HEAVY METALS DISTRIBUTION IN VEGETATIVE AND NON-VEGETATIVE PART OF *SENNA ITALICA* GROWN IN ARID CLIMATIC CONDITIONS

JALAL, R. S.

Department of Biological Sciences, College of Science, University of Jeddah, Jeddah 21493, Saudi Arabia
(e-mail: rsjalal@uj.edu.sa)

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Abstract. Heavy metals (HMs) contamination in soil and their potential transfer to the food chain have been raised since last three decades due to urbanization and industrialization in developing and developed world. The study aimed to investigate the concentration and distribution of trace elements in the *Senna italica* plant grown in soil contaminated with HMs. This study also elaborates the concentration of elements in vegetative and non-vegetative soil. For this study, an area of Asfan in Saudi Arabia was selected, and soil and *Senna italica* plant samples were collected from various spots by randomly. After collection the soil and plant samples were brought to laboratory and prepared for digestion to check the contents of various HMs in root, shoot and soil samples. The results showed a significant variation in the concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn of *Senna italica* in arid climate. The results revealed that plant have the ability to bioaccumulate a higher concentration (more than 60%) of these HMs in their root and shoot part. Moreover, the vegetative soil sample contain a reduced level of HMs that shows the importance of *Senna italica* as a hyperaccumulator and their proposed use as phytoremediation plant. These findings could be beneficial for reclamation of HMs contaminated soil and their possible usage for sustainable agricultural production.
Keywords: *at least five, excluding words from the title*

Introduction

Globally, heavy metals (HMs) contamination has major consequences on environmental components due to anthropogenic activities (Seleiman et al., 2019, 2013). In recent years, the HMs exposures has been increased throughout the world as a result of expanded industrialization that increased the negative impacts of HMs on human health (Proshad et al., 2018). Soil normally contains lesser amount of HMs due to natural weathering processes (Ding et al., 2017), but their amount has been increased in soil due to anthropogenic activities such as mining, fossil fuel burning, inappropriate fertilizer usage, and sewage sludge application (Vareda et al., 2019). Rapid urbanization along the industrial zones of Saudi Arabia increases the risk of HMs contamination in biological spheres (Almohisen et al., 2020). Moreover, the specific climatic and geospatial location of the country worsen the impacts of HMs pollution (Metwaly et al., 2023). The arid climate and limited water supply increases the buildup of HMs in soil for extended period of time (Naorem et al., 2023). These circumstances threaten the local biodiversity and present a substantial health impact to local human population through contaminated food chain (Adnan et al., 2024).

Continuous exposure of HMs to plants, animals and human beings causes physiological imbalance as the buildup of HMs in the body (Mitra et al., 2022). These HMs acts as a substitute for important nutrients (Manzoor et al., 2018). So, their inclusion in soil-plant system disturbs the entire food chain due to higher bioaccumulation and biomagnification characteristics (Kumar et al., 2020). In soil, HMs toxicity decreases

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seed germination, initial seedling growth, and plant biomass (Noor et al., 2022).It also causes reduced relative water contents, photosynthesis, stomatal conductance, and transpiration rate in many plants (Hafeez et al., 2023). In response to HMs stress, plants produce reactive oxygen species (ROS), hydrogen peroxide (H2O2) and ethylene which inhibit the physiological processes inside the cells by affecting the membranous structures (Sachdev et al., 2021). The HMs also disturbs the carbohydrates manufacturing that regulates the total soluble sugars and proteins metabolic pathways that regulate various activities and metabolic pathways in plant nitrogen metabolism (Emamverdian et al., 2015). Therefore, strategies are required to remove HMs from the contaminated environment and restricts its entry to the food chain.

Phytoremediation has gained the attention of scientists in the last few decades to overcome problems in removal of inorganic and organic elements from land and water bodies (Adnan et al., 2024; Shen et al., 2022). It is an efficient, eco-friendly, less expensive and easy technique with great public acceptance to combat HMs pollution Asante-Badu et al. 2020. In phytoremediation, plant roots release different enzymes to degrade, accumulate and adsorb the contaminants from soil solution and transferred to body tissues where they can be stabilized or volatilized (Abdel-Shafy and Mansour, 2018; Nasir et al., 2023).Various plant species have been known for their phytoremediation potential, however, *Senna italica* is also known for higher tolerance and more accumulation of multiple HMs under various climatic conditions (Mehta et al., 2017).

Senna italica, a perennial and leguminous crop widely known due to its medicinal properties (Lal et al., 2023). This plant exhibits a robust growth in harsh climatic conditions and its deeper root system with symbiotic microbes not only increase the biomass production but also increase the uptake and bioaccumulate HMs from contaminated environment (Veerapagu et al., 2023). Moreover, the widespread availability in desert areas and lower maintenance requirements increases the suitability of the native plant species for phytoremediation of several HMs from the contaminated soil (Alsaedi et al., 2022). The present study aims to explore the potential of *Senna italica* plant to phyto-accumulate HMs from suburbs of Saudi Arabian industrial zones. The findings of the study pave the ways for better understanding of current contaminant levels in selected sites as well the phytoremediation capability of *Senna italica* plant and the possible use of reclaimed soil for sustainable farming.

Materials and Methods

Study location

Asfan, northeast of Jeddah, Saudi Arabia (latitude: 21.53'13.3" N, longitude: 39.15'06.6" E, and elevation: 2.8m) was selected as an experimental site for the collection of soil and plant samples. The weather in Asfan is hot and dry as well as the texture of the soil is sandy. The area has lesser rainfall events for supporting proper plant growth.

Sample collection

The soil samples were collected from the planted and non-planted area from 18–25 cm depth from soil surface. Basically, the sampling was done below the surface to maximize the chances of HMs accumulation in that area due to limited chances of erosion. The sampling was done with the help of a soil sampling auger and the soil samples were collected in plastic zip lock bag and stored in refrigerated box before further

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experimentation. Similarly, the plant samples of *Senna italica* were also collected from nine random sites and root and shoots were separated at same time. These samples were also cleaned gently and after drying stored in zip lock bags and transported to laboratory for HMs analysis.

Sample preparation and heavy metals extraction

Ammonium bicarbonate-DTPA procedures were used to determine the levels of extractable Pb, Cr, Co, Zn, Ni, Mn, Fe and Cd in soil samples. Briefly, 1.97 g of DTPA and 79.06 g of NH4HCO3 was dissolved in 1000 mL distilled water and the pH of the solution was adjusted to 7.60 by adding appropriate amount of NH4OH and HCl. The solution was prepared in an Erlenmeyer flask. After that, 20 mL of the solution was added in 10 g of soil of each soil sample. The flasks were shake well for a period of 15 minutes in a reciprocating shaker at a rate of 180 rpm. Then, samples were digested using a microwave digestion system (Milestone, Ultra-wave), which was programmed as follows: t max = 230 C, Pmax = 1400(W), t time = 20 and 10 minutes. Afterwards, the samples were filtered through Whatman filter paper, and the volume was prepared upto 50 mL mark. These samples were then run on inductively coupled plasma optical emission spectroscopy (ICP-OES) to check the presence of extent of the selected HMs in samples.

For plants, the harvested plants parts (roots and shoots) are oven-dried at 65 °C until the obtainment of constant weight. These samples were then crushed and ground to get fine powder. Thereafter, 0.5 g from each plant samples were added in digestion flask and a di-acid solution of HNO3 and HClO4 (2:1, v/v) was poured in it. These samples were kept overnight for partial dissolution of the organic material. Afterwards, plants samples were digested in microwave digester (a closed digestion system). Thereafter, the digested samples were filtered through Whatmann filter paper, and the volume was marked till 50 mL by adding distilled water in it. These samples were then run on inductively coupled plasma optical emission spectroscopy (ICP-OES) to check the presence of extent of the selected HMs in samples. The translocation factor (TF) values were also determined by observing the total amount of HMs in shoot and root parts by using the following equation (Yu et al., 2011).

$$TF = \text{shoot metal concentration}/\text{root metal concentration} \qquad \text{(Eq.1)}$$

Statistical analysis

The data obtained in this experiment were statistically analyzed using STATISTIX 8.1 software. The analysis of variance (ANOVA) was applied on data to check the level of significance 0.05%. For means comparison, least significance difference test (LSD) was applied to check the significance of metals distribution in various plant parts.

Results

The present study showed a significantly higher concentration of HMs in the soil and plant tissues. The soil samples collected from non-planted area showed significantly higher amount of Cd (7.86 mg/kg) when we compared them with vegetative soil samples (0.47 mg/kg). Moreover, a significant amount of Cd was also observed in root

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(2.32 mg/kg) and shoot (3.19 mg/kg) of *Senna italica* plant. This showed that the plant shoots and roots accumulate 40.7% and 29.5%, respectively of the Cd present in planted soil as compared to non-planted soil (*Fig. 1*). In case of Cd, it was also observed that plant roots translocate more Cd into aerial parts with the TF of 1.38. Similarly in case of Cr contents, the soil samples collected from non-vegetative sites showed substantial amount of Cr (102.1 mg/kg) as compared to vegetative soil samples (20.5 mg/kg) as presented in *Fig. 1*. Likewise, a significant concentration of Cr was also observed in root (i.e., 43.3 mg/kg) and shoot (i.e., 59.0 mg/kg) samples of *Senna italica* plant. Moreover, the plant accumulates 57.8% and 42.4% of the Cr in their shoots and roots parts, respectively with a TF of 1.36 than that of non-vegetative soil.

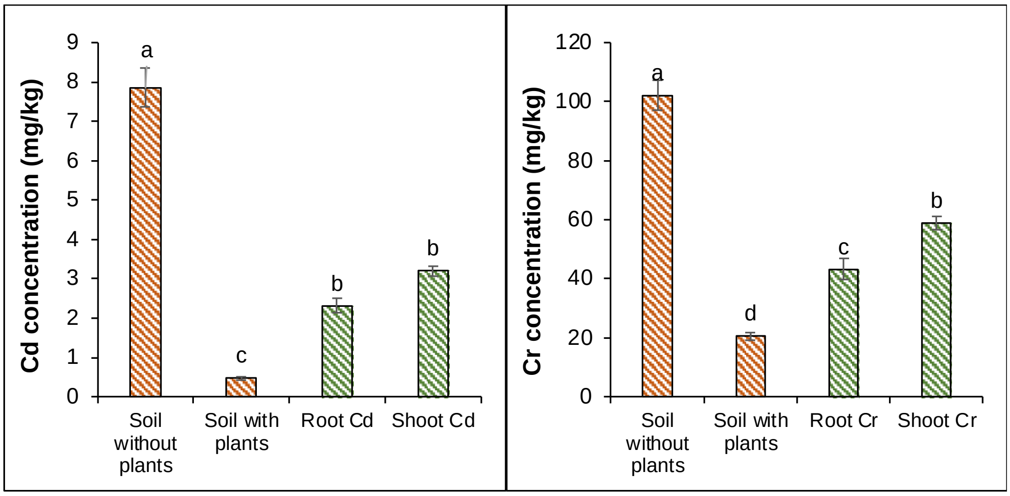

Figure 1. *Concentration of Cd and Cr in soil collected from non-vegetative and vegetative area along with the phytoaccumulation of Cd and Cr uptake in root and shoot of Senna italica plant. Columns represent the mean value of three replicates and error bars on each column present the variation within each mean value. The alphabetical letters on each column denoted the level of significance among different columns by using lease significance difference (LSD) test*

When we talk about the Zn concentration, the soil samples from non-planted sites showed significantly higher amount of Zn (69.1 mg/kg) in comparison to vegetative soil samples (22.3 mg/kg). Likewise, a 49.1 mg/kg and 64.4 mg/kg of Zn was also observed in root and shoot of targeted plant samples (*Fig. 2*). This showed that the plant shoots (93.1%) and roots (70.9%) accumulate higher Zn contents present in vegetative soil as compared to non-planted soil and the TF for Zn was 1.31. Similarly, a higher amount of Cu was also seen in the sampling sites as the soil samples collected from non-planted area contained significantly higher amount of Cu (142 mg/kg) than that of vegetative soil samples (24.0 mg/kg) as shown in *Fig. 2*.

Moreover, a substantial quantity of Cu was also obtained in roots (69.0 mg/kg) and shoots (56.1 mg/kg) samples of *Senna italica* plant. Plant shoots and roots accumulate 39.5% and 48.6% of the Cu from planted soil when compared them with non-planted soil. The TF observed for Cu in this study was 0.81.

In the current study a significantly higher concentration of Fe was found in the soil and plant tissues. However, the soil samples collected from non-vegetative area showed significantly higher amount of Fe (4854 mg/kg) when compared with vegetative soil samples (695 mg/kg) as presented in *Fig. 3*. Moreover, a significant amount of Fe was

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also found in roots (3123 mg/kg) and shoots (1853 mg/kg) of *Senna italica* plant. This indicated that plant shoots and roots accumulate 38.1% and 64.4%, respectively of the Fe available in planted soil as compared to non-planted soil. Whether the TF for Fe was 0.59 in this study. In case of Mn contents, the soil samples from non-planted area showed maximum amount of Mn (227 mg/kg) as compared to vegetative soil samples (25.7 mg/kg). More specifically, a significant amount of Mn was seen in root (134 mg/kg) and shoot (46 mg/kg) of selected plant as presented in *Fig. 3*. This indicated that plant shoots and roots accumulate 20.4% and 59.4% of the Mn present in planted soil as compared to non-planted soil and the TF for Mn was 0.34.

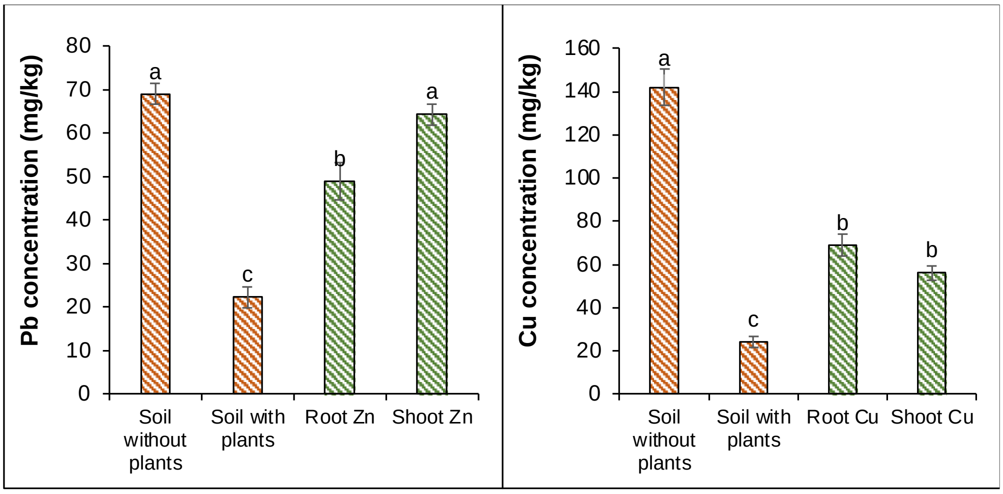

Figure 2. *Concentration of Pb and Cu in soil collected from non-vegetative and vegetative area along with the phytoaccumulation of Pb and Cu uptake in root and shoot of Senna italica plant. Columns represent the mean value of three replicates and error bars on each column present the variation within each mean value. The alphabetical letters on each column denoted the level of significance among different columns by using lease significance difference (LSD) test*

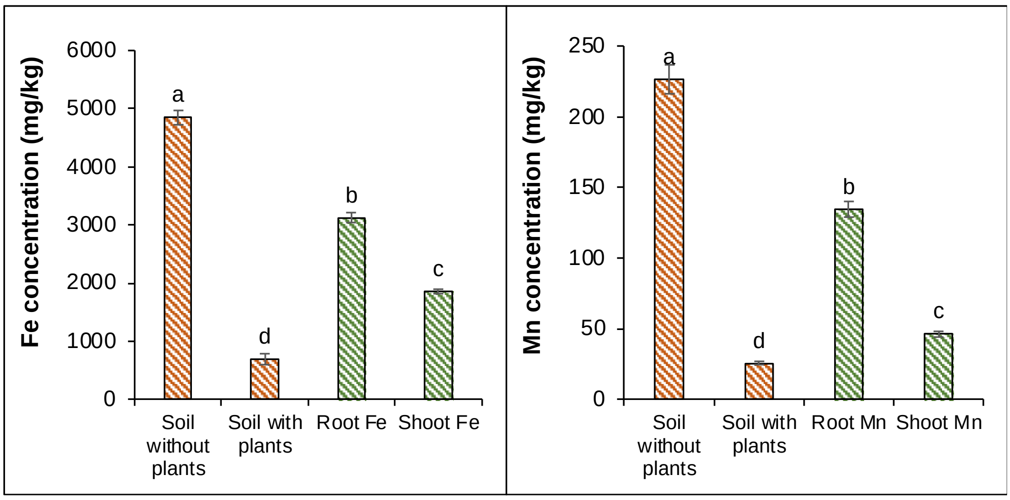

Figure 3. *Concentration of Fe and Mn in soil collected from non-vegetative and vegetative area along with the phytoaccumulation of Fe and Mn uptake in root and shoot of Senna italica plant. Columns represent the mean value of three replicates and error bars on each column present the variation within each mean value. The alphabetical letters on each column denoted the level of significance among different columns by using lease significance difference (LSD) test*

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The concentration of Ni was significantly higher in the soil and plant tissues. The soil samples collected from non-planted area showed significantly higher amount of Ni (88.9 mg/kg) when we compared them with vegetative soil samples (37.0 mg/kg). Moreover, a significant amount of Ni was also observed in root (82.0 mg/kg) and shoot (65.0 mg/kg) of plant. This showed that the plant shoots and roots accumulate 73.2% and 92.2%, respectively of the Ni present in planted soil as compared to non-planted soil (*Fig. 4*). It was also observed that plant roots translocate lesser amount of Ni into aerial parts (TF 0.79). In case of Pb contents, the present study showed a significantly higher concentration of Pb in the soil and plant parts. Moreover, a non-planted soil samples showed significantly higher amount of Pb (82.0 mg/kg) when compared them with planted soil samples (19.3 mg/kg). Moreover, a significant amount of Pb was also observed in root (66.6 mg/kg) and shoot (43.8 mg/kg) of *Senna italica* plant as shown in *Fig. 4*. This showed that the plant shoots and roots accumulate 53.5% and 81.2% of the Pb, respectively present in planted soil as compared to non-planted soil with the TF of 0.65.

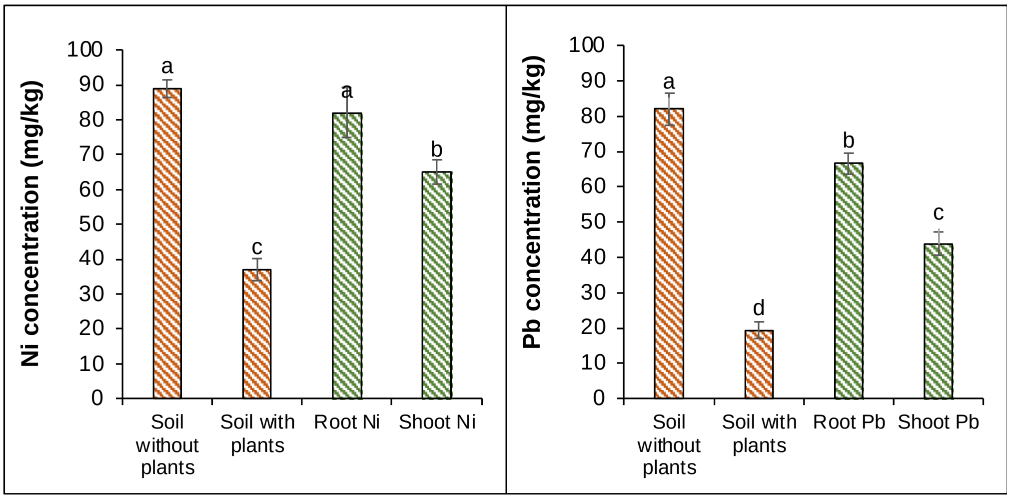

Figure 4. *Concentration of Ni and Pb in soil collected from non-vegetative and vegetative area along with the phytoaccumulation of Ni and Pb uptake in root and shoot of Senna italica plant. Columns represent the mean value of three replicates and error bars on each column present the variation within each mean value. The alphabetical letters on each column denoted the level of significance among different columns by using lease significance difference (LSD) test*

Discussion

Although plants face toxicity when pollutants are above a certain level, they may also have ability to remove certain amount of organic and inorganic pollutants from the medium (i.e., soil and water) through a number of processes such as phytoextraction, phytostabilization. Here, in the present study, *Senna italica* plant was found as a suitable candidate to accumulate a higher concentration of Cd, Ni, Cr, Cu, Zn, Fe, Mn and Pb from contaminated soil in arid climatic conditions.

Cadmium has been shown to have negative impacts on plant growth, development, and metabolism in several studies (Asgher et al., 2015; Shanmugaraj et al., 2019). According to Kieffer et al. (2009), high Cd levels in the rhizosphere can inhibit nutrient absorption, inhibit plant development, and disturb physiological processes (Zulfiqar et

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al., 2022). Similarly, depending on the concentration, the trace element Cr can be either beneficial or poisonous to plants. Chromium is necessary for plant development and metabolism at low concentrations, but excessive amounts can cause phytotoxicity and oxidative stress and limit root development, hinder photosynthesis, and interfere with cellular functions (López-Bucio et al., 2022; Srivastava et al., 2021).

Copper is also a vital micronutrient, necessary for numerous enzymatic reactions and metabolic functions in plants (Mir et al., 2021). Adequate Cu is necessary for plant growth, chlorophyll synthesis, and reproductive development (Hemant Saini et al., 2019; Rehman et al., 2019). However, excessive Cu concentrations can be toxic to plants, leading to oxidative damage and disruptions in cellular functions (Dutta et al., 2018; Mir et al., 2021).

Similar to Cu, Fe is also crucially important for various physiological processes in plants, including chlorophyll synthesis, electron transport, and enzyme activities. Iron deficiency can result in chlorosis and reduced plant growth (Li et al., 2021). Conversely, excessive iron concentrations can induce oxidative stress and impair nutrient uptake in plants (Delias et al., 2022; Galaris et al., 2019). Manganese plays a vital role in plant physiology, including photosynthesis, respiration, and enzyme activation (Schmidt and Husted, 2019). Rhizosphere samples around *Senna italica* showed higher Mn contents relative to non-vegetative control. Adequate Mn levels are crucial for plant growth and development (Arif et al., 2016; Rashed et al., 2021). However, higher Mn concentrations can lead to toxicity symptoms, such as leaf chlorosis and tissue necrosis (Li et al., 2019).

Nickel, despite being considered a trace element, plays a crucial role in cellular redox balance, and in numerous biochemical, physiological, and growth responses (Ameen et al., 2019). Lead is not required by plant for any physiological activities that's why it is poisonous for both plants and animals (Zulfiqar et al., 2019). It reduced the root, shoot lengths and biomass production (Bashmakov et al., 2017). However, *Senna italica* accumulate a higher amount of Pb in their root and shoot part and proved beneficial for rhizoremediation projects. Zinc as an essential micronutrient is very beneficial for the production of DNA, proteins, and hormones in plants (Elshayb et al., 2022). Stunted growth and irregular leaf development can be caused by a Zn shortage (Bhantana et al., 2020). Unbalanced nutrient levels and oxidative stress can result from high Zn concentrations, which can be hazardous to plants (Kaur and Garg, 2021).

Senna italica was able to remove substantial amounts of selected HMs from soil and the results supports the finding of Nasir et al. (2023) who also described more uptake of HMs by leaves and store them in their tissues when initial metal contents were higher in rhizosphere. Pirsarandib et al. (2022) also reported that improved shoot uptake higher amount of HMs in plant tissues when higher contents were present in medium (as seen in the case of Cd, Cr and Zn in current study). Similarly, Riyazuddin et al. (2021) described that root of plants hinders the metallic ions movement towards the stem and leaves and absorb moderate concentration of HMs in shoot than that of root tissues (as seen in Pb, Mn, Ni, Fe, and Cu in the present study).

In this study, *Senna italica* was proved best hyperaccumulator of these HMs as they accumulate more than 60% of the HMs contents in their different plant parts when compared with non-vegetative soil. The roots of *Senna italica* absorbs more Pb, Cu, Fe, Mn and Ni in respect to other parts and these results are in direct match with the findings of Mahfooz et al. (2020) who also stated more buildup of metals in below ground parts. *Senna italica* was also able to remove substantial amounts of Cd, Cr and Zn from soil, but more of these metals were found in aerial parts rather than the underground tissues

(Raza et al., 2020). Gupta et al. (2019) referred plant uptake more Cd, Cr and Zn in their tissues and it might be due to mobile nature of Cd, Cr and Zn elements and they were translocated to the upper plant parts i.e, shoots and leaves. Edelstein and Ben-Hur (2018) expressed same results, when Pb and Ni concentration is increased in medium, the plant roots absorb maximum from it and a moderate quantity of these metals were transported to the above ground parts. This might be due to the fact that soil might contain several kinds of cation and anions that make complexes and retains a sufficient amount of these HMs and make them unavailable for plant uptake (Derakhshan Nejad et al., 2021; Kamran et al., 2021).

Results from this study confirmed that *Senna italica* is hyperaccumulator for Pb, Ni, Cu and Fe. These findings are in match with the results of Raza et al. (2020) who expressed Cassia italica hyperaccumulate Pb and Ni in their plant parts. There could be justify that soil acidity from nitrogen fixation may be accountable for this rise in HMs concentration (Yu et al., 2020). Cation absorption could be boosted by a net outflow of H+ ions into the soil. Even a slight change in pH can affect growth and nutrient availability. The higher bioaccumulation of HMs in plans could be due to the presence of symbiotic bacteria in the rhizosphere that can stimulate plant growth through the production of plant growth-promoting substances such as phytohormones and enzymes (Nazli et al., 2020; Qin et al., 2024). These substances can enhance root development, nutrient uptake, and overall plant vigor, potentially counteracting the negative effects of drought stress on the availability and uptake of elements.

Conclusions

Soil contamination with HMs is a serious threat for global food security and effective phytoremediation strategies are a helpful too for reclamation of soil and sustainable food production. The HMs such as Cd, Ni, Cr, Pb, Zn, Fe, Mn and Cu are highly toxic for plants in excessive quantities. However, the phytoremediation potential of *Senna italica* plant is quietly important for better reclamation of the soil. The plants accumulate higher amount of these HMs in their root and shoots parts. The metals like Cd, Cr and Zn were found higher in aerial parts while the other metals like Cu, Pb, Ni, Fe and Mn were found higher in roots tissues. The soil having the vegetation (grown *Senna italica* plant) on it, showed lesser remaining contents of HMs, which highlight the importance of this plant as a hyperaccumulator for various HMs polluted soils even in harsh environmental conditions. After reclamation the soil could be used for sustainable food production to meet the higher food demand of nation.

Conflict of Interests. The author declares that there are no conflicts of interest related to this article.

Data availability statement. The data that support the findings of this study are available upon reasonable request from the author.

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