc contamination include plant toxicity, water pollution through leaching into groundwater and surface water bodies, bioaccumulation in the food chain, and disruption of soil microbial communities and ecosystem health (Ferrarini et al. 2021). Human health risks associated with zinc exposure include acute symptoms such as nausea and diarrhea, as well as chronic effects like neurological disorders and reproductive toxicity, necessitating mitigation strategies like soil remediation and regulatory measures to safeguard both the environment and public health (Hussain et al. 2022). Recognizing the adverse impact of zinc contamination on soil health and ecosystem integrity, our research endeavors aimed to explore effective plant-based remediation strategies to alleviate its detrimental effects and restore environmental balance.

Lead (Pb): The degradation of ecological conditions due to lead in the soil necessitated its inclusion in our phytoremediation studies. Our research focused on developing effective phytoremediation strategies to mitigate the adverse impact of lead contamination on soil quality, ecosystem health, and human well-being. Lead contamination poses significant risks, including soil degradation, water pollution, and toxicity to plants and animals (Raj et al. 2023). Lead contamination in soil presents multifaceted challenges with far-reaching environmental and human health implications. This pervasive issue stems from various sources, including historical and current industrial activities, urbanization, agricultural practices, and improper waste disposal (Oorts et al. 2021). The environmental effects of lead contamination are extensive, encompassing soil quality degradation, plant toxicity, water contamination, bioaccumulation, and ecological disruption (Collin et al. 2022). Moreover, lead exposure poses significant health risks to humans, particularly vulnerable populations such as children and pregnant women, leading to neurological, developmental, cardiovascular, renal, and reproductive disorders (Srivastava et al. 2022). Addressing lead contamination demands holistic management strategies focused on prevention, remediation, and regulatory measures to safeguard environmental integrity and public health. By investigating the efficacy of phytoremediation techniques, we aimed to restore environmental balance and protect ecosystems and human health from the harmful effects of lead contamination.

Cadmium (Cd): The adverse change in ecological conditions, exacerbated by heavy metal cadmium contamination in the soil, highlights the critical need to address its adverse impacts. Cadmium contamination threatens soil biodiversity, ecosystem integrity, and overall ecological health (Raza et al. 2020). Its presence can disrupt soil microbial communities, inhibit plant growth, and impair nutrient cycling, leading to cascading effects throughout the ecosystem (Soubasakou et al. 2022). Industrial activities, agricultural practices, urbanization, and improper waste disposal contribute to cadmium contamination in soil, highlighting the need for comprehensive management strategies (Li et al. 2023). The environmental effects of cadmium contamination include soil degradation, plant toxicity, water contamination, bioaccumulation, and ecological disruption, with adverse impacts on soil health, aquatic ecosystems, and biodiversity (Mahajan et al. 2018). Furthermore, cadmium exposure poses serious health risks to humans, including respiratory problems, kidney damage, bone disorders, and cancer. To address these challenges, preventive measures, remediation efforts, and regulatory interventions are crucial for mitigating the environmental and human health impacts of cadmium contamination and safeguarding ecosystems and public health. In response to these challenges, our research focused on employing phytoremediation as a viable solution. By utilizing cadmium in our phytoremediation studies, we aimed to mitigate the detrimental effects of cadmium contamination and restore ecological balance. Through comprehensive investigations and experiments, we sought to develop effective strategies for remediating cadmium-contaminated environments and safeguarding the health and resilience of affected ecosystems.

Plant Species For The Process Of Phytoremediation

In this research, aromatic plants such as Tulsi (Ocimum tenuiflorum), Aloe Vera (Aloe barbadensis Miller), and Vetiver (*Chrysopogon zizanioides*) were utilized for the phytoremediation process. These plant species were chosen for their resilience to environmental stresses and their potential to thrive in contaminated soil conditions (Pang et al. 2023, Rungwa et al. 2013, Haller et al. 2023). To initiate the phytoremediation process, saplings of the selected plant species were obtained and pruned to ensure uniformity, with a height of 20 cm and roots measuring 8 cm in length. This standardization ensured consistency in the experimental setup and facilitated accurate monitoring of plant growth and metal uptake over time. The collected saplings were then planted in pots filled with soil contaminated with specific levels of heavy metals. Using aromatic plants for phytoremediation presents a promising solution to heavy metal contamination in soil. Unlike traditional crops, aromatic plants are primarily cultivated for their essential oils and industrial applications, not for direct consumption by humans or animals (Gupta et al. 2013). This significantly reduces the risk of heavy metal accumulation in the food chain, providing a sense of security for our ecosystems and human health.

Experimental Investigations

pH Test: The pH level of the soil is essential to ensure optimal plant growth. pH test results will guide the decision whether and how much the soil needs supplements, like fertilizers and soil pH adjusters (Zhang et al. 2022). To measure soil pH accurately, a clean trowel was used to collect soil samples from multiple locations within the testing area to ensure a representative sample. Clumps, debris, or organic matter were removed from the samples. Approximately 15-30 grams of soil was placed into a clean container, and distilled water was added to create a slurry. The mixture was then stirred thoroughly with a clean stirring rod to ensure an even distribution of soil particles in the water. The mixture is then allowed to settle for 30 minutes, letting the soil particles settle at the bottom. A clean pH test paper is dipped into the suspension for a few seconds, then removed, and the color is allowed to develop. The resulting color is compared to the provided pH color chart, and the pH value is recorded once it stabilizes. Control measures include using clean equipment and distilled water to prevent contamination, ensuring consistent sample collection methods, and performing tests under similar environmental conditions to maintain accuracy. These steps ensure reliable pH measurements, providing insights into soil conditions and the effectiveness of phytoremediation.

Moisture content test: The moisture content of a soil sample is determined by measuring the amount of water present in the sample as a percentage of its dry mass. A representative soil sample was collected from the desired depth to determine soil moisture content accurately, ensuring it is free from contaminants, large organic materials, and rocks. Clumps were crushed to ensure uniform drying. An empty drying dish was weighed, the soil sample was added to the dish, and the combined mass was recorded. The dish with the soil sample was then placed in an oven set at a constant temperature of around 105°C for 24 hours to evaporate all moisture. After drying, the dish was transferred to a desiccator to cool, preventing moisture absorption from the air. Once cooled, the dish with the dried soil was weighed, and the mass was recorded. The moisture content was calculated based on the weight difference before and after drying. Control measures include ensuring the oven maintains a constant temperature, using a desiccator to prevent rehydration, and conducting multiple trials to verify consistency. These steps ensure accurate measurement of soil moisture content, which is crucial for assessing soil health and the effectiveness of phytoremediation processes.

AAS Test: The identification of heavy metal absorption by plant species was conducted using Atomic Absorption Spectroscopy (AAS), a widely employed analytical technique for quantifying metal elements. An AA500 instrument was utilized for the AAS test. For the AAS test, soil samples were collected from the desired location using appropriate sampling techniques to ensure representativeness and capture the variability in soil composition. The collected samples were then air-dried to remove moisture and any volatile organic compounds, with the option to grind the dried samples into a fine powder for homogeneity. Then, the soil samples underwent a digestion process, where a measured amount was transferred into a digestion vessel, and an appropriate digestion method, such as acid digestion, was chosen to extract the metals from the solid matrix into a solution for analysis. Calibration standards were prepared by diluting known concentrations of metal standard solutions, covering the expected concentration range of the metals in the soil samples. The AAS instrument was calibrated using these standards, establishing a linear relationship between the absorbance of metal ions and their concentrations. Specific absorption wavelengths for the metals of interest were selected, and instrument parameters were optimized for sensitivity and resolution. The absorbance of calibration standards and digested soil sample solutions was measured using the AAS instrument, and concentrations of metals in the soil samples were calculated based on the calibration curve obtained from the standards. The analysis was repeated for each metal of interest, and quality control checks, including blank measurements and replicate analyses, were performed to ensure the accuracy and precision of the results. By following these rigorous procedures, accurate data on the levels of heavy metals in soil samples was collected using Atomic Absorption Spectroscopy (AAS), therefore determining the specific metals absorbed by the plant.

RESULTS AND DISCUSSION

pH Test

Phytoremediation, using plants to remove, degrade, or stabilize contaminants in the environment, often involves various mechanisms that can influence soil pH. The pH measurements conducted after the phytoremediation process provide valuable insights into the effectiveness of these plant species in remediation efforts and their potential impact on soil conditions. Table 1 shows, that the consistent pH values observed for tulsi and Aloe vera before and after the phytoremediation process (both at 7.1) suggest that these plant species maintain their near-neutral pH preferences even after exposure to contaminated soil. This resilience in pH levels indicates the stability of their physiological processes and their capacity to thrive in environments with potentially elevated levels of contaminants. The pH of vetiver remaining at 7.2 post-phytoremediation indicates that this plant species was able to maintain the alkaline pH of the soil. Vetiver is well-known for its ability to tolerate and even thrive in alkaline soil conditions. Its root system, which can penetrate deeply into the soil, facilitates the uptake and sequestration of contaminants while also contributing to soil stabilization and pH regulation. The consistent pH level of 7.2 suggests that vetiver effectively mitigated any potential soil acidification resulting from the phytoremediation process. The stable pH levels observed across all three plant species post-phytoremediation reflect the effectiveness of these plants in facilitating the remediation of contaminated soil without causing significant alterations to soil pH. This is crucial for maintaining the overall health and fertility of the soil ecosystem, as fluctuations in pH can impact nutrient availability, microbial activity, and plant growth. Furthermore, the ability of these plants to maintain their pH preferences underscores their suitability for phytoremediation applications in various environmental settings. By selecting plant species compatible with the existing soil conditions and can thrive throughout the remediation process, phytoremediation efforts can achieve sustainable and long-lasting results while minimizing adverse effects on soil quality. Hence, it is inferred that the stable pH values seen in vetiver, tulsi, and aloe vera throughout the phytoremediation process demonstrate their potential as excellent phytoremediators for polluted soil while maintaining soil health and integrity.

Moisture Content Test

Moisture content is a crucial parameter in assessing soil health and plant performance, particularly in phytoremediation, where plants are utilized to mitigate soil contaminants.

Table 1: pH test results on soil.

S. No.	Plant name	Metal name	Before Contamination	After Contamination	After Phytoremediation (4 weeks later)
1.	Vetiver	Zn	7	8	7.2
2.	Tulsi	Zn	7	8.2	7.1
3.	Aloe Vera	Zn	7	8.2	7.1
4.	Vetiver	Pb	7	8.1	7.1
5.	Tulsi	Pb	7	8	7.1
6.	Aloe Vera	Pb	7	8.2	7.3
7.	Vetiver	Cd	7	8	7.2
8.	Tulsi	Cd	7	8.3	7.2

Table 2: Moisture Content after one month of plantation.

S. no.	Metal	Plant name	Empty wt. of tier (g)	Wt. of tier with wet soil (g)	Wt. of tier with dry soil (g)	Moisture content (%)
1.	Zn	Vetiver	7	12	11	25
2.	Zn	Tulsi	6	13	12	16.67
3.	Zn	Aloe vera	7	19	17	20
4.	Pb	Vetiver	6	14	12	33.3
5.	Pb	Tulsi	11	18	17	16.67
6.	Pb	Aloe vera	12	24	22	20
7.	Cd	Vetiver	7	13	12	20
8.	Cd	Tulsi	7	14	13	16.67

The moisture content measurements obtained after the phytoremediation process offer insights into the soil's water retention capacities and the physiological responses of the plant species involved.

Vetiver: Vetiver, with a moisture content ranging from 20% to 25%as shown in Table 2, demonstrates a moderate level of water retention capacity following the phytoremediation process. This moisture range indicates adequately hydrated soil, essential for sustaining plant growth and facilitating the biochemical processes involved in remediation. Vetiver's fibrous root system, known for its ability to absorb water and nutrients efficiently, likely contributed to maintaining optimal moisture levels in the soil while aiding in contaminant uptake and stabilization.

Tulsi: Tulsi, with a moisture content of 15-17% as shown in Table 2, exhibits a slightly lower moisture range compared to vetiver. However, this moisture level is still acceptable for supporting plant growth and remediation activities. Tulsi is recognized for its drought tolerance and adaptive capacity, enabling it to thrive in diverse soil conditions. Despite the lower moisture content, tulsi's resilience may have contributed to its effective participation in the phytoremediation process, albeit with slightly reduced water availability.

Aloe Vera: Aloe vera, displaying the highest moisture content ranging from 25% to 35%, indicates a significant water retention capacity in the soil post-phytoremediation. Aloe vera is renowned for its succulent leaves and water-storing capabilities, making it well-suited for arid environments and water-stressed conditions. The elevated moisture content observed in the soil associated with Aloe vera suggests efficient water uptake and retention by the plant, which may have facilitated its remediation performance while ensuring its own hydration needs.

The variation in moisture content among the three plant species reflects their distinct physiological characteristics and adaptive strategies in response to environmental conditions. Thus, the moisture content measurements obtained after the phytoremediation process reflect the role of vetiver, tulsi, and aloe vera in maintaining soil moisture balance while actively participating in the remediation of contaminated soil. By effectively managing water availability and uptake, these plant species contribute to sustainable soil restoration efforts and ecosystem resilience in contaminated environments.

AAS Test

Soil-metal remediation: The Atomic Absorption Spectroscopy (AAS) content test results for zinc (Zn), lead (Pb), and cadmium (Cd) concentrations in the contaminated soil before and after the phytoremediation process, using tulsi

Table 3: Soil Contamination with various metals.

S.No.	Metals	Plant	Contaminated soil Mg/kg	After phytoremediation process Mg /kg
1.	Zn	Tulsi	300	188.3
2.	Zn	Vetiver	300	179.3
3.	Zn	Aloe vera	300	158.3
4.	Pb	Tulsi	600	188.3
5.	Pb	Vetiver	600	164.3
6.	Pb	Aloe vera	600	179.6
7.	Cd	Tulsi	80	18.62
8.	Cd	vetiver	80	17.62

(Ocimum sanctum), vetiver (*Chrysopogon zizanioides*), and aloe vera, are summarized in Table 3.

Zinc remediation in soil: All three plant species, vetiver, and aloe demonstrated considerable efficacy in reducing Zn concentrations in the soil post-phytoremediation. Tulsi exhibited the highest reduction, with Zn levels decreasing from 300 mg/kg to 188.3 mg/kg as shown in Fig. 2. This reduction indicates the effectiveness of tulsi in remediation, as it absorbed and accumulated zinc within its tissues. Tulsi possesses efficient mechanisms for heavy metal uptake and translocation, making it effective in reducing the bioavailability of zinc in the soil. The decrease in zinc concentration post-phytoremediation suggests that Tulsi successfully remediated the contaminated soil, thereby mitigating the potential adverse effects of zinc pollution on the environment. Vetiver and aloe vera also significantly reduced Zn concentrations to 179.3 mg/kg and 158.3 mg/kg, respectively. Vetiver's extensive root system, characterized by deep penetration and high surface area, enables effective uptake and accumulation of heavy metals like zinc.

While the reduction in zinc concentration is substantial, it indicates that vetiver contributed significantly to the remediation process, albeit to a slightly lesser extent compared to tulsi. Aloe vera also demonstrated remediation potential, although its efficiency in reducing zinc concentration appeared to be lower compared to tulsi and vetiver. Aloe vera's succulent leaves and efficient water uptake mechanisms contribute to its ability to absorb and accumulate heavy metals. While the reduction in zinc concentration is notable, it suggests that Aloe vera may have a lower uptake capacity or affinity for zinc compared to tulsi and vetiver. However, Aloe vera's contribution to remediation should not be discounted, as even marginal reductions in heavy metal concentrations can significantly improve soil quality and environmental health. These results show the potential of these plant species to uptake and accumulate Zn, thereby mitigating its adverse effects on soil and environmental health. Each plant species exhibited varying remediation efficiency, with tulsi demonstrating the highest efficacy followed by vetiver and aloe vera.

Lead remediation in soil: Similar to Zn, all three plant species exhibited notable remediation capabilities for Pb contamination. Tulsi, vetiver, and aloe vera reduced Pb concentrations from 600 mg/kg to 188.3 mg/kg, 164.3 mg/kg, and 179.6 mg/kg, as shown in Fig. 3 respectively. Following the intervention of tulsi, the Pb concentration significantly decreased to 188.3 mg/kg. This remarkable reduction indicates the high remediation efficiency of tulsi in mitigating Pb contamination. Tulsi possesses mechanisms for Pb uptake, translocation, and accumulation within its tissues, thereby effectively removing Pb from the soil matrix. The substantial decrease in Pb concentration post-phytoremediation highlights the effectiveness of tulsi as a potential candidate for Pb remediation in contaminated sites. Vetiver also exhibited notable remediation capabilities for Pb contamination. The Pb concentration in the soil decreased from 600 mg/kg to 164.3 mg/kg after the phytoremediation process involving vetiver. Vetiver's extensive root system, characterized by deep penetration and high surface area, facilitates the uptake and sequestration of Pb from the soil. The significant reduction in Pb concentration reflects the effectiveness of vetiver in immobilizing Pb and reducing its bioavailability in the soil, thereby minimizing potential risks to human health and the environment. Aloe vera demonstrated efficacy in reducing Pb concentrations in the contaminated soil, albeit slightly less than tulsi and vetiver. The Pb concentration decreased from 600 mg/kg to 179.6 mg/kg following the phytoremediation process involving Aloe vera. Aloe vera's succulent leaves and efficient water uptake mechanisms contribute to its ability to absorb and accumulate

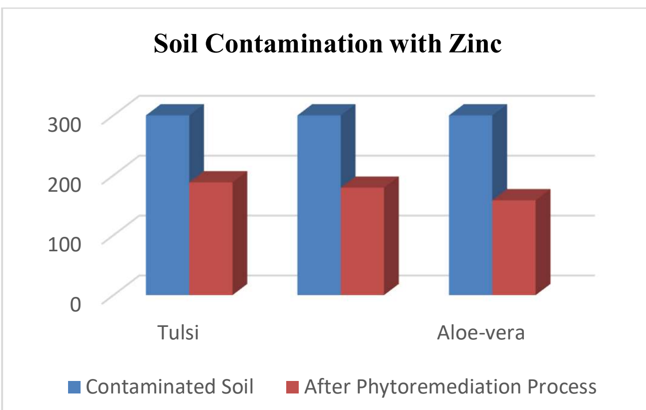

Fig. 2: AAS test results of soil contaminated with zinc.

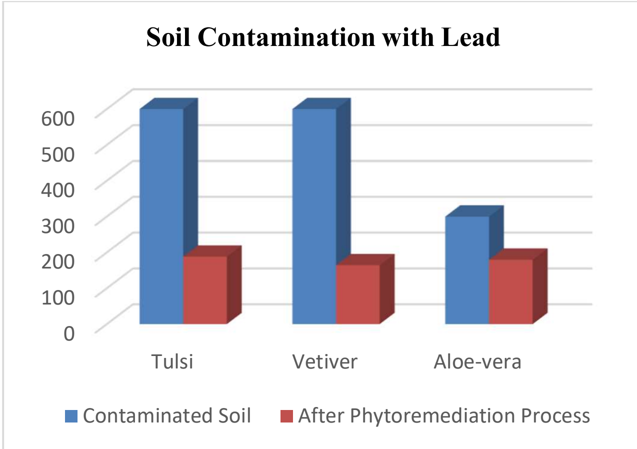

Fig. 3: AAS test results of soil contaminated with Lead.

Pb, thereby aiding in its remediation potential. While the reduction in Pb concentration is significant, it suggests that Aloe vera may have a lower uptake capacity or affinity for Pb compared to tulsi and vetiver. The efficacy of tulsi in Pb remediation is particularly noteworthy, indicating its strong affinity for Pb uptake and sequestration. Vetiver and aloe vera also demonstrated substantial reductions in Pb levels, further highlighting their potential for phytoremediation of Pb-contaminated soils.

Cadmium remediation in soil: Before the phytoremediation process, the Cd concentration in the soil was recorded as 80 mg/kg. Following the intervention of Tulsi, the Cd concentration significantly decreased to 18.62 mg/kg as shown in Fig. 4. This substantial reduction indicates the high remediation efficiency of Tulsi in mitigating Cd contamination. Tulsi possesses mechanisms for Cd uptake, translocation, and accumulation within its tissues, thereby effectively removing Cd from the soil matrix. The significant decrease in Cd concentration post-phytoremediation highlights the effectiveness of Tulsi as a potential candidate for Cd remediation in contaminated sites. Similarly, vetiver demonstrated notable remediation capabilities for Cd contamination. The Cd concentration in the soil decreased from 80 mg/kg to 17.62 mg/kg after the phytoremediation process involving vetiver. Vetiver's extensive root system, characterized by deep penetration and high surface area, facilitates the uptake and sequestration of Cd from the soil. The significant reduction in Cd concentration reflects the effectiveness of vetiver in immobilizing Cd and reducing its bioavailability in the soil, thereby minimizing potential risks to human health and the environment. These findings suggest that both tulsi and vetiver are capable of effectively sequestering Cd, thereby mitigating its environmental impact. The significant reduction in Cd levels underscores the importance of utilizing these plant species in Cd-contaminated soil remediation efforts.

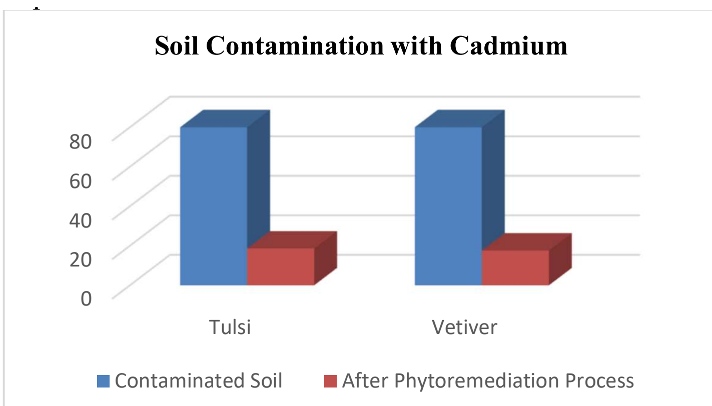

Fig. 4: AAS test results of soil contaminated with cadmium.

Plant-Metal Accumulation

a) Tulsi:

- Zinc (Zn): Table 4 suggests tulsi accumulated a moderate amount of zinc, with a concentration of 44.92 mg/kg. This suggests that tulsi can uptake and store zinc from the soil, contributing to its potential role in phytoremediation efforts targeting zinc pollution.
- Cadmium (Cd): The concentration of cadmium in tulsi was below the quantification limit (BQL), indicating that it either did not absorb significant amounts of cadmium from the soil or accumulated it below detectable levels. This could suggest that tulsi may not be as effective in accumulating cadmium compared to other metals.
- Lead (Pb): Tulsi accumulated a relatively high concentration of lead, with a value of 66.32 mg/kg. This indicates tulsi's ability to uptake and sequester lead from the soil, making it potentially useful for phytoremediation of lead-contaminated environments.

b) Vetiver:

- Zinc (Zn): Vetiver accumulated a relatively lower concentration of zinc compared to tulsi, with a value of 19.62 mg/kg. While lower, this still indicates the vetiver's capacity to uptake and store zinc, albeit to a lesser extent.
- Cadmium (Cd): Similar to tulsi, vetiver's cadmium concentration was below the quantification limit (BQL), suggesting limited accumulation or accumulation below detectable levels.
- Lead (Pb): Vetiver accumulated a moderate concentration of lead, with a value of 46.9 mg/kg. This indicates its potential for phytoremediation of lead-contaminated soils, although it may be less effective than tulsi in this regard.

c) *Aloe vera*:

- Zinc (Zn): Aloe vera accumulated a significantly higher concentration of zinc compared to both tulsi and vetiver, with a value of 176.3 mg/kg. This suggests that Aloe vera has a strong affinity for zinc uptake and may be highly effective in phytoremediation of zinc-contaminated environments.
- Cadmium (Cd): Similar to tulsi and vetiver, Aloe vera's cadmium concentration was below the quantification limit (BQL), indicating limited accumulation or accumulation below detectable levels.

Table 4: Phytoremediation of plant species.

S.No.	Plant	Metals	Amount of metal absorbed
1.	Tulsi	Zn	44.92
2.	Tulsi	Cd	BQL(LOQ:0.1)
3.	Tulsi	Pb	66.32
4.	vetiver	Zn	19.62
5.	Vetiver	Cd	BQL(LOQ:0.1)
6.	vetiver	Pb	46.9
7.	Aloe vera	Zn	176.3
8.	Aloe vera	Cd	BQL(LOQ:0.1)
9.	Aloe vera	Pb	11.3

- Lead (Pb): Aloe vera accumulated a relatively low concentration of lead, with a value of 11.3 mg/kg. While lower than tulsi and vetiver, this still suggests Aloe vera's potential for phytoremediation of lead-contaminated soils, particularly in conjunction with its other beneficial properties.

Overall, the AAS test results highlight the varying metal accumulation capacities of tulsi, vetiver, and Aloe vera, indicating their potential roles in phytoremediation efforts targeting specific heavy metal pollutants.

CONCLUSIONS

Revegetating heavy metal-polluted land using phytoremediation offers a promising and sustainable solution to mitigate environmental contamination while promoting ecosystem restoration. Through the utilization of metal-accumulating plants, known as hyperaccumulators, and the natural processes of phytoextraction, rhizofiltration, and phytostabilization, phytoremediation effectively removes, stabilizes, or transforms heavy metals in contaminated soils, reducing their bioavailability and potential ecological and human health risks. The phytoremediation project utilizing tulsi (*Ocimum sanctum*), vetiver (*Chrysopogon zizanioides*), and Aloe vera (*Aloe barbadensis*) have yielded promising results in addressing soil contamination. Through this innovative approach, these plant species have demonstrated their effectiveness in mitigating heavy metal pollution while contributing to overall soil health and ecosystem restoration. Hence, it was concluded that:

- **pH Stability:** The consistent pH values observed for tulsi, Aloe vera, and vetiver before and after the phytoremediation process indicate their resilience in maintaining near-neutral or alkaline soil pH levels. This stability is crucial for preserving soil health and supporting plant growth in contaminated environments.
- **Moisture Regulation:** Vetiver, tulsi, and Aloe vera

exhibited varying moisture content levels post-phytoremediation, reflecting their adaptive strategies to environmental conditions. Vetiver's moderate moisture retention, tulsi's drought tolerance, and Aloe vera's water-storing capabilities contribute to soil moisture balance and plant health during remediation processes.

- **Metal Remediation:** The phytoremediation process effectively reduced zinc, lead, and cadmium concentrations in contaminated soil. Tulsi demonstrated the highest efficacy in reducing metal concentrations, followed by vetiver and Aloe vera. These findings highlight the potential of these plant species in mitigating heavy metal pollution and restoring soil quality.
- **Metal Accumulation:** Tulsi, vetiver, and Aloe vera exhibited varying capacities for metal accumulation, with Tulsi accumulating the highest concentrations of zinc and lead. These differences in metal accumulation profiles underscore the importance of selecting appropriate plant species based on specific remediation requirements and metal pollutants present in the soil.
- **Soil Health and Ecosystem Restoration:** The findings suggest that phytoremediation not only mitigates heavy metal pollution but also contributes to soil health and ecosystem restoration. By stabilizing soil pH, regulating moisture content, and reducing heavy metal concentrations, tulsi, vetiver, and Aloe vera play a pivotal role in improving soil fertility, promoting plant growth, and enhancing biodiversity in polluted environments.
- **Long-Term Viability:** The long-term viability of phytoremediation as a sustainable remediation strategy is underscored by the resilience of the selected plant species and their ability to thrive in contaminated soil conditions. However, ongoing research is needed to assess the persistence of remediation effects over time and to address any potential limitations or challenges associated with the prolonged use of phytoremediation in diverse environmental settings.

Thus, the stable pH levels, effective moisture regulation, and significant metal remediation capabilities exhibited by tulsi, vetiver, and Aloe vera demonstrate their potential as efficient phytoremediators for polluted soil environments, while also emphasizing the need for tailored plant selection in remediation efforts. Future research directions in this field are crucial for advancing our understanding and optimizing the efficacy of phytoremediation. Critical areas for further investigation can include:

- **Enhancing Remediation Efficiency:** Research efforts should focus on identifying and optimizing the factors influencing the remediation efficiency of phytoremediation, such as plant selection, soil conditions, and pollutant concentrations. Developing tailored approaches and employing genetic engineering techniques to enhance the metal uptake and tolerance of plants can improve remediation outcomes.
- **Understanding Plant-Metal Interactions:** Further studies are needed to elucidate the mechanisms underlying plant-metal interactions, including metal uptake, translocation, and sequestration within plant tissues. Investigating the physiological and biochemical processes involved in metal accumulation and detoxification can inform the selection of hyperaccumulator species and the development of strategies to enhance metal remediation.
- **Long-Term Monitoring and Evaluation:** Longitudinal studies are essential to assess the long-term effectiveness and sustainability of phytoremediation over extended periods. Monitoring changes in soil quality, plant health, and ecosystem dynamics over time can provide insights into the persistence of remediation effects and potential ecological impacts, guiding management decisions and remediation strategies.
- **Integration with Sustainable Land Management Practices:** Future research should explore the integration of phytoremediation with other sustainable land management practices, such as agroforestry, biochar application, and soil amendment techniques. Evaluating the synergistic effects of combining phytoremediation with these practices can enhance remediation outcomes, improve soil fertility, and promote ecosystem resilience.

In conclusion, the practical significance of phytoremediation lies in its potential to address heavy metal pollution in soil effectively while promoting environmental sustainability as presented in this research work. By advancing our understanding of plant-soil interactions, optimizing remediation strategies, and integrating phytoremediation with sustainable land management practices, future research can contribute to harnessing the full potential of this eco-friendly remediation technique for environmental restoration and protection.

STATEMENT OF PERMISSION

This research project involves the collection and study of plant species including aloe vera (*Aloe barbadensis*), vetiver (*Chrysopogon zizanioides*), and tulsi (*Ocimum sanctum*). The collection of these plant specimens has been conducted in accordance with all applicable regulations and permissions.

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