Jordan Journal of Civil Engineering, Volume **19**, No. **4**, **2025**
DOI: https://doi.org/10.14525/JJCE.v19i4.03

Jordan Journal of Civil Engineering

Journal homepage: https://jjce.just.edu.jo

Removal of Heavy Metal Contamination from Soil Using Vetiver Grass

***Jiji Krishnan* [1)*]*, Ammu Dileep* [1)]*, Rena Farzana* [1)]*, S., Ruksana Faizal* [1)]*, Siyana Siyad* [1)]**

[1)] *Carmel College of Engineering and Technology, Alappuzha, India. * Corresponding Author. E-Mail: jijiktu@gmail.com*

ARTICLE INFO

Article History:
Received: 3/2/2025
Accepted: 7/6/2025

ABSTRACT

The current study explores the phyto-remediation potential of vetiver grass (*Chrysopogon zizanioides*) for mitigating heavy metal contamination in soil through pot and column leaching experiments. In 60-day pot trials, soil samples collected at intervals of 30, 45, and 60 days revealed significant reductions in Iron (Fe), Zinc (Zn), Copper (Cu), and Lead (Pb), with Fe showing the highest reduction. Metal uptake analysis showed higher accumulation in vetiver roots than in leaves, with Zn (58.78 mg/kg) and Cu (38.64 mg/kg) exhibiting the highest concentrations. The BCF exceeded 1 for Zn (1.95) and Cu (1.29), indicating effective root uptake, while Pb remained below detection limits in plant tissues. The translocation factor (TF) for all metals was below 1, confirming limited movement from roots to shoots and showing vetiver's suitability for phyto-stabilization, effectively locking metals in roots to prevent ecosystem spread. Vetiver's root-dominant uptake and low TF highlight its suitability for phyto stabilization in industrial soils. In the column leaching experiment, Vetiver grass was compared with *Triticum aestivum* (wheat) as a control. The leachate from vetiver-planted columns showed markedly reduced concentrations of Zn and Cu, demonstrating vetiver's effectiveness in promoting downward metal mobility and stabilizing contaminated soils. These results highlight the potential of vetiver grass as an effective phyto-remediation species, particularly for Zn and Cu, and recommend further research into optimizing uptake through soil amendments and genetic enhancements.

Keywords: Vetiver grass, Phyto-remediation, Heavy metals, Bio-concentration factor (BCF), Inductively coupled plasma mass spectrometry (ICP-MS).

INTRODUCTION

Heavy metal contamination in soil has become a critical environmental issue, particularly in industrial regions, where the accumulation of metals, such as Lead (Pb), Cadmium (Cd), and Zinc (Zn) poses significant threats to ecosystems, agricultural productivity, and human health. Elevated concentrations of these metals degrade soil by altering its physical and chemical properties, leading to reduced fertility, poor soil structure, and impaired water and nutrient retention (Chandra & Kumar, 2023; Gregory et al., 2015; Lwin et al., 2018; Nguyen et al., 2023; Pande et al., 2022; Wuana et al., 2011). Soil micro-organisms, which play an essential role in nutrient cycling and organic matter decomposition, are also adversely affected by metal toxicity, leading to reduced microbial activity and impaired soil processes (Angon et al., 2024; Zeng et al., 2024). Beyond the soil, heavy metals can leach into groundwater or contaminate surface water through run-off, posing risks to aquatic ecosystems and human health due to bio-accumulation in food chains (Maurya et al., 2019; Saravanan et al., 2024; Xu et al., 2024).

Among available remediation strategies, phyto-remediation has emerged as a sustainable and widely accepted technique (Iqbal et al., 2018; Mocek-Płóciniak et al., 2023; Nedjimi, 2021a; Yan et al., 2020). Vetiver grass (Chrysopogon zizanioides), known for its extensive root system and high tolerance to metal toxicity, has emerged as a promising candidate for the phyto-remediation of contaminated soils (Banerjee et al., 2016a, 2019; Dorafshan et al., 2023a; Holanda et al., 2022; Nguyen et al., 2023; Roychowdhury et al., 2020). It is particularly effective in absorbing and stabilizing heavy metals, thereby reducing their mobility and bio-availability. In parallel, the leaching of contaminants through water movement plays a crucial role in metal transport in soils, particularly under high-rainfall or irrigated conditions. Thus, assessing phyto-remediation efficiency under controlled leaching scenarios is essential for developing comprehensive remediation solutions (Bijay-Singh & Craswell, 2021; Briffa et al., 2020; Hou et al., 2020; Rashid et al., 2023; Wuana et al., 2011).

Heavy-metal pollution from industrial activities, mining, and agricultural practices has led to widespread soil and water contamination, posing threats to ecosystems and human health. Metals, such as Cadmium (Cd), Lead (Pb), Copper (Cu), Nickel (Ni), and Zinc (Zn), persist in the environment, bio-accumulate in organisms, and cause adverse health effects, including neuro-toxicity and organ damage (Alzboon & Alzboon, 2022; Angon et al., 2024; Mitra et al., 2022; Nedjimi, 2021; Odat & Alshammari, 2011; Rashid et al., 2023; Wuana et al., 2011; Yaseen & Al-Hawari, 2015). Remediating heavy metal-contaminated sites is a priority, and phyto-remediation has emerged as an eco-friendly, cost-effective solution (Awasthi et al., 2022; Priya et al., 2023; Tan et al., 2023).

Phyto-remediation involves the use of plants to extract, stabilize, or immobilize heavy metals from contaminated environments. It is considered a sustainable alternative to traditional methods, like soil excavation and chemical treatments due to its minimal environmental disturbance, aesthetic appeal, and lower operational costs. Vetiver grass (*Chrysopogon zizanioides*) and Indian mustard (*Brassica juncea*) are two plants that have shown great potential for phyto-remediation because of their adaptability, high biomass production, and tolerance to various heavy metals (Chandra & Kumar, 2023; Deng et al., 2024).

Several studies have demonstrated the phyto-remediation potential of vetiver grass and Indian mustard in removing heavy metals from soils and water. Many researchers (Chen et al., 2004a; Dorafshan et al., 2023a; Nedjimi, 2021a; Yan et al., 2020) highlighted the effectiveness of vetiver grass, with its deep root system, in stabilizing heavy metals and preventing their leaching into groundwater, while Indian mustard, a known hyper-accumulator, efficiently extracts metals from agricultural lands. Many investigators (Liu et al., 2024; Nepal et al., 2024; Otunola et al., 2022) also found that soil amendments, like attapulgite and bentonite, further enhanced vetiver's uptake of metals, such as Nickel, Chromium, and Cobalt, although Indian mustard showed limited response to these amendments. Jampasri & Saeng-Ngam (2019) demonstrated that both *Vetiveria nemoralis* and *V. zizanioides* tolerated Cadmium and salinity well, with *V. nemoralis* proving a better Cadmium accumulator in saline conditions, showing that vetiver grass can stabilize Cadmium-contaminated soils in challenging environments. Further research by (Gravand et al., 2021) demonstrated vetiver grass's ability to remove Lead (Pb), Cadmium (Cd), Manganese (Mn), and Nickel (Ni) from contaminated soils. Many researchers revealed that vetiver grass absorbed significant amounts of these metals, particularly Lead, with higher concentrations accumulating in the roots than in the shoots. The biological concentration factor (BCF) was greater than one for all metals, confirming vetiver grass's effectiveness in stabilizing heavy metals in contaminated soils. While prior studies established vetiver's ability to absorb heavy metals, this work advances the field by (1) quantifying its efficacy in multi-metal industrial soils, (2) demonstrating its role in reducing leaching risks, and (3) identifying time-dependent uptake patterns critical for remediation planning.

While vetiver grass shows promising results, its phyto-remediation efficiency can be improved through soil amendments or the application of chelating agents. For example, Ng et al. (2019) explored the use of ethylenediaminetetraacetic acid (EDTA) to enhance Lead uptake by vetiver grass. While EDTA significantly increased the translocation of Lead from roots to shoots, it also posed environmental risks, as EDTA-heavy metal complexes may leach into groundwater. This highlights the need for caution when using chemical amendments in phyto-remediation.

Recent advancements in phyto-remediation have focused on the role of rhizosphere microbiota and genetic engineering. Many researchers (Ali et al., 2013; Alves et al., 2022; Marques et al., 2009; Sahoo et al., 2024) reviewed the potential of rhizosphere micro-organisms to enhance plant tolerance and metal uptake. These micro-organisms can solubilize heavy metals and improve phyto-extraction by interacting with plant roots. Genetic engineering is another area being explored to develop plants with enhanced metal-accumulation traits. Vetiver grass is a highly promising plant for the remediation of heavy metal-contaminated soil due to its adaptability, tolerance to metal stress, and extensive root system. However, optimizing its phyto-remediation efficiency remains essential. The current study focuses on evaluating the phyto-remediation potential of vetiver grass (*Chrysopogon zizanioides*) in reducing heavy metal contamination in soils near an industrial area in Cherthala South, Alappuzha District, Kerala.

MATERIALS AND SOIL COLLECTION

Location of Study Area

Soil samples were collected from the vicinity of an industrial facility located in Cherthala South, Alappuzha District, Kerala. The site is situated at Latitude 9.63234° N and Longitude 76.33378° E, near National Highway-66. The facility is one of the largest foundry units in south India, involved in the production of ductile Iron, grey Iron, and steel castings.

Soil Samples

Soil samples collected from the vicinity of the industrial area revealed elevated concentrations of heavy metals, such as Lead (Pb), Iron (Fe), Copper (Cu), and Zinc (Zn), all exceeding WHO permissible limits. A systematic grid-based sampling pattern (e.g. 5 m × 5 m) was employed to ensure spatial representation across the study area. Sampling was conducted at a depth of 15 cm-30 cm to capture soil conditions relevant to root development and contaminant distribution. All samples were placed in polyethylene zip lock bags, properly labeled, and transported to the laboratory. In the laboratory, soils were air-dried, sieved through a 2-mm mesh, and stored for further analysis. The basic soil properties of the soil used in the current study are given in Table 1.

Table 1. Soil properties

Property	Result
% gravel (>4.75mm)	3.4%
% coarse sand (4.72-2.36mm)	9.5%
% medium sand (2.36-0.425mm)	32.3%
% fine sand (0.425-0.075mm)	45.7%
% fines (silt and clay <0.075mm)	9.1%
Uniformity coefficient $C_u = \frac{D_{60}}{D_{10}}$	0.045
Coefficient of curvature $C_C = \frac{{D_{30}}^2}{D_{60} \times D_{10}}$	2.125
Coefficient of permeability ($^{cm}/_{sec}$)	0.271
Specific gravity	1.9

This methodology ensured representative and consistent soil sampling for evaluating the effectiveness of phyto-remediation. Elevated levels of these heavy metals pose toxicity risks to plants, animals, and soil micro-organisms, adversely affecting soil health and ecosystem functionality. High concentrations of these metals can disrupt nutrient uptake in plants, resulting in deficiencies and reduced agricultural productivity. Moreover, these metals can bio-accumulate in plant and animal tissues, potentially leading to bio-magnification through the food chain, posing significant health risks to higher trophic organisms, including humans. Overall, the excessive presence of Lead, Iron, Copper, and Zinc in soil can have severe environmental and public health consequences, underscoring the urgent need for remediation efforts to reduce contamination and mitigate associated risks. The original metal concentrations in the soil are mentioned in Table 2.

Table 2. Original metal concentrations

Metal	Permissible Limit (mg/kg) (WHO standards)	Metal Concentration (mg/kg)
Lead (Pb)	2	7.52
Iron (Fe)	425.5	57677
Copper (Cu)	10	92.8
Zinc (Zn)	0.6	122.60

Vetiver Grass

Chrysopogon zizanioides, commonly known as vetiver, is a perennial bunchgrass of the family *Poaceae*. Figure (1) shows a vetiver plant, which is a large, tufted bunchgrass and can reach up to 1.5 meters (5 feet) in height.

Figure (1): Vetiver plant, a large, tufted bunchgrass

The thin leaves and stems are erect and rigid, and the plant bears small brown-purple flowers in long spikes. The fragrant roots grow downward in the soil and can attain depths of more than 3 meters (10 feet). More details of the vetiver grass are listed in Table 3.

Table 3. Vetiver grass essential plant details

Taxonomic Classification	Details
Kingdom	Plantae
Phylum	Angiosperms
Class	Monocots
Order	Poales
Family	Poaceae
Genus	Chrysopogon
Species	*Chrysopogon zizanioides*

EXPERIMENTAL METHODS

Pot Experiment

Vetiver grasses (*Chrysopogon zizanioides*) were initially established in soil for approximately one month before being transplanted into three separate pots for a duration of 60 days under natural climatic conditions. The 60-day period was selected based on prior evidence of vetiver’s peak metal uptake during early growth (Goren et al., 2021) and practical constraints of pot/column experiments. Following the transplantation of vetiver grass, the plants were monitored at intervals of 30, 45, and 60 days to assess their growth and health.

Soil samples were collected from each pot at these specified intervals and subjected to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis to evaluate changes in the concentration patterns of heavy metals over time. This analytical technique allows for the precise quantification of heavy metals in the soil, providing insights into the effectiveness of Vetiver grass in remediating heavy metal contamination. Soil samples were air-dried, sieved (2 mm), and digested with HNO_3:$HClO_4$ (5:1) *via* micro-wave-assisted digestion (180°C, 30 min.). Filtered digests were analyzed by ICP-MS with internal standardization and calibration of the apparatus. At each interval (30, 45, 60 days), ~100 g of soil was collected from the top 15 cm of each pot, homogenized to form a composite sample, and analyzed *via* ICP-MS.

Figure (2) illustrates the experimental setup for the pot experiment, showcasing the arrangement of pots and the environmental conditions maintained throughout the study. The systematic monitoring and sampling process aimed to elucidate the relationship between the growth of vetiver grass and the reduction of heavy-metal concentrations in the soil, thereby contributing to our understanding of phyto-remediation dynamics.

Figure (2): Pot experiment setup

Each pot used in the experiment measured approximately 65 cm in diameter and 35 cm in height, capable of holding an estimated 20 kg of soil, as observed in the setup shown in the pot experiment setup (Figure (2)). Soil was filled up to around 5 cm below the rim to allow for irrigation. One vetiver plant was transplanted per pot, maintaining a uniform plant density across all test conditions. The experimental layout was arranged in an open rooftop space with natural light exposure and monitored to maintain consistent moisture through periodic watering.

Throughout the 60-day experiment, the pots were maintained under natural daylight conditions on an open rooftop. Plants received an average of 11-12 hours of sunlight per day, with ambient temperatures ranging from 27°C to 33°C, which contributed to natural humidity. To ensure consistent moisture levels, manual watering was carried out once every two days, supplying approximately 500 mL-700 mL of water per pot, depending on rainfall and soil moisture status. Care was taken to avoid both waterlogging and drought stress, ensuring optimal conditions for vetiver growth and metal uptake.

Column Leaching Experiment

Initially, vetiver grass (*Chrysopogon zizanioides*) was planted in soil for a duration of approximately one month before being transplanted into three soil columns. Additionally, two control groups containing triticum (wheat) were established for comparative analysis. The experimental setup for the column leaching process is illustrated in Figure (3). Each soil leaching column was vegetated with one vetiver seedling (20-30 cm tall, roots pre-rinsed) planted centrally. Roots grew through the mesh into the gravel layer, simulating natural metal uptake and stabilization. On the 45th day of the experiment, three soil columns with vetiver grass were subjected to water drenching to facilitate leaching. In contrast, the plants in the control group demonstrated stunted growth after two weeks, indicating their inability to thrive under metal-stressed conditions.

After the 60th day, an additional water application was performed on the three soil columns to further induce leaching. Leachate solutions were subsequently collected from the bottom of the columns, and soil samples were analyzed for heavy metal concentrations using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Soil Extraction and Plant Harvesting

Soil samples from the pots were collected at 30, 45, and 60 days and subsequently analyzed for elemental composition using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). This technique enabled the precise quantification of heavy metals and other elements present in the soil, facilitating a comprehensive assessment of changes in metal concentrations over time.

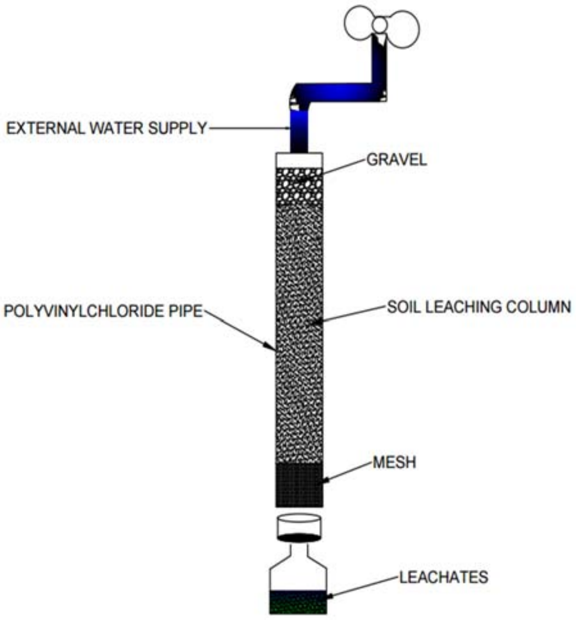

Figure (3): Soil column setup

In parallel, soil samples from the column experiment were analyzed to assess vetiver's dual role in (1) immobilizing metals (root uptake) and (2) limiting their leaching into effluent under saturated conditions.

After 60 days, the plants from the column experiment were harvested, and the plant parts were carefully separated into roots and leaves for further analysis. After harvesting, the roots and leaves of vetiver grass were separated, oven-dried at 60°C for 48 hours, and weighed to determine their dry biomass. The average dry weights per plant were 12.5 g for roots and 8.3 g for leaves. These values were used to scale metal concentrations (from ICP-OES analysis) to total metal uptake per plant for mass balance calculations. 1-g sub-samples were digested for ICP-OES analysis. This division allows for a detailed examination of metal uptake and translocation within the vetiver grass, providing insights into the plant's efficiency in accumulating heavy metals from the soil. By analyzing both the roots and leaves, we can better understand the dynamics of metal absorption and the overall phyto-remediation potential of vetiver grass in contaminated environments.

Digestion of Sample

The digestion of samples was conducted at Cochin Test House (accredited by NABL, India; ISO/IEC 17025:2017). The roots and leaves of the plants were air-dried for two days and subsequently placed in a hot-air oven for 8 hours to ensure complete moisture removal. Once dried, the samples were ground using a grinder to obtain a fine powder.

The ground samples were then digested with concentrated nitric acid (HNO_3) to facilitate the extraction of metal ions. Figures 4a and 4b illustrate the dried and powdered leaves and roots of vetiver grass. For the digestion process, an amount of 2.5 mL of concentrated nitric acid was added to 1 g of the ground, oven-dried sample in a 100-mL beaker.

Digestion of plant materials in hot concentrated nitric acid at temperatures ranging from 130°C to 140°C is a standard procedure (U.S. EPA Method 3051A: Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils) for assessing nutrient content and metal concentrations. The digestion process involves converting solid samples into a liquid form through the application of chemical reagents, which often include strong acids and bases. Heat is applied to accelerate the digestion process, ensuring that both the sample and the reagent are adequately mixed and react to release metal ions effectively.

Figure (4a): Dried leaves and roots

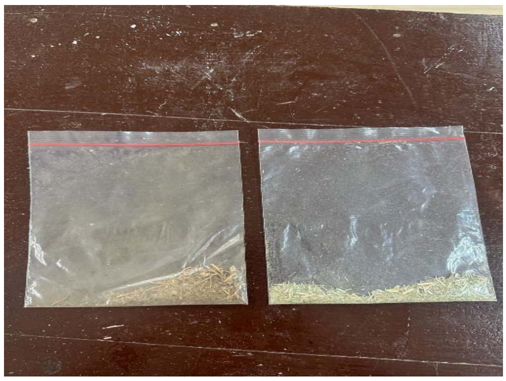

Figure (4b): Powdered leaves and roots

DATA ANALYSIS

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was performed at CSIR-NIIST in Trivandrum. ICP-MS is an advanced analytical technique utilized to measure and identify elements within a sample matrix through the ionization of its components. The mass spectrometer (MS) separates ions based on their mass-to-charge ratio after passing through the inductively coupled plasma (ICP). The detector then counts the number of selected ions per second, enabling the instrument to determine the concentration of each target element. This technique is employed across various industries, including routine environmental monitoring, consumer product testing, food and pharmaceutical safety, life sciences, clinical research, mining, metal analysis, geo-chemistry, nuclear applications, and petro-chemicals, to detect trace metal contaminants.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was conducted at Cochin Test House. This analytical technique is utilized in chemistry to determine the elemental composition of samples. ICP-OES combines two powerful methodologies—inductively coupled plasma (ICP) and optical emission spectroscopy (OES)—to achieve highly accurate and precise elemental analyses. Known for its ability to analyze a wide range of elements across the periodic table simultaneously and with high sensitivity, ICP-OES is applicable in numerous fields, including environmental monitoring, pharmaceuticals, metallurgy, geology, and food analysis. It is particularly beneficial for detecting trace elements in complex matrices, making it an invaluable tool in analytical chemistry and research. The technique is widely employed in numerous sectors, such as environmental monitoring, metallurgy and mining, pharmaceuticals, food and beverage, chemical manufacturing, geology, and water treatment.

The collected soil samples and plant parts were analyzed to observe temporal changes in metal concentrations. The results indicate a significant decrease in metal concentrations in soil samples over time. The accumulation of metals in plant tissues is attributed to both uptake and translocation mechanisms. Specifically, soil samples extracted from pots after 60 days exhibited the lowest metal concentrations compared to those collected at 30 days and 45 days. Additionally, soil samples collected from the bottom of each column also showed reduced metal quantities, reflecting the effective metal uptake by vetiver grass. To confirm that the observed metal removal was not due to other factors, ICP-OES testing is also being conducted to ascertain the atomic composition of the samples.

To evaluate the phyto-remediation potential of vetiver grass, bio-concentration factor (BCF) and translocation factor (TF) were calculated. The BCF can be used to evaluate the contents of heavy metals in plant parts, while the TF can be used to measure the amounts of heavy metals transferred from one plant part to another.

The calculation of BCF and TF was performed to assess whether plants could be categorized as accumulators.

$$BCF = \frac{\text{Concentration in leaf or root}}{\text{Concentration in sediment}} \tag{1}$$

$$TF = \frac{\text{BCF of shoot (stem or leaf)}}{\text{BCF of root}} \tag{2}$$

Heavy metals are commonly found in roots and leaves. The process of heavy metal binding in plants occurs *via* the mechanisms of heavy metal accumulation and tolerance. Vetiver roots can thus contribute to the prevention of heavy metal accumulation in the surrounding environment; high concentrations of heavy metal contaminants in sediments may be stored/extracted by the roots and leaves of plant (Ali et al., 2013; Chintani et al., 2021; Ketaubon et al., 2024).

RESULTS AND DISCUSSION

Variation in Metal Concentration with Time

Pot Experiment

A visible decrease in metal concentrations can be observed in Iron, Copper and Zinc after 30 days of transplantation to pots. Lead also decreases its concentration, but in less amounts.

Pb, Zn, Fe, and Cu concentrations in soil were assessed to evaluate their bio-availability in soil. The results obtained showed a decrease of Lead, Iron, Copper and Zinc concentrations in soil at uptake by vetiver for a period of one month. Iron content in soil decreased from 57677 to 28695 mg/kg (by 28982) and Copper from 92.8 to 48.5 mg/kg (by 44.3), Zinc from 122.6 to 97.6 mg/kg (by 25) and Lead from 10.52 to 9.96

(by 0.56). The original test was conducted at NIIST, Trivandrum. After a period of 45 days, concentrations of Lead, Zinc, Copper and Iron were assessed. The results showed a decrease in metal concentrations from those at 30 days. Iron content in soil decreased from 28695 to 23432(by 5263), Copper from 48.5 to 21.1 (by 27.4), Zinc from 97.6 to 31.6 (by 66) and Lead from 9.96 to 6.69 (by 3.27). Soil assessed after a period of 60 days showed a decrease in metal concentrations as compared to those obtained from 45days.Iron content in soil decreased from 23432 to 7447 (by 15985), Copper from 21.1 to 14.1 (by 7), Zinc from 31.6 to 21.7 (by 9.9) and Lead from 6.69 to 4.42 (by 2.27).

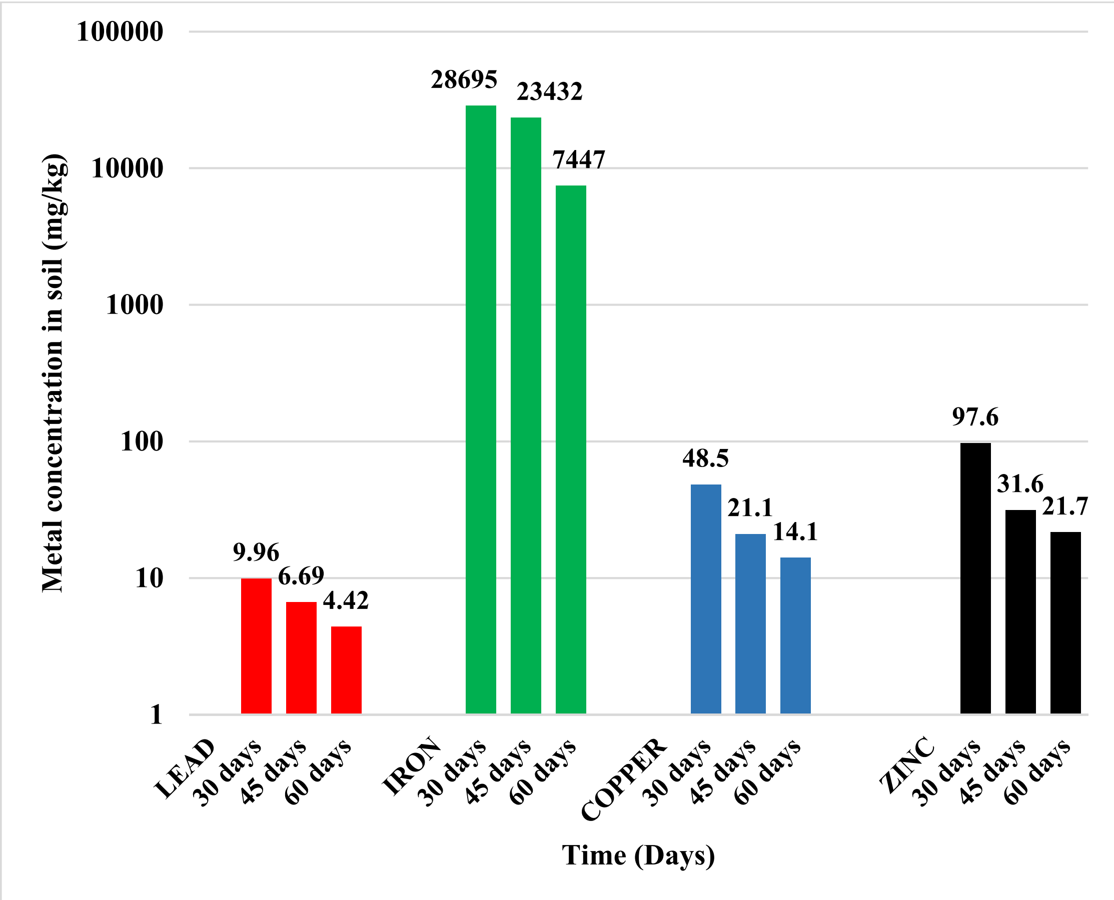

Figure (5): Graph of variations in metal concentrations with time

Figure (5) shows variations in metal concentrations detected in soil samples collected from pots during a period of 30,45 and 60 days. The results indicated that the amounts of metals in soil decrease with an increase in time. The lowest values for metal concentrations were detected for the soil samples subjected to ICP-MS test after the 60th day.

The analysis of soil samples over 30, 45, and 60 days reveals a consistent and progressive decline in the concentrations of heavy metals, particularly Iron (Fe), Copper (Cu), and Zinc (Zn), indicating the effectiveness of vetiver grass (*Chrysopogon zizanioides*) in phyto-remediation. Over the 60-day period, Fe concentration decreased drastically from 57,677 to 7,447 mg/kg, reflecting the plant's strong ability to stabilize and absorb Iron, which aligns with prior findings that highlight vetiver's extensive root system and metal-binding capabilities (Banerjee et al., 2016b; Otunola et al., 2023). Copper levels similarly declined from 92.8 to 14.1 mg/kg, and Zinc levels from 122.6 to 21.7 mg/kg, further supporting the species' high metal uptake potential, especially for Zn, which showed high BCF values in earlier studies (Roe & MacFarlane, 2022). While Lead (Pb) exhibited a gradual decrease from 10.52 to 4.42 mg/kg, its reduction was comparatively lower, likely due to its lower solubility and strong sorption to soil particles at a near-neutral pH of 6.8, which limits its bio-availability (Ur Rahman et al., 2024). The consistent downward trend in metal concentrations across all sampling intervals underscores the cumulative effect of phyto-remediation over time. These findings suggest that longer vegetation periods could further enhance metal removal, especially for metals, like Pb, that exhibit slower uptake kinetics. Overall, the data validates vetiver grass as an effective phyto-stabilizer, particularly for Cu and Zn, while also highlighting the need for soil or plant amendments to improve Pb remediation efficiency.

Column Leaching Experiment

After a period of 2 months of transplantation, soil from columns with actively grown vetiver was subjected to ICP -MS test for analysis of variation in metal concentration with time. The results showed that there was a significant decrease in Iron, Copper, and Zinc contents and a slight decrease in Lead content. Concentration of Iron decreased from 57677 to 36775, that is, by 20902. Copper concentration decreased from 92.8 to 13.36, that is, by 79.44. Concentration of Zinc decreased from 122.6 to 17.21, that is, a significant decrease of 105.39. Lead exhibited a slight decrease from 7.52 to 4.12, that is, by about 3.4. From this decrease, it can be conducted that except Iron all other metals' concentrations exhibited a decrease in value in comparison with the 60-day ICP-MS results of pot experiment. This reduction is attributed to the significant leaching of metals as leachates by providing water to induce leaching. Iron content exhibited minimal decreases in comparison with 60-day ICPMS results of pot experiment, which can be due to factors, like soil pH, soil moisture, absence of Iron-eating bacteria, temperature and organic matter. The original test was conducted at NIIST, Trivandrum. Figure (6) shows the ICP-MS results of column leaching experiment after 60 days.

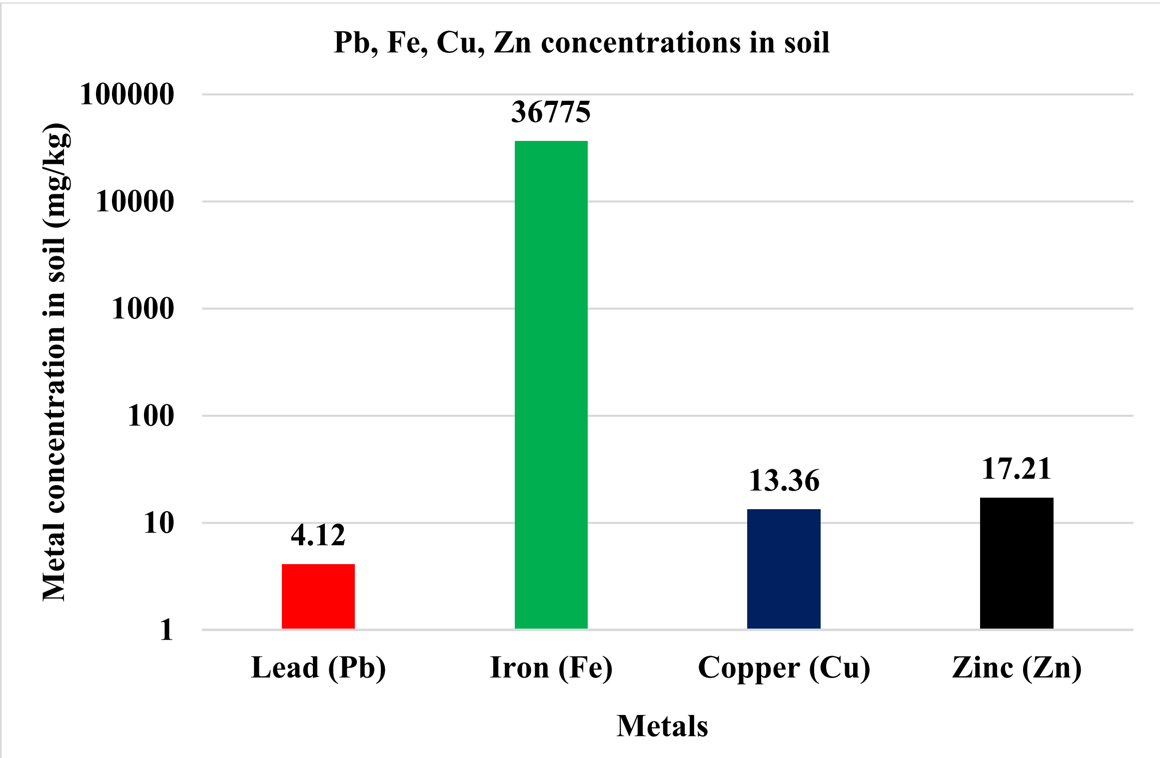

Figure (6): Graph of ICP-MS results of column leaching experiment after 60 days

The results of the column leaching experiment after 60 days further demonstrate the phyto-remediation potential of vetiver grass, particularly when combined with water-induced leaching. Significant reductions were observed in the concentrations of Copper (from 92.8 to 13.36 mg/kg) and Zinc (from 122.6 to 17.21 mg/kg), confirming that these metals are not only absorbed by the vetiver roots, but also effectively mobilized downward as leachates. This aligns with previous findings that highlighted vetiver's ability to promote the downward movement and stabilization of metals due to its dense root system and tolerance to high metal loads (Banerjee et al., 2016a; Dorafshan et al., 2023b). While Lead (Pb) exhibited a moderate reduction (from 7.52 to 4.12 mg/kg), the decrease was more substantial than in the pot experiment, suggesting enhanced mobility under leaching conditions. However, the relatively smaller decrease in Iron (from 57,677 to 36,775 mg/kg) compared to the pot experiment (down to 7,447 mg/kg) may be attributed to its lower solubility and complex interactions with soil properties. Factors, such as near-neutral soil pH (6.8), limited microbial activity (e.g. absence of Iron-oxidizing bacteria), and high organic matter content, can restrict Fe leaching and availability. These findings underscore the complementary role of leaching in augmenting phyto-remediation and point to the influence of environmental conditions on metal mobility and uptake. Overall, the column setup simulates real-field drainage more effectively than the pot experiment and highlights the combined utility of phyto-stabilization and phyto-leaching in removing contaminants from soil.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

The results for ICP-OES testing conducted on the roots and leaves of the cultivated plants after a period of 60 days are as follows. From the sediments, good concentrations of Copper, Zinc and Iron had been taken up by the root part of the plant. In comparison with the uptake by the root part, leaves showed less uptake ability. That is, uptake from sediments to the root part had been done actively, while translocation of metals from the root to aerial parts of plants, like leaves, had occurred in smaller amounts. The original test was conducted at Cochin Test House.

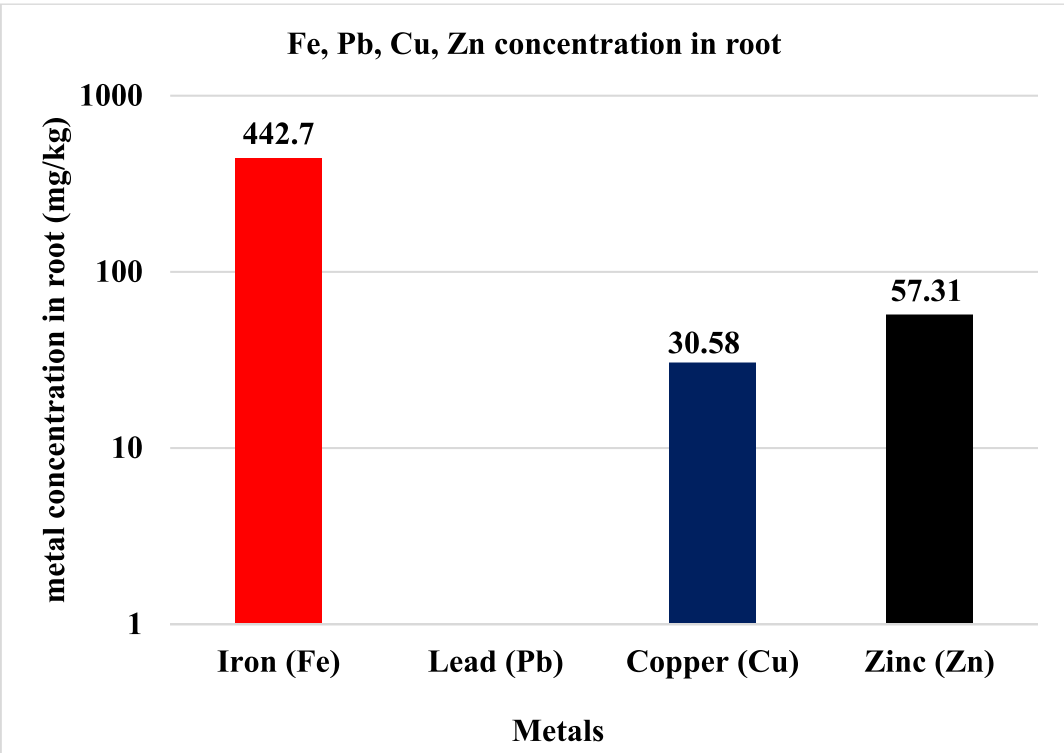

Figure (7): Graph of ICP-OES results of roots

Based on the results obtained for ICP - OES on roots and leaves, it can be concluded that a good percentage of metal uptake was shown by the root part as compared to that of leaves. Leaves also exhibited metal uptake but in lower concentrations. The concentration of Iron is 442.7mg /kg in roots while it is 85.18 in leaves. The concentration of Lead was not visibly shown in both parts, as it was below the detection limit. The amount of Lead absorbed by plants is affected by pH, organic matter of the soil. pH of the soil was found to be 6.8, which significantly affected the amount of Lead uptake by plants (Steingräber et al., 2022). The concentration of Copper is 30.58 mg/kg in roots and 7.29 mg/kkg in leaves. Zinc content in roots is 57.31 mg /kg and 25.02 in leaves (Figures (7, 8)).

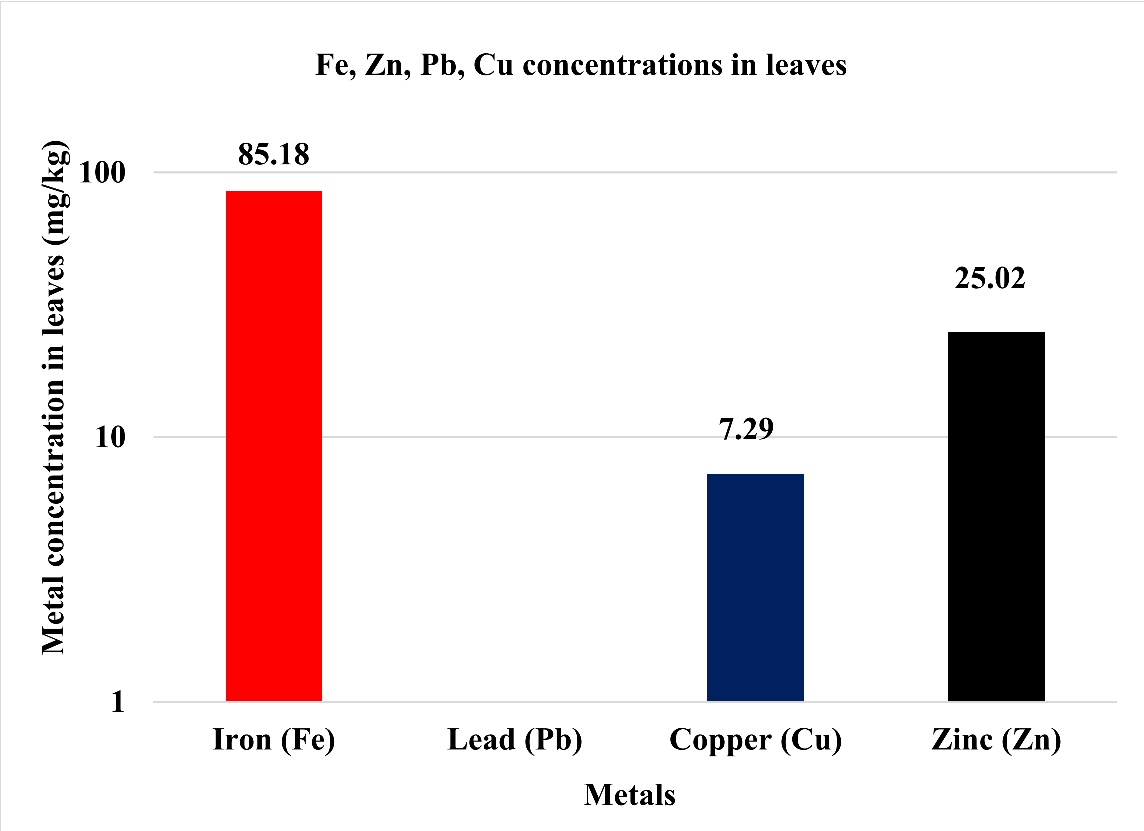

Figure (8): Graph of ICP-OES results of leaves

The ICP-OES analysis of vetiver grass after 60 days revealed a clear preference for metal accumulations in the roots over those in the aerial parts, indicating a dominant phyto stabilization mechanism. The root tissues recorded significantly higher concentrations of Iron (442.7 mg/kg), Copper (30.58 mg/kg), and Zinc (57.31 mg/kg) compared to the respective leaf concentrations of 85.18, 7.29, and 25.02 mg/kg, respectively. This root-dominant uptake aligns with the findings of several studies that reported vetiver's limited translocation of heavy metals to shoots due to its strong metal-binding capacity in roots and a low TF (Chen et al., 2004b; Nedjimi, 2021b). The limited metal transfer from roots to leaves suggests that vetiver functions primarily as a phyto-stabilizer rather than a phyto-extractor. Notably, Lead (Pb) was below detection limits in both plant parts, which is consistent with previous observations that Pb exhibits low bio-availability under near-neutral pH conditions (6.8) and tends to remain bound to soil particles and organic matter (Steingräber et al., 2022). This further confirms the influence of soil chemistry—particularly pH and organic content—on the uptake efficiency of different metals. These results reinforce vetiver's role as an effective candidate for *in-situ* stabilization of heavy metals, especially Zn and Cu, and point to the need for soil modifications or chelating agents if enhanced translocation or phyto-extraction of less mobile metals, like Pb is desired.

Bioconcentration Factor (Bcf) and Translocation Factor(TF)

Based on the data, BCF values of more than 1 were found for Copper and Zinc in roots. Meanwhile, BCF of less than 1 was found for Iron in roots. Also, Lead was found to be below the detection limit, which concludes that Lead was not absorbed significantly from the sediments of vetiver plant. BCF value of more than 1 was found for Zinc in leaves and BCF values of less than 1 were found for Iron and Copper in leaves. Hence, the content of heavy metals accumulated is more in the root part than in leaves (Figures (9, 10)).

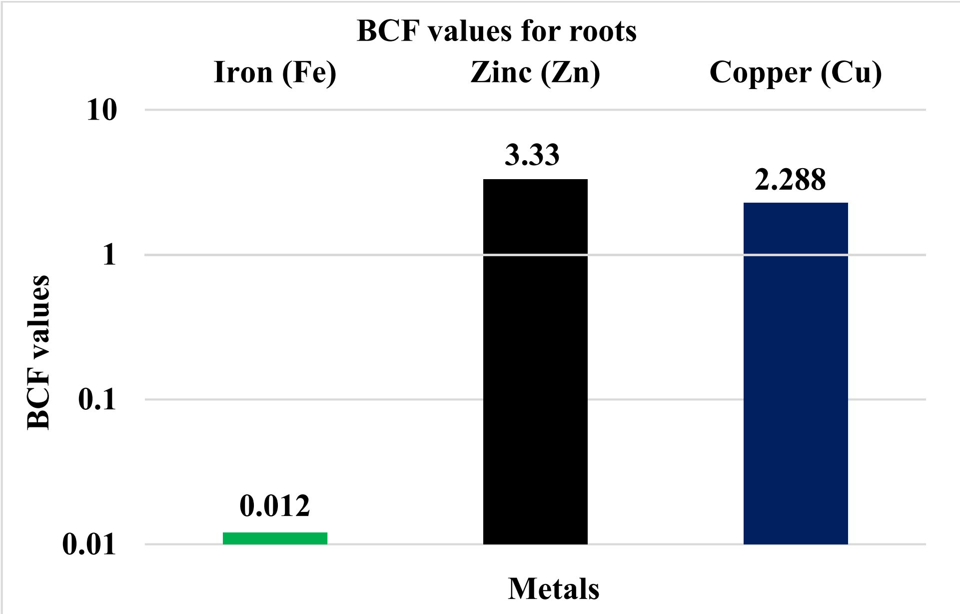

Figure (9): Graph of bio-concentration factors for roots

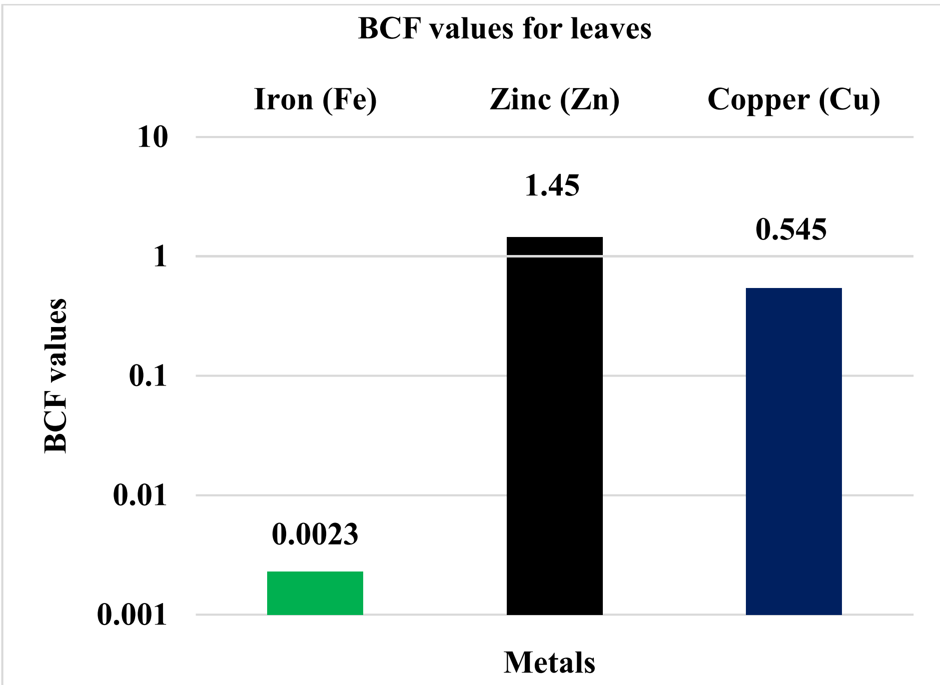

Figure (10): Graph of bio-concentration factors for leaves

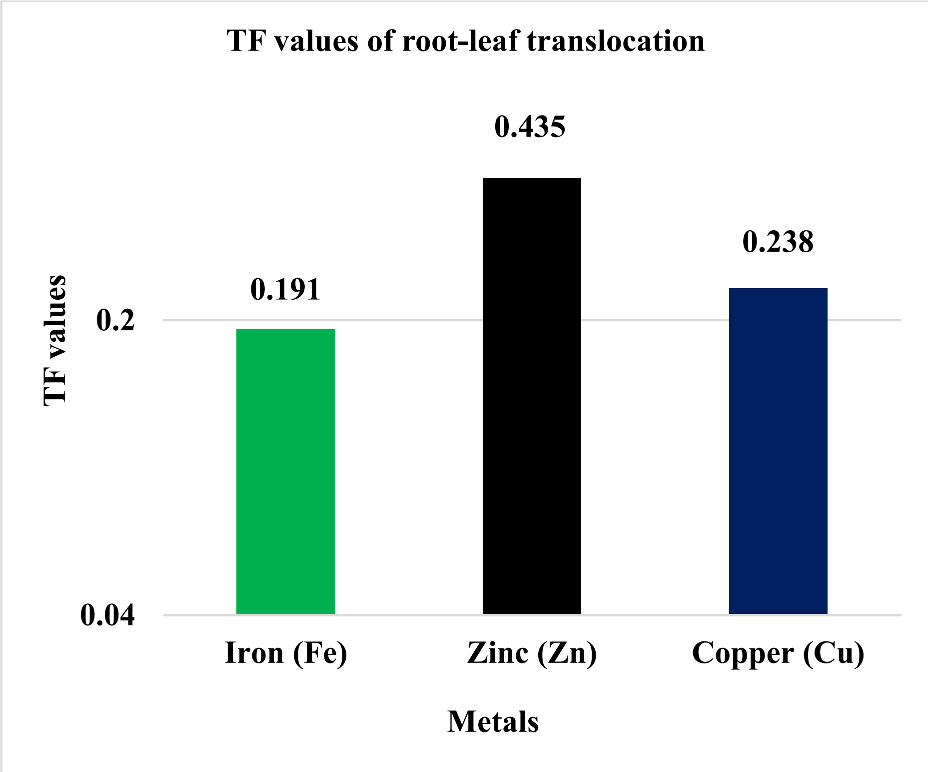

Figure (11): Graph of translocation factors for root-leaf

TF of more than 40% was found in root-leaf translocation. This means that vetiver translocated heavy metals from the root to leaves at a rate of less than 50%. This finding indicates that vetiver was capable of translocating metals from the root to leaves, but not at a significant rate. TF for heavy metals in plants should be more than one to be considered as a bio-accumulator. Hyper-accumulator plants can accumulate high concentrations of heavy metals at the tissue surface (above ground) when found in their natural habitat. Here, Zinc was found to be translocated at a good rate from the root to leaves in comparison to other heavy metals. Plants that have BCF and TF >1 can be used as bio-accumulators. BCF > 2 are high values. Plants can be used as phyto-stabilizers if they have BCF >1 and TF < 1 (Ngoc Ba et al., 2024; Usman et al., 2013) (Figure 11).

The analysis of BCF and TF provides valuable insights into the phyto-remediation behavior of vetiver grass. The BCF, which indicates the plant's ability to absorb metals from the sediment, was greater than 1 for both Zinc (Zn) and Copper (Cu) in the roots, suggesting effective uptake and accumulation. In contrast, the BCF for Iron (Fe) in roots was less than 1, and Lead (Pb) remained below the detection limit, indicating poor absorption, likely due to the metal's low mobility and soil pH constraints. In the aerial parts, Zn was the only metal with a BCF greater than 1, while Cu and Fe showed lower values, reaffirming that vetiver preferentially accumulates heavy metals in the root zone rather than translocating them to the shoots. The translocation factor (TF) values were below 0.5 for all metals, implying that metal movement from roots to leaves was limited. This trend is particularly useful for phyto-stabilization, where metal containment in the rhizosphere is desired to prevent entry into the food chain. Zinc, although better translocated than other metals, still exhibited a TF below 1, confirming that vetiver does not behave as a hyper-accumulator. These results are consistent with prior studies indicating that plants with BCF >1 and TF <1 act as effective phyto-stabilizers, while hyper-accumulators require both values to exceed 1 (Ngoc Ba et al., 2024; Usman et al., 2013). Therefore, vetiver grass demonstrates strong potential as a phyto-stabilizing agent, particularly for Zn and Cu, with limited risk of metal transfer to above-ground biomass.

Mass Balance Analysis of Metal Removal

To quantify vetiver's phyto-remediation efficiency, a comprehensive mass balance analysis (Table 4) was conducted by comparing the metal removal from soil with the metal accumulation in plant tissues. The metal removal from soil was determined by calculating the difference between initial and final metal concentrations in sediment samples, as measured by ICP-MS over the 60-day experimental period. Concurrently, metal accumulation in vetiver grass was assessed through ICP-OES analysis of root and leaf tissues, with values scaled to the total plant bio-mass using average dry weights of 12.5 g for roots and 8.3 g for leaves per plant. The unaccounted metal losses, potentially attributable to leaching or adsorption processes, were derived by subtracting the percentage of metals accounted for by plant uptake from the total metal removal from soil. This approach provided a systematic evaluation of vetiver 's phyto-remediation performance by distinguishing between biological uptake and other potential metal removal pathways in the soil-plant system. Soil loss (mg/kg) represents the difference between initial (Table 2) and final (60-day) metal concentrations in soil, as measured by ICP-MS. Unaccounted losses (attributed to leaching/adsorption) were inferred as:

$$\text{Unaccounted (\%)} = 100\% - (\text{\% Accounted by Plant Uptake}) \tag{3}$$

Table 4. Mass balance of heavy metal distribution during vetiver phyto-remediation

Metal	Soil Loss (mg/kg)	Plant Uptake (mg/kg)	% Accounted	% Unaccounted
Fe	50,230	527.9	1.05%	98.95%
Zn	100.9	82.3	81.60%	18.40%
Cu	78.7	37.9	48.10%	51.90%
Pb	3.1	BDL	0%	100%

The mass balance analysis revealed distinct patterns in vetiver's metal remediation capabilities. For zinc (Zn), the high uptake efficiency (81.6%) corresponds with its bio-concentration factor (BCF) exceeding 1, strongly supporting vetiver's phyto-extraction potential for this metal. Copper (Cu) showed an intermediate behavior, with 48.1% accounted for by plant uptake, suggesting a dual mechanism combining phyto-stabilization and leaching, as corroborated by column effluent data. In contrast, Iron (Fe) and Lead (Pb) demonstrated minimal absorption (1.05% and 0%, respectively), indicating that these metals primarily follow non-plant pathways, such as leaching or rhizosphere adsorption.

These findings have important implications for phyto-remediation applications. Vetiver exhibits metal-specific efficacy, with performance following the order Zn > Cu ≫ Fe/Pb. The substantial unaccounted losses (particularly 98.95% for Fe) underscore the limitations of current analytical methods in tracking all metal fate pathways. Future research should employ advanced techniques, like SEM-EDS and sequential extraction, to better quantify root-surface adsorption and other non-uptake processes. These results highlight both the promise and limitations of vetiver grass for site-specific remediation strategies, emphasizing the need for complementary approaches when dealing with less bio-available metals, like Fe and Pb.

The small fraction of unaccounted metals may result from adsorption to column materials, microbial transformations, or precipitation as insoluble complexes—processes not quantified in this study, but common in phyto-remediation systems (Burton et al., 2019). Future experiments using tracer isotopes or inert materials could clarify these losses.

CONCLUSIONS

- Vetiver grass is effective in removing certain heavy metals. It showed good uptake of Zinc (Zn), Copper (Cu), and Iron (Fe), but had limited effectiveness in absorbing Lead (Pb) from contaminated sediments.
- In the pot experiment, the order of reduction in soil was Fe < Zn < Cu < Pb, while in the column experiment, metal uptake followed Zn > Cu > Fe, highlighting the influence of experimental conditions on metal removal efficiency. The bio-concentration factor (BCF) values for Zn and Cu were greater than 1, confirming efficient metal uptake and vetiver's potential for remediating these metals.
- Pb levels remained below detectable limits in both roots and leaves, likely due to its toxicity and the root’s role as a barrier preventing translocation to

aerial parts.

- After 60 days, columns showed substantial metal accumulation in roots, affirming vetiver's suitability for stabilizing metals in sediment environments.

Plants with BCF and TF greater than 1 can be considered bio-accumulators. A BCF value greater than 2 is regarded as high. Plants with BCF >1 and TF <1 function as Phyto-stabilizers, while those with BCF <1 and TF >1 act as phyto-extractors. Typically, plants under metal stress exhibit a TF less than 1. A TF greater than 1 indicates that the plant can tolerate and effectively use the contaminant, a characteristic of hyper-accumulators. Overall, it appears that vetiver roots have a higher capacity for absorbing heavy metals than accumulating them in the aerial parts, highlighting their role in effective phyto stabilization of contaminated soils.

While this study demonstrates the phyto-remediation potential of vetiver grass, it was conducted without experimental replicates due to practical limitations. This restricts the statistical validation of the findings.

REFERENCES

Ali, H., Khan, E., and Sajad, M.A. (2013). "Phyto-remediation of heavy metals: Concepts and applications". Chemosphere, 91 (7), 869-881. https://doi.org/ 10.1016/J.CHEMOSPHERE.2013.01.075

Alves, A.R.A., Yin, Q., Oliveira, R.S., Silva, E.F., and Novo, L.A.B. (2022). "Plant growth-promoting bacteria in phyto-remediation of metal-polluted soils: Current knowledge and future directions". Science of The Total Environment, 838, 156435. https://doi.org/ 10.1016/J.SCITOTENV.2022.156435

Alzboon, Kamel. (2022). "Single and ternary removal of heavy metals from aqueous solution using Fe_3O_4 magnetic nano-particles". Jordan Journal of Civil Engineering, 17 (2).

Angon, P.B., Islam, M.S., KC, S., Das, A., Anjum, N., Poudel, A., and Suchi, S. A. (2024). "Sources, effects and present perspectives of heavy metal contamination: Soil, plants and human food chain. Heliyon, 10 (7), e28357. https://doi.org/10.1016/J.HELIYON.2024.E28357

Awasthi, G., Nagar, V., Mandzhieva, S., Minkina, T., Sankhla, M.S., Pandit, P.P., Aseri, V., Awasthi, K.K., Rajput, V.D., Bauer, T., and Srivastava, S. (2022). "Sustainable amelioration of heavy metals in soil ecosystem: Existing developments to emerging trends". Minerals, 12 (1), 85. https://doi.org/ 10.3390/MIN12010085

Banerjee, R., Goswami, P., Lavania, S., Mukherjee, A., and Lavania, U.C. (2019). "Vetiver grass is a potential candidate for phyto-remediation of iron ore mine spoil dumps". Ecological Engineering, 132, 120-136. https://doi.org/10.1016/J.ECOLENG.2018.10.012

Banerjee, R., Goswami, P., Pathak, K., and Mukherjee, A. (2016a). "Vetiver grass: An environment clean-up tool for heavy metal contaminated iron ore mine-soil. Ecological Engineering, 90, 25-34. https://doi.org/ 10.1016/J.ECOLENG.2016.01.027

Banerjee, R., Goswami, P., Pathak, K., and Mukherjee, A. (2016b). "Vetiver grass: An environment clean-up tool for heavy metal contaminated iron ore mine-soil". Ecological Engineering, 90, 25-34. https://doi.org/ 10.1016/J.ECOLENG.2016.01.027

Bijay-Singh, and Craswell, E. (2021). "Fertilizers and nitrate pollution of surface and ground water: An increasingly pervasive global problem". SN Applied Sciences, 3 (4), 1-24. https://doi.org/10.1007/ S42452-021-04521-8

Briffa, J., Sinagra, E., and Blundell, R. (2020). "Heavy metal pollution in the environment and their toxicological effects on humans". Heliyon, 6 (9), e04691. https://doi.org/10.1016/J.HELIYON.2020.E04691

Burton, G.A., Hudson, M.L., Huntsman, P., Carbonaro, R.F., Rader, K.J., Waeterschoot, H., Baken, S., and Garman, E. (2019). "Weight-of-evidence approach for assessing removal of metals from the water column for chronic environmental hazard classification". Environmental Toxicology and Chemistry, 38 (9), 1839-1849. https://doi.org/10.1002/ETC.4470;JOURNAL:JOURNAL:15528618;REQUESTEDJOURNAL:JOURNAL:15528618;WGROUP:STRING:PUBLICATION

Chandra, R., and Kumar, V. (2023). "Phyto-remediation: A green sustainable technology for industrial waste management". Phyto-remediation of Environmental Pollutants, 1-42. https://doi.org/10.4324/9781315161549-1/PHYTOREMEDIATION-GREEN-SUSTAINABLE-TECHNOLOGY-INDUSTRIAL-WASTE-MANAGEMENT-RAM-CHANDRA-VINEET-KUMAR

Chen, Y., Shen, Z., and Li, X. (2004b). "The use of vetiver grass (*Vetiveria zizanioides*) in the phyto-remediation of soils contaminated with heavy metals". Applied Geochemistry, 19 (10), 1553-1565. https://doi.org/10.1016/J.APGEOCHEM.2004.02.003

Chintani, Y.S., Butarbutar, E.S., Nugroho, A.P., and Sembiring, T. (2021). "Uptake and release of chromium and nickel by vetiver grass (*Chrysopogon zizanioides* (L.) Roberty). SN Applied Sciences, 3 (3), 1-13. https://doi.org/10.1007/S42452-021-04298-W/TABLES/5

Deng, S., Zhang, X., Zhu, Y., and Zhuo, R. (2024). "Recent advances in phyto-combined remediation of heavy metal pollution in soil. Biotechnology Advances, 72, 108337. https://doi.org/10.1016/J.BIOTECHADV. 2024.108337

Dorafshan, M.M., Abedi-Koupai, J., Eslamian, S., and Amiri, M.J. (2023a). "Vetiver grass (*Chrysopogon zizanoides* L.): A hyper-accumulator crop for bioremediation of unconventional water". Sustainability, 15 (4), 3529. https://doi.org/10.3390/SU1 5043529

Goren, A.Y., Yucel, A., Sofuoglu, S. C., and Sofuoglu, A. (2021). "Phyto-remediation of olive mill wastewater with *Vetiveria zizanioides* (L.) *Nash and Cyperus alternifolius* L. *Environmental Technology & Innovation*, 24, 102071. https://doi.org/10.1016/J.ETI.2021.102071

Gravand, F., Rahnavard, A., and Pour, G.M. (2021). "Investigation of vetiver grass capability in phyto-remediation of contaminated soils with heavy metals (Pb, Cd, Mn, and Ni)". Soil and Sediment Contamination, 30 (2), 163-186. https://doi.org/10.1080/15320383.2020.1819959

Gregory, A.S., Ritz, K., Mcgrath, S.P., Quinton, J.N., Goulding, K.W.T., Jones, R.J.A., Harris, J.A., Bol, R., Wallace, P., Pilgrim, E.S., and Whitmore, A.P. (2015). "A review of the impacts of degradation threats on soil properties in the UK". Soil Use and Management, 31, 1-15. https://doi.org/10.1111/SUM.12212

Holanda, F.S.R., Santos, L.D.V., Pedrotti, A., de Araújo Filho, R.N., Sartor, L.R., Santos-Sobrinho, V.R.A., de Jesus, R.J.S., de Oliveira Silva, P.A., and Andrade, K.M.A. (2022). "Evaluation of the root system of vetiver grass (*Chrysopogon zizanioides* L. *Roberty*) using different sampling methods". Environmental Systems Research, 11 (1), 1-15. https://doi.org/10.1186/S40068-022-00262-8/TABLES/5

Hou, D., Bolan, N.S., Tsang, D.C.W., Kirkham, M.B., and O'Connor, D. (2020). "Sustainable soil use and management: An inter-disciplinary and systematic approach". Science of the Total Environment, 729. https://doi.org/10.1016/j.scitotenv.2020.138961

Iqbal, N., Hayat, M.T., Zeb, B.S., Abbas, Z., and Ahmed, T. (2018). "Phyto-remediation of Cd-contaminated soil and water". Cadmium Toxicity and Tolerance in Plants: From Physiology to Remediation, 531-543. https://doi.org/10.1016/B978-0-12-814864-8.00021-8

Jampasri, K., and Saeng-Ngam, S. (2019). "Phyto-remediation and accumulation of cadmium from contaminated saline soils by vetiver grass". Environmental Control in Biology, 57 (3), 61–67. https://doi.org/10.2525/ECB.57.61

Ketaubon, P., Ritthikasem, N., Tanheng, P., and Prapagdee, B. (2024). "Enhancing heavy metal phyto-remediation in landfill soil by *Chrysopogon zizanioides* (L.) Roberty through the application of bacterial-biochar pellets". Environmental Technology & Innovation, 35, 103738. https://doi.org/10.1016/J.ETI.2024.103738

Liu, N., Zhao, J., Du, J., Hou, C., Zhou, X., Chen, J., and Zhang, Y. (2024). "Non-phyto-remediation and phyto-remediation technologies of integrated remediation for water and soil heavy metal pollution: A comprehensive review". Science of the Total Environment, 948, 174237. https://doi.org/10.1016/J.SCITOTENV.2024.174237

Lwin, C.S., Seo, B.H., Kim, H.U., Owens, G., and Kim, K.R. (2018). "Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality: A critical review". Soil Science and Plant Nutrition, 64 (2), 156-167. https://doi.org/10.1080/00380768.2018.1440938

Marques, A.P.G.C., Rangel, A.O.S.S., and Castro, P.M.L. (2009). "Remediation of heavy metal contaminated soils: Phyto-remediation as a potentially promising clean-up technology". Critical Reviews in Environmental Science and Technology, 39 (8), 622-654. https://doi.org/10.1080/10643380701798272

Maurya, P.K., Malik, D.S., Yadav, K.K., Kumar, A., Kumar, S., and Kamyab, H. (2019). "Bio-accumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation". Toxicology Reports, 6, 472-481. https://doi.org/10.1016/J.TOXREP.2019.05.012

Mitra, S., Chakraborty, A.J., Tareq, A.M., Emran, T. Bin, Nainu, F., Khusro, A., Idris, A. M., Khandaker, M. U., Osman, H., Alhumaydhi, F.A., and Simal-Gandara, J. (2022). "Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity". Journal of King Saud University - Science, 34 (3), 101865. https://doi.org/10.1016/J.JKSUS.2022.101865

Mocek-Płóciniak, A., Mencel, J., Zakrzewski, W., and Roszkowski, S. (2023). "Phyto-remediation as an effective remedy for removing trace elements from ecosystems". Plants, 12 (8), 1653. https://doi.org/10.3390/PLANTS12081653

Nedjimi, B. (2021a). "Phyto-remediation: A sustainable environmental technology for heavy metal decontamination. SN Applied Sciences, 3 (3), 1-19. https://doi.org/10.1007/S42452-021-04301-4

Nedjimi, B. (2021b). "Phyto-remediation: A sustainable environmental technology for heavy metal decontamination. SN Applied Sciences, 3 (3), 1-19. https://doi.org/10.1007/S42452-021-04301-4

Nepal, A., Antonious, G.F., Bebe, F.N., Webster, T.C., Gyawali, B.R., and Neupane, B. (2024). "Heavy metal accumulation in three varieties of mustard grown under five soil management practices". Environments, 11 (4), 77. https://doi.org/10.3390/ENVIRONMENTS11040077

Ng, C.C., Boyce, A.N., Abas, M.R., Mahmood, N. Z., and Han, F. (2019). "Phytoassessment of vetiver grass enhanced with EDTA soil-amendment grown in single and mixed heavy metal–contaminted soil". Environmental Monitoring and Assessment, 191 (7), 1-16. https://doi.org/10.1007/S10661-019-7573-2/METRICS

Ngoc Ba, V., Thien, B.N., Phuong, H.T., Thi Hong Loan, T., and Anh, T.T. (2024). "Bio-concentration and translocation of elements from soil to vegetables and associated health risk". Journal of Food Composition and Analysis, 132, 106296. https://doi.org/10.1016/J.JFCA.2024.106296

Nguyen, M.K., Hung, N.T.Q., Nguyen, C.M., Lin, C., Nguyen, T.A., and Nguyen, H.L. (2023). "Application of vetiver grass (*Vetiveria zizanioides* L.) for organic matter removal from contaminated surface water". Bioresource Technology Reports, 22, 101431. https://doi.org/10.1016/J.BITEB.2023.101431

Odat, S., and Alshammari, A.M. (n.d.). "Seasonal variations of soil heavy metal contaminants along urban roads: A case study from the city of hail, Saudi Arabia". Jordan Journal of Civil Engineering, 5(4), 2011-2581.

Otunola, B.O., Aghoghovwia, M.P., Thwala, M., Gómez-Arias, A., Jordaan, R., Hernandez, J.C., and Ololade, O.O. (2022). "Influence of clay mineral amendment characteristics on heavy metal uptake in vetiver grass (chrysopogon zizanioides L. Roberty) and Indian Mustard (*Brassica juncea L. Czern*)". Sustainability, 14 (10), 5856. https://doi.org/10.3390/SU14105856

Otunola, B.O., Aghoghovwia, M. P., Thwala, M., Gómez-Arias, A., Jordaan, R., Hernandez, J.C., and Ololade, O.O. (2023). "Improving capacity for phyto-remediation of vetiver grass and Indian mustard in heavy metal (Al and Mn)- contaminated water through the application of clay minerals". Environmental Science and Pollution Research, 30 (18), 53577-53588. https://doi.org/10.1007/S11356-023-26083-5/FIGURES/4

Pande, V., Pandey, S.C., Sati, D., Bhatt, P., and Samant, M. (2022). "Microbial interventions in bioremediation of heavy metal contaminants in agroecosystem. Frontiers in Microbiology, 13. https://doi.org/10.3389/FMICB.2022.824084

Priya, A.K., Muruganandam, M., Ali, S.S., and Kornaros, M. (2023). "Clean-up of heavy metals from contaminated soil by phyto-remediation: A multi-disciplinary and eco-friendly approach". Toxics, 11 (5). https://doi.org/10.3390/TOXICS11050422

Rashid, A., Schutte, B.J., Ulery, A., Deyholos, M.K., Sanogo, S., Lehnhoff, E.A., and Beck, L. (2023). "Heavy metal contamination in agricultural soil: Environmental pollutants affecting crop health". Agronomy, 13 (6), 1521. https://doi.org/10.3390/AGRONOMY13061521/S1

Roe, R.A.L., and MacFarlane, G.R. (2022). "The potential of saltmarsh halophytes for phyto-remediation of metals and persistent organic pollutants: An Australian perspective". Marine Pollution Bulletin, 180, 113811. https://doi.org/10.1016/J.MARPOLBUL.2022.113811

Roychowdhury, A., Mukherjee, P., Panja, S., Datta, R., Christodoulatos, C., and Sarkar, D. (2020). "Evidence for phyto-remediation and phyto-excretion of NTO from industrial wastewater by vetiver grass". Molecules, 26 (1), 74. https://doi.org/10.3390/MOLECULES26010074

Sahoo, R., Sow, S., Ranjan, S., Dharminder, Kumar, R., Roy, D.K., Kumar, S., Kumar, A., Srivastava, R.K., Prasad, R., Padhan, S.R., and Nath, D. (2024). "Unveiling the potential of plant growth promoting rhizobacteria (PGPR) in phyto-remediation of heavy metal". Discover Applied Sciences, 6 (6), 1-20. https://doi.org/10.1007/S42452-024-06024-8

Saravanan, P., Saravanan, V., Rajeshkannan, R., Arnica, G., Rajasimman, M., Baskar, G., and Pugazhendhi, A. (2024). "Comprehensive review on toxic heavy metals in the aquatic system: Sources, identification, treatment strategies, and health risk assessment". Environmental Research, 258, 119440. https://doi.org/10.1016/J.ENVRES.2024.119440

Steingräber, L.F., Ludolphy, C., Metz, J., Kierdorf, H., and Kierdorf, U. (2022). "Uptake of lead and zinc from soil by blackberry plants (*Rubus fruticosus* L. agg.) and translocation from roots to leaves". Environmental Advances, 9, 100313. https://doi.org/10.1016/J.ENVADV.2022.100313

Tan, H.W., Pang, Y.L., Lim, S., and Chong, W.C. (2023). "A state-of-the-art phyto-remediation approach for sustainable management of heavy metal recovery". Environmental Technology & Innovation, 30, 103043. https://doi.org/10.1016/J.ETI.2023.103043

Ur Rahman, S., Qin, A., Zain, M., Mushtaq, Z., Mehmood, F., Riaz, L., Naveed, S., Ansari, M. J., Saeed, M., Ahmad, I., and Shehzad, M. (2024). "Pb uptake, accumulation, and translocation in plants: Plant physiological, bio-chemical, and molecular response: A review". Heliyon, 10 (6), e27724. https://doi.org/10.1016/ J.HELIYON..2024.E27724

U.S. EPA Method 3051A: Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils. (n.d.). Retrieved on 26 April 2025, from: https://www.epa.gov/esam/us-epa-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-and-oils

Usman, A.R.A., Alkredaa, R. S., and Al-Wabel, M.I. (2013). "Heavy metal contamination in sediments and mangroves from the coast of Red Sea: *Avicennia marina* as potential metal bio-accumulator". Ecotoxicology and Environmental Safety, 97, 263-270. https://doi.org/10.1016/J.ECOENV.2013.08.009

Wuana, R.A., Okieimen, F.E., Montuelle, B., and Steinman, A.D. (2011). "Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation". International Scholarly Research Notices, 2011 (1), 402647. https://doi.org/10.5402/2011/402647

Xu, W., Jin, Y., and Zeng, G. (2024). "Introduction to heavy metal contamination in water and soil: A review on source, toxicity and remediation methods". Green Chemistry Letters and Reviews, 17 (1). https://doi.org/10.1080/17518253.2024.2404235

Yan, A., Wang, Y., Tan, S.N., Mohd Yusof, M.L., Ghosh, S., and Chen, Z. (2020). "Phyto-remediation: A promising approach for revegetation of heavy metal-polluted land". Frontiers in Plant Science, 11, 513099. https://doi.org/10.3389/FPLS.2020.00359/BIBTEX

Yaseen, I.A.B., and Al-Hawari, Z. (n.d.). "Assessment of heavy metal pollution of surface sediments in Wadi Shu'ayb, Jordan". Jordan Journal of Civil Engineering, 9 (3).

Zeng, K., Huang, X., Guo, J., Dai, C., He, C., Chen, H., and Xin, G. (2024). "Microbial-driven mechanisms for the effects of heavy metals on soil organic carbon storage: A global analysis". Environment International, 184, 108467. https://doi.org/10.1016/J.ENVINT.2024.108467.