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## Assessment of heavy metals uptake of native plant species naturally grown in soil irrigated with treated wastewater at Serapium agroforest, Ismailia City

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In the Egyptian desert, Serapium Agroforest uses sustainable land management and eco-friendly phytoremediation. The present study examined the phytoremediation efficacy of native plants growing naturally on soil irrigated with treated wastewater in Serapium agroforest, Ismailia City. The native plants were *Hyoscyamus muticus*, *Zygophyllum album*, *Alhagi gracourum*, *Chenopodium mural*, *Malva parviflora*, *Senecio vulgaris*, and *Withania somnifera*. All the analyzed heavy metals (HMs) (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Co) were under safe levels in recorded species. The seven native plants were grown on pH 7.9 soil with a PLI of 0.87. Pb increases with soil pH. Seven species' phytoremediation effectiveness (BCF), translocation factor (TF), and shoot enrichment coefficient (TC) were considered. *Malva parviflora* had the highest BCF and TC for Pb (1.15), whereas all other species had BCF and TC below unity. TF of HMs was found higher-than-unity in reported species. Results show that all species can grow in slightly alkaline soil with low HMs. *Malva parviflora* was best for phytoextraction and Pb cleanup.

**Keywords:** Native plants- Heavy metals- Soil, Serapium agroforest, Ismailia City

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### INTRODUCTION

Water contamination can take several forms of which wastewater is a major form (Owhonka & Otto, 2023). The growth of human activities, including urbanization, industry, and agriculture, has led to an escalation in the discharge of wastewater (Sun *et al.*, 20168; Koop & van Leeuwen, 2017, Rastmanesh *et al.*, 20188; Borah *et al.*, 20208; Lam *et al.*, 2022). Significant damages and other difficulties can result from industrial effluent being discharged into nature (Bora *et al.*, 2023). Untreated wastewater supplies contain persistent, non-biodegradable, toxic heavy metals. Heavy metals remain in the environment and can be accumulated by living organisms, posing a severe public health risk and causing significant damage to the surrounding flora, fauna, and people (Xie *et al.*, 20198; Pandey & Tripathi, 2014). Ominously, metals persist in soil for substantially longer durations than in other biosphere compartments. According to Sajinkumar & Rani, 2017, metal pollution can harm local plants, animals, and humans. This can happen due to metallurgical processes, untreated industrial effluent, mining, pesticide use, or sewage sludge. Arsenic, cadmium, chromium, nickel, lead, and other heavy metals are classified as carcinogenic to both humans and animals by the International Agency for Research on Cancer (Sainger *et al.*, 2011). Natural and industrial processes have quickly increased soil heavy metal contamination (Xie *et al.*, 20198; Pandey & Tripathi, 2014). Many nations now require environmentally friendly technologies, and natural wastewater treatment systems like built wetlands (CWs) are included in their planning processes. Egypt's municipal, industrial, and agricultural operations

produce wastewater due to the country's rapidly expanding population (Elbana & Elbana, 2017). Strict discharge rules lower the permitted number of contaminants in waste streams by reducing the expense of disposing of the generated wastewater (Bora *et al.*, 2023). Some scientists (Birost & Palahi, 2011; Giuseppe *et al.*, 2010) found that agroforests improve regional water cycles. Advanced bioengineering technology must be used to treat raw sewage to recover valuable water for diverse uses. Sewage treatment removes most organic matter and dangerous bacteria. Significant steps are being taken to reduce heavy metal concerns in sewage effluents.

Egypt faces significant challenges due to water scarcity, limited freshwater resources, and rapid population growth. However, scientists see that one of the potential solutions that can help address the problems of water scarcity and deforestation in Egypt is the use of treated wastewater for planting and irrigating tree forests. Several projects have been implemented in Egypt to explore the feasibility of using treated wastewater in afforestation. One of the most prominent projects is the "green Sinai" initiative which aims to plant 2 million acres of trees in the Sinai Peninsula using treated wastewater. In addition, another project, the "New Delta" project, aims to plant one million acres of trees in the Nile Delta region (E1-Kady & E1-Shibini, 2001; Elbana *et al.*, 2017; Gaber *et al.*, 2021, Nikiel & Eltahir, 2021; Fouad *et al.*, 2023). Egypt hurred the "National Program for the Safe Use of Treated Wastewater for Afforestation." This program aims to recover the environment by exploiting wastewater to plant trees in deserts and

then to encourage the long-standing practice of watering tree plantations with sewage (El Kateb & Abdallah, 2022). Trees may absorb trace elements from soil, water, or air and store them for an extended period, making them useful in phytoremediation. This technique delays soil pollutant-induced ecological degradation (Bhat *et al.*, 2022b). Many methods to use plant metal removal can be followed via exclusion or accumulation, depending on the plant (Malik & Qader, 2023). The five most efficient strategies for treating sites with little organic, nutritional, or metal contamination are phyto transformation, rhizosphere bioremediation, phytostabilization, phytoextraction, and rhizofiltration (Raskin & Salt, 1997).

Zhu *et al.*, 2016 discovered that biodiversity and a favorable climate for species make phytoremediation good treatment for contaminated areas. Serapium forest as an example of afforestation, located two hours from Cairo (30°28'44.72" N & 32°11'32.47" E), is considered part of the program of the Egyptian government in the nineties. The 200-hectare farm contains natural and non-native plants of commercial importance, including eucalyptus and mahogany. The researchers noted that watering the trees with sewage helps them grow in nutrient-poor soils. Wastewater provides enough nutrients to avoid fertilizers. The wastewater used for watering those trees is at the second stage of purification. (Ghorab & Abd El-Lah, 2017) reported that, after irrigation with primary drainage water, some species of trees of the Serapium forest in Ismailia have increased biomass, stem size, and basal area. Organic matter particles bind and hold positively charged cations, making treated wastewater irrigation more successful than freshwater irrigation. This ensures that the roots have plenty of water and nutrients. Egypt yielded 4.5 times more particular tree species from forest plantations than Germany, Europe's largest forest country (Paschen *et al.*, 2023).

Studying vegetation dynamics in existing regions, community structure, and vegetative function are the most essential environmental aspects of forests that respond differently to environmental and anthropogenic activities. Restoration and re-vegetation planning must include annual plants because they can store a lot of water and have higher organic matter and phosphorus content for plant uptake, providing more biomass for perennial plants

(Gairola & Todaria, 2008). Floristic composition and forest structure can also identify ecologically and commercially important plants, according to (de Aledo *et al.*, 2023). This makes it crucial to examine the phytoremediation abilities of native plants that are grown naturally in agroforestry in arid regions. Heavy metals can accumulate in plant tissues without generating toxicity after phytoremediation (YaşarOzyigit & Serin, 2010; Nsanganwimana *et al.*, 2014). The key benefits are its low cost and universal acceptance. (Al-Moshaddak & El-Zohri, 2020), and (Kumar *et al.*, 2016) reported that natural halophytes, including *Sporobolus virginicus*, *Phragmites australis*, and *Aeluropus lagopoides* are excellent phyto-stabilizers. While *Malva parviflora* is a good candidate for a phytoremediation strategy because it can absorb nearly all HMs, including Pb, Zn, Cu, Ni, and Cd. Immediate timely scientific interventions are needed to support the conservation of native plants in Serapium agroforestry and assess their phytoremediation potential. Additionally, studies on floristic composition and management strategies for desert afforestation using wastewater for irrigation are lacking. This study aimed to determine how well native plants thriving in soil watered with treated wastewater performed as phytoremediators in the Serapium Agroforestry site in Ismailia City.

## MATERIALS AND METHODS

### Study sites

The investigation region is situated in the desert landscape of the northern Egyptian Governorate of Ismailia, at coordinates 30°28'44.72" N and 32°11'32.47" E. At a height of about 30 m above sea level, the estimated precipitation is 29 mm/year, with annual temperatures of 21.6 °C, relative humidity of 53.9%, and winds of 2.5 m/s. The total evapotranspiration (ET<sub>o</sub>) is 1821 mm /year.

### Plant identification and phytochemical analysis

Fifty plots, each measuring 1.5 m<sup>2</sup>, were randomly chosen in three places (A1, A2, and A3) within the Serapium agroforest in Ismailia City. These plots were selected following regular evaluations of the growing areas of young *Eucalyptus* sp. and *Kahya* sp. during the winter and spring seasons of 2022-2023. The plants were classified based on the taxonomic system developed by (Täckholm, 1974; Täckholm, 1976) and updated by (Boulos, 1989; Boulos, 1995). The Botany

Department of the Faculty of Women for Arts, Science & Education of Ain Shams University houses all voucher specimens. The shoots and roots of the species under investigation were gathered from multiple locations inside the cultivated fields of the Serapium Agroforest in Ismailia Governorate, Egypt. A mixed-acid digestion approach was employed to remove heavy metals from samples weighing between 0.5 and 1 g. Analytical testing was conducted on Co, Al, Cd, Cr, Cu, Fe, Mn, Ni, and Pb using inductively coupled plasma mass spectrometry (ICP-MS).

### Water and soil analysis

The irrigation water was mostly treated wastewater extracted from Serapium oxidation pond. It contained industrial and domestic influence. Each stand had three composite soil samples taken 0–50 cm below the soil surface. Inductively coupled plasma mass spectrometry was used to detect heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Co) in soil and water samples. Three composite soil samples from each stand captured a vertical profile from 0 to 50 cm below the soil surface. The assessment of soil texture was conducted using the approach described by Hendry and Grime (1993). The quantification of oxidizable organic carbon (OM) was performed using the rapid titration method developed by Walkely and Black, as described by Gelman et al. (2012). Salinity (EC) and pH measurements were obtained from soil water extracts using a 1:5 ratio, following the guidelines of APHA (2008).

### Analysis of data

The mean, range, and standard deviation of plant and soil samples were calculated statistically. ANOVA was used to compare heavy metal averages across the species. High soil heavy metal concentrations are linked to organic matter (OM), electrical conductivity (EC), pH, sand %, silt %, and clay %. The significance of the results was assessed using a P-value of < 0.05. The statistical analyses were done using SPSS 20 (SPSS Inc., Chicago, IL, USA).

### Contamination Factor (CF)

The contamination factor (CF) of the soil samples was calculated using the following equation:

$$CF = C_{\text{metal}} / C_{\text{background}}$$

Where CF: is the ratio of the concentration of each metal in the soil (C metal) to the baseline or background value (C metal), which is the average concentration of the metal in unpolluted soil. 0 indicates no pollution, 1 indicates low to moderate pollution, 2 indicates moderate pollution, 3 indicates moderate to high pollution, 4 indicates high pollution, 5 indicates high to very high pollution, and 6 indicates very high pollution (Muller, 1969; Ajeh et al., 2022; Bhuyan et al., 2023; Zhang et al., 2023).

### Pollution Load Index (PLI)

This study calculated the Pollution Load Index (PLI) for the entire sampling site by taking the nth root of the product of the n CF values. As mentioned by Tomlinson et al., 1980, the PLI for a site is calculated by multiplying the nth root of n contamination factor (CF) values. The equation for CF is:

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \dots \times CF_n)^{1/n}$$

This simplifies and compares heavy metal contamination assessment. Taking the nth root of the product of the n CFs yielded the sampling location's PLI (Usero et al., 1997; Morillo et al., 2004).

### Phytoremediation factors

The bioconcentration factor (BCF), translocation factor (TF), and transfer coefficient (TC) were calculated to assess the absorption and storage of heavy metals in various plant components, as detailed in references (Antoniadis et al., 2017; Subramanian & Shanks, 2003; EPA, 2000; Shaheen et al., 2013; Baker & Galloway, 2014). The calculation of these factors was performed in the following manner:

*Translocation Factor (TF)*

$$= \text{Metal concentration in shoots} / \text{Metal concentration in roots}$$

*Bioconcentration factor (BCF)*

$$= \text{Metal concentration in plant} / \text{Metal concentration in soil}$$

*Transfer coefficient (TC)*

$$= \text{heavy metal concentration in shoot} / \text{the same heavy metal in soil}$$

## RESULTS AND DISCUSSION

### Plant identification and phytochemical analysis (Heavy metals concentrations in the studied species)

The families, life forms, and floristic groups of the species documented in the 50 stands that were examined are detailed in Table 1. Seven genera and six families comprised the seven species that were reported. There are two species in the Solanaceae

family: *Withania somnifera* and *Hyoscyamus muticus*. In contrast, the following families and orders are represented by one species each: Fabaceae, Zygophyllaceae, Chenopodiaceae, Malvaceae, and Compositela (*Alhagi grocourum*, *Zygophyllum album*, *Malva parviflora*, and *Senecio vulgaris*, respectively).

Numerous studies have emphasized that the accumulation of heavy metals in plant tissues results in the suppression of growth and decreased yield. In severe instances, it might lead to a lack of permanency in plants (Ashraf & Foolad, 2007; Morkunas et al., 2018; Bhat et al., 2022a; Briffa & Blundell, 2020). Resilient and tolerant weed species thrive in severely contaminated environments. Phytoremediation which involves employing these and other non-consumable plants effectively prevents toxins from entering the food chain (Gautam et al., 2023; Wang et al., 2021; Jaskulak et al., 2021 and Xu et al., 2022). Table 2 shows the different heavy metal concentrations in terrestrial plants and the examined species. Terrestrial plant heavy metal concentrations typically range: Al (200-1000 mg/kg) by Chenery et al., 2019, Cr (0.006 -18 mg/kg) by Kostic et al., 2019, Co (0.1-10 mg/kg) by Palit and Talukder, 1994, Cd (0.2-2.4 mg/kg), Cu (2-20 mg/kg), Ni (1-5 mg/kg), Fe (70-2486 mg/kg), Pb (1-13 mg/kg), and Mn (20-700 mg/kg) by Kostic et al., 2019. In recorded species, aluminum (Al) ranged from 80.25 to 615 (mg/kg), cadmium (Cd) was not detected (under the analysis system), chromium (Cr) ranged from 1.75 to 4.06 mg/kg, copper (Cu) from 3.56 to 12.06 mg/kg, iron (Fe) from 107.6 to 952.5 mg/kg, manganese (Mn) from 8.7 to 34.25 mg/kg, nickel (Ni) from 1.23 to 5.51 mg/kg, and lead (Pb) from 0.75 to 5. Fe and Al were the most abundant heavy metals in all plant species. The quantities of heavy metals (HMs) in the examined indigenous plants are displayed in Table 3. According to Kostic et al., (2019), Zayed and Terry (2003); Palit et al., (1994), and Chenery et al., (1949) all heavy metals in normal natural concentration intervals for land plants, except Ni concentration (5.51 mg kg<sup>-1</sup>) in *Zygophyllum album*, which was above the normal levels in plants. Statistically, (HMs) showed a highly significant difference in each concentration between the studied native plants (Table 3). Plant species exhibited that the maximum concentration of metals (Fe, Mn, and Pb) was in *Malva parviflora*, (Al and Cr) in *Hyoscyamus muticus*, (Cu and Co) in *Chenopodium murale*, and (Ni) in *Zygophyllum album*.

Typically, most plant species, particularly those used for farming, interpret aluminum tolerance as the ability to disregard aluminum (Chenery, 1949; Zhu et

al., 1996; Giani et al., 2005; Harrison, 2005, Dassey & Theegala, 2014, Vetrimurugan et al., 2016; VetrimuruganKarthikeyan & Lakshmanan, 2017; Nazarudin et al., 2022). The aluminum (Al) content across the sampled native plants displayed a wide range, spanning from 80.25 mg kg<sup>-1</sup> to an impressive 615 mg kg<sup>-1</sup>. Among these, *Hyoscyamus muticus* and *Malva parviflora* emerged as the frontrunners, showcasing Al levels of 615 mg kg<sup>-1</sup> and 614.5 mg kg<sup>-1</sup>, respectively. At the opposite end of the spectrum, *Alhagi grocourum* recorded the lowest Al concentration, setting a contrasting baseline. These results provide compelling evidence that the studied plant species fall comfortably into the category of non-accumulators of aluminum, as none breached the threshold of 1000 mg Al kg<sup>-1</sup> required for classification as Al accumulators. This categorization aligns with the seminal work of Chenery (1949), who proposed a clear dichotomy: plants with Al concentrations of ≥1000 mg kg<sup>-1</sup> were deemed aluminum accumulators, while those below this level were considered non-accumulators. While the numbers tell a straightforward story, the ecological implications are intriguing. The modest Al levels in these plants may reflect adaptive strategies to thrive in aluminum-laden soils without allowing toxic accumulation. It also invites further exploration into the biochemical mechanisms regulating Al uptake and distribution in non-accumulators, offering a fertile ground for research.

The Cr concentration varied between 1.25 and 4.06 mg kg<sup>-1</sup>, with the greatest levels in *Hyoscyamus muticus* and the lowest in *Senecio vulgaris*. Excessive concentrations of Cr, over 2 mg kg<sup>-1</sup>, can lead to plant toxicity (Bonanno & Lo Giudice, 2010; Hassan & Aarts, 2011). Chromium (Cr) levels in *Alhagi grocourum*, *Senecio vulgaris*, and *Withania somnifera* were within the safe limit, while they exceeded the safe limit in *Zygophyllum album* *Hyoscyams muticus*, *Chenopodium mural*, and *Malva parviflora*. The absorption of copper (Cu) by plants is contingent upon their capacity to transport the metal across the interface between the soil and roots, as well as the overall quantity of copper in the soil (Kumar et al., 2021; AzoozAbou-Elhamd & Al-Fredan, 2012; Shabbir et al., 2020). Plants require low quantities of Cu for optimal growth. Cu is a crucial component of enzymes and is absorbed in the form of the divalent cation (Cu<sup>2+</sup>) or as a copper complex. Copper frequently occurs in elevated amounts, which causes harm to living organisms according to Sulaiman and his colleagues (2014).



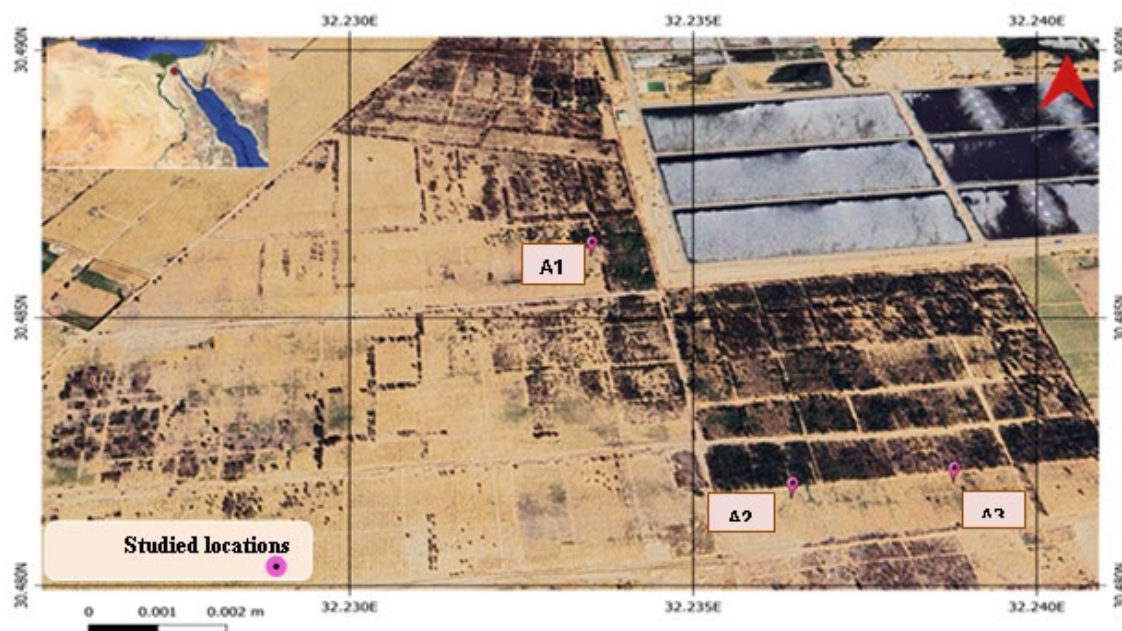


Figure 1. Map of three investigated areas in Serapium agroforestry, Ismailia.

Table 1. The families, life forms, and floristic groups of the studied species

	Family	Life span	Life Form	chorotype	Salty resistance
<i>Alhagi grocourum</i>	Fabaceae	perennating	hemicryptophyte	Med - Irano-Turanian	grows in salty and non-salty habitats
<i>Zygophyllum album</i>	Zygophyllaceae	perennating	chamaephyte	Saharo-Arabian	halophyte
<i>Hyoscyamus muticus</i>	Solanaceae	perennating	chamaephyte	Saharo-Arabian	glycophyte
<i>Chenopodium mural</i>	chenopodiaceae	Annual	Therophyte	COSM	glycophyte
<i>Malva parviflora</i>	Malvaceae	Annual	Therophyte	ME+IR-TR	glycophyte
<i>Senecio vulgaris</i>	Compositae	Annual	Therophyte	ME+SA-SI+IR-TR	glycophyte
<i>Withania somnifera</i>	Solanaceae	perennial	chamaephyte	Med - Irano-Turanian	glycophyte

Table 2. The typical ranges of heavy metal concentrations in terrestrial plants and ranges of heavy metal concentrations in the investigated native plants were presented

	Range in studied plants (mg/kg)	The normal range in the plant (mg/kg)	Ref
Al	80.25 - 615	200 - ≥1000	81
Cd	n.d	0.2-2.4	73
Cr	1.25 - 4.06	0.006 -18	76
Cu	3.56 - 12.06	2.0 -20	73
Fe	107.66 - 952.56	70 - 2486	73
Mn	8.7 -34.26	20-700	73
Ni	1.23 - 5.51	1-5	73
Pb	0.75 - 5.76	1_13	73
Co	1.82 - 3.32	0.1-10	74

Table 3. Heavy metal values with standard deviation in the investigated native plants, grown at Serapium Agro Forest, Ismailia city

Species	Heavy metals (mg/Kg)							
	Al	Cr	Cu	Fe	Mn	Ni	Pb	Co
<i>Alhagi grocourum</i>	80.20 ±0.10	2.±0.1	3.57±0.67	107.67±2.08	16.63±0.64	1.23±0.06	1±0.10	1.82±0.11
<i>Zygophyllum album</i>	185.17±5.80	3.07±0.25	5.08±0.45	270. ±13.23	8.70±1.14	5.52±0.95	3.10±0.2	2.05±0.09
<i>Hyoscyamus muticus</i>	615.00±8.89	4.07±1.05	10.27±1.97	687.50±12.60	21.33±6.11	2.70±0.2	1.03±0.15	2.88±0.16
<i>Chenopodium mural</i>	436.90±64.64	2.02±0.32	12.07±1.05	435±12.77	21±4.00	2.37±0.32	1.53±0.35	3.30±0.2
<i>Malva parviflora</i>	614.60±5.41	3.50±0.43	10±2.65	952.57±26.64	34.27±0.84	3.03±0.9	5.77±1.21	2.73±0.65
<i>Senecio vulgaris</i>	180 ±27.84	1.25±0.21	5.10±1.06	220.07±18.14	12.07±2.23	1.25±0.02	0.75±0.06	2.03±0.25
<i>Withania somnifera</i>	322.53±0.36	1.75±0.01	7.03±1	515±9.54	28±1.35	1.70±0.22	2.75±0.15	2.48±0.18
F value	190.53***	14.66***	14.69***	1106.78***	26.41***	24.25***	39.75***	9.99***

Exposure to dosages over  $20 \text{ mg kg}^{-1}$  can lead to the manifestation of toxicity (KlokeSauerbeck & Vetter, 1984). The concentration of Cu in all plant species varied between  $3.56$  and  $12.06 \text{ mg kg}^{-1}$ , indicating that no hazardous levels of copper were detected. The maximum value was observed in *Chenopodium murale*, while the minimum was found in *Alhagi grocourum*. However, both values are within the normal range, as indicated in Table 2. Iron (Fe) content varied between  $107.6$  and  $952.56 \text{ mg kg}^{-1}$ . The greatest concentration ( $952.56 \text{ mg kg}^{-1}$ ) was found in *Malva parviflora*. The iron concentration in *Malva parviflora* exceeded the reported values by Kostic and his colleague (2019) for the typical natural range of iron concentration in land plants ( $70$ – $2486 \text{ mg kg}^{-1}$ ).

Additionally, the iron concentration in *Malva parviflora* was higher than that reported in *Malva sylvestris* ( $568 \text{ mg kg}^{-1}$ ) by (Kostic et al., 2019), collected from an urban area contaminated around Niš city, Republic of Serbia. Aloud et al. (2022) documented significantly elevated Fe concentrations in *Malva parviflora*, measuring  $2308 \text{ mg/kg}$ , when cultivated in industrial environments in Saudi Arabia. The surrounding soil in these areas exhibited Fe levels ranging from  $1303.5$  to  $6478.5 \text{ mg/kg}$ . These examples support the observation that Fe concentrations in *Malva parviflora* vary based on regional environmental factors and anthropogenic influences. The Fe concentration of  $952.56 \text{ mg/kg}$  in our study exceeds most reported values, which may indicate a localized increase in Fe levels due to specific soil ( $11745 \text{ mg/kg}$ ) or wastewater conditions.

Manganese (Mn) is vital for plant development, but excessive amounts of Mn in the soil can significantly impede plant growth (Ghori et al., 2016, Kumar et al., 1995, Hashmi & Varma, 2018). According to Stanojković-Sebić et al. (2017), Manganese (Mn) accumulates in plant organs when there is a high concentration of this element in the soil, along with low pH values and strong redox potential (Hashmi & Varma, 2018; Stanojković-Sebić et al., 2017). The Mn concentrations varied between  $8.7$  and  $34.26 \text{ mg kg}^{-1}$ , and no dangerous levels of Mn ( $>400 \text{ mg kg}^{-1}$ ) were detected. This suggests that the proximity of the soil did not lead to an elevated Mn concentration in the plant material. *Malva parviflora* exhibits a peak value, while *Zygophyllum album* demonstrates a trough. Nevertheless, the concentration range discovered was comparable to the one predicted by Glavač et al., (2017) for wild medicinal plants ( $7.8$ – $75.4 \text{ mg kg}^{-1}$ ) in Meža Valley (Slovenia), where the soil was

contaminated. Nickel (Ni), like chromium, is a non-essential metal. However, certain plants, known as hyperaccumulators, can accumulate nickel in the vacuoles of their leaves. This helps as a shielding mechanism in contradiction of nickel toxicity in these plants (Sainger et al., 2011).

The typical concentrations of heavy metals in plants growing in unpolluted soil ranged from  $0.1$  to  $3.7 \text{ mg kg}^{-1}$  for Nickel (Ni), according to McComb et al., (2015). The concentrations of nickel varied from  $1.23$  to  $5.51 \text{ mg kg}^{-1}$ . The highest observed value of the *Zygophyllum album* exceeded the normal values, surpassing  $5 \text{ mg kg}^{-1}$ . This outcome could be attributed to resilient species originating from the *Zygophyllum* genus, which have adapted to harsh and arid environments, according to Amini-Chermahini (Amini-Chermahini et al., 2014). The Ni content ( $1.23$  and  $1.25 \text{ mg kg}^{-1}$ ) was found to be the lowest in *Alhagi Grocourum* and *Senecio vulgaris* respectively. The possible cause for this could be the low nickel concentration in the soil, which is  $20 \text{ mg kg}^{-1}$ , below the typical range of  $50 \text{ mg kg}^{-1}$ . Lead exhibits a toxic and carcinogenic effect on animals, resulting in the development of liver, skin, and lung cancer, as well as alterations in hematological parameters (Kochare & Tamir, 2015). Plants can gather lead from the soil or air (Collin et al., 2022, Sharma & Dubey, 2005). The amounts of lead (Pb) in the studied species were not higher than normal threshold in terrestrial plants. The range of Pb concentrations was between  $0.75$  and  $5.76 \text{ mg kg}^{-1}$ , with the highest concentration found in *Malva parviflora* and the lowest in *Senecio vulgaris*. The data fall below the critical concentration range for animal nutrition ( $10$ – $30 \text{ mg kg}^{-1}$ ) and the hazardous level ( $20 \text{ mg kg}^{-1}$ ). The present data agreed with those obtained by Collin and his colleague (2022) as well as Sharma & Dueby (2005). They reported environmental lead deposition is rising due to human activity. The normal Cobalt (Co) levels in plants are reported to range from  $0.1$  to  $10 \text{ mg kg}^{-1}$  of dry weight; its significant function as a trace element has been elucidated by some researchers (Kalaivanan & Ganeshamurthy, 2015, Palit et al., 1994). Table 2 shows that Co values were within the normal range of  $1.82$ – $3.3 \text{ mg kg}^{-1}$ . *Chenopodium murale* had the highest value, while *Alhagi Grocourum* had the lowest.

This study revealed that the investigated native plants are capable of accumulating heavy metals (Al, Cr, Pb, Fe, Cu, Mn, Ni, and Co) within the allowable limits set for plants. The release of immobilized metal(loid) into

the aqueous phase is mostly controlled by soil environmental variables. The primary parameters governing the possible release of immobilized metal(loid) into the aqueous phase are pH and redox conditions. Root uptake of heavy metals depends on soil physico-chemical characteristics, which may impact metal availability. Acidic soils contain more easily transportable heavy metals. Heavy metals are free ions or soluble organometals at low pH [44,45]. Native plants in arid soil are exposed to heavy metals (HMs) and high pH and electrical conductivity, which create alkaline and saline environments. The process of adsorption is widely acknowledged for its cost-effectiveness and efficacy in removing metal (loids) from soils. However, the lack of specificity for numerous pollutants can be viewed as a drawback (Abou Jaoude et al., 2019; Li et al., 2023).

#### Water and soil physicochemical parameters

The levels of accessible heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Co) in the primary treated wastewater were within the permitted range according to the recommendations provided by the FAO (Table 4). This result corroborates the findings of Mohamed et al., 2007; Mohamed & Rahman, 2022; DrechselZadeh & Salcedo, 2023 for the identical location. Table 5 displays the essential physical and chemical characteristics of the soil. The topsoil was obtained from various sample sites in the region where *Eucalyptus* sp., and *Khaya* sp exist. The examination revealed that the sample consisted of 92.73% sandy particles, 3.36% silt particles, and 4.21% clay particles. The sandy soil was averaged across all sites. Organic matter (OM) = 0.043%; EC = 1.1 dS/m. The sodium adsorption ratio (SAR) determines agricultural irrigation water suitability. SAR of soil samples is 3.03. According to USSS, the SAR of soil samples is classified as low  $\text{Na}^+$  water. High sodium levels in irrigation water will generally reduce the penetration rate due to soil dispersion and structural degradation (Shainberg & Letey, 1984). Specifically, pH is one of the primary parameters controlling the possible release of immobilized metal(loid) to the aqueous phase (Li et al., 2022, Fan et al., 2023). According to Dietrich, (2013), soil with a pH of 7.9 is somewhat alkaline. Some studies found that irrigation with wastewater raised soil pH (Mohammad & Rousan, 2007). These findings are consistent with those of several others for the same site (Shawky & Mohamad, 2022, Ghorab et al., 2017).

Table 6 illustrates the soil's average heavy metal concentrations and plant-harming values. The soil at

the specific location had elevated levels of Cd ( $0.50 \text{ mg kg}^{-1}$ ) and Co ( $14.43 \text{ mg kg}^{-1}$ ) due to heavy metals in wastewater used for irrigation, surpassing the typical soil concentrations (Nagarajan et al., 2023; Tomasek et al., 2022; Lam et al., 2022) as in (Table 4), but below the threshold considered toxic for plants as reported by (Ahmed et al., 2023; SharmaSingh & Tong, 2022; Alamri et al., 2021). Bowen (1997) found soil Cu, Cr, Pb, Mn, and Ni below a normal range. The WHO report's maximum iron level is  $50,000 \text{ mg kg}^{-1}$  although Fe concentrations were below that level. Table 7 shows no significant relationship between soil parameters (EC, sandy soil %, and clay soil %) and heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb & Co). OM content correlated negatively with Cr. OM affects soil chromium availability and structure by adsorption, direct reduction, and indirect reduction (Xia et al., 2019). The result showed that Pb correlates positively with pH (7.9). That means the solubility of Pb in soil samples increased with pH soil raised. Keran et al. (2008) recorded that soil pH affects lead solubility. Our results agree with the finding of many Pb-contaminated soils in the world which are alkaline with a soil pH above 7.6, including urban soil in Australia (Halim et al., 2005), a soil located near a smelting site in China (Zhu et al., 2004), a soil exposed to heavy fallout of traffic exhausts in Egypt (Abouloos et al., 2006), and a Pb mine-impacted soil in the USA (Weber et al., 2015).

#### Contamination factor and pollution load index

According to Lacatusu (2000), ten contamination categories are identified based on contamination factors (CF): < 0.1 very slight contamination; 0.10-0.25, slight contamination; 0.26-0.5, moderate contamination; 0.51-0.75, severe contamination; 0.76-1.00, very severe contamination; 1.1-2.0, slight pollution; 2.1-4.0, moderate pollution; 4.1-8.0, severe pollution; 8.1-16.0, very severe pollution; and >16 excessive pollution. In Table 8, the highest CF value for Co indicates substantial contamination of this metal (CF = 0.76) while Al (0.2), Cd (0.17), and Mn (0.24) indicate very small contamination. Nevertheless, CF values of Cr (0.41), Cu (0.50), Fe (0.25), Ni (0.29), and Pb (0.24) were slight contaminations in the studied site. The contamination factors indicated low metals contamination for the studied heavy metals (CF < 1). The following sequence was visible in terms of the average CF values obtained in the present study: Co > Cu > Cr > Ni > Pb > Mn > Cd > Al. As represented in Table 6, PLI (0.87) was found less than one demonstrating that the soil site has not been exposed



**Table 4.** Water analysis in 2022 and FAO recommendation values of heavy metals.

Parameter	pH	SAR	Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Co
Certain study	6.6	0.5355	0.27	<0.001	0.01	0.06	1.77	0.08	<0.001	<0.001	<0.001
Mohamed <i>et al.</i> (2022)	6.6	4.87	-	0.004	0.006	-	4.5	-	0.03	0.08	0.01
FAO, 1999	6.0-6.5	0.2-0.7	-	0.01	0.1	0.20	5	0.20	0.2	5	0.05

**Table 5.** Basic soil physical and chemical properties Eucalyptus and Khaya-grown soils from multiple test sites

	PH	E.C	SAR	Sand%	silt %	clay%	OM
average	7.95	1.13	3.03	92.73	3.36	4.22	0.04
St. deviation	0.19	0.15	0.45	0.32	0.12	0.02	0.01

**Table 6.** The total heavy metal concentration in analyzed soil, normal soil, and plant-toxic soils.

	normal range in soils <sup>a</sup> (mg/kg <sup>-1</sup> )	Toxic soil for plants <sup>b</sup> (mg/kg <sup>-1</sup> )	present study(mg/kg <sup>-1</sup> )
Al	n.d	n.d	16050±9
Cd	0.35	3 – 8	0.50±0.02
Cr	70	75-100	37.33±1.89
Cu	30	60-125	22.50±0.79
Fe	50000 c	n.d	11745±13
Mn	1000	1500-3000	205±17.52
Ni	50	100	20.07±2.15
Pb	35	100-400	5.03±0.98
Co	8	25-50	14.43±1.11

a: (Bowen H.,1997) b:(Ross SM 1994) C: WHO n.d: (not detected)

**Table 7.** The correlation between heavy metal content and soil physicochemical properties.

	Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Co
PH	-0.78	0.62	0.73	0.47	-0.60	0.20	0.94	0.999*	0.48
EC	-0.98	-0.19	-0.06	-0.37	0.23	-0.62	0.34	0.69	-0.36
Sandy %	-0.78	-0.63	-0.52	-0.76	0.66	-0.91	-0.14	0.26	-0.76
Silt %	0.82	-0.57	-0.67	-0.40	0.54	-0.13	-0.91	-1.000*	-0.41
Clay %	0.98	0.19	0.06	0.37	-0.23	0.62	-0.34	-0.69	0.36
O.M %	0.10	-1.00	-0.999*	-0.95	0.99	-0.84	-0.91	-0.66	-0.96
SAR %	-0.38	-0.93	-0.87	-0.98	0.94	-1.00	-0.60	-0.23	-0.98

**Table 8.** Heavy metals Contamination factors and pollution load index of study soil .

	Al	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Co
CF	0.20	0.17	0.41	0.50	0.25	0.24	0.29	0.25	0.76
PLI	0.87								

to the types of pollution that cause sediment disintegration quality (Tomlinson *et al.*, 1980). Suggesting low contamination levels in the Serapium soil, which may favor plant growth and effective remediation. The observed low HM levels in the plants support the view that the treated wastewater irrigation employed in this agroforest provides an ecologically sustainable water source while minimizing soil contamination. This outcome underscores the importance of wastewater management in enhancing phytoremediation efforts mentioned by many researchers (Mazher 2017; Afaf *et al.* 2021; Amedi *et al.* 2021)

### The phytoremediation efficiency Bioaccumulation Factor (BCF)

Our bioconcentration factor (BCF) offered in Figure 2 measures plants' soil metal absorption. This is the ratio of plant and soil metal concentration (Tomlinson *et al.*, 1980). It is crucial for phytoremediation data on plant heavy metal accumulation efficiency (metal absorption, mobilization into plant tissues, and shoot section storage) (Newman, 2014). If the BCF value exceeds one, it suggests a potential heavy metal hyperaccumulator species (Zhang *et al.*, 2023). Cd levels were below the detection limit (<0.03) in all the species studied and localities. The BCF values for all tested HMs in the current investigation ranged from

0.01 to 1.15. The BCF values of all the studied HMs were less than one, except for *Malva parviflora*, which had a BF of 1.15. That could be because soil alkalinity reduces the availability of metals for transfer from soil to plants. BCF values less than one are typically unfavorable for phytoextraction. Therefore, most of the plants that were studied were restricted to becoming phytoremediators for heavy metals in contaminated soil (Ahmed et al., 2023, El-Metwally et al., 2022, Lipy et al., 2021). *Malva parviflora* exhibited the greatest Bioconcentration Factor (BCF) value and demonstrated a greater uptake of Pb, suggesting that this species has a higher capacity to collect Pb compared to the other species that were identified. Out of the 7 indigenous plant species, *Malva parviflora* displayed the highest BF values of 0.09, 0.08, 0.17, and 1.15 for the elements Cr, Fe, Mn, and Pb, respectively. Overall, the analysis of bio-concentration factors (BCF) suggests that *Malva parviflora* has the potential to effectively remove lead (Pb) from mildly alkaline soil through phytoextraction. Soil physical and chemical properties affect heavy metal availability and plant root uptake. We found that distinct plant species hyperaccumulate certain metals, which is consistent with earlier study (Kumar et al., 2022; Ali et al., 2020).

#### Transfer coefficient (TC)

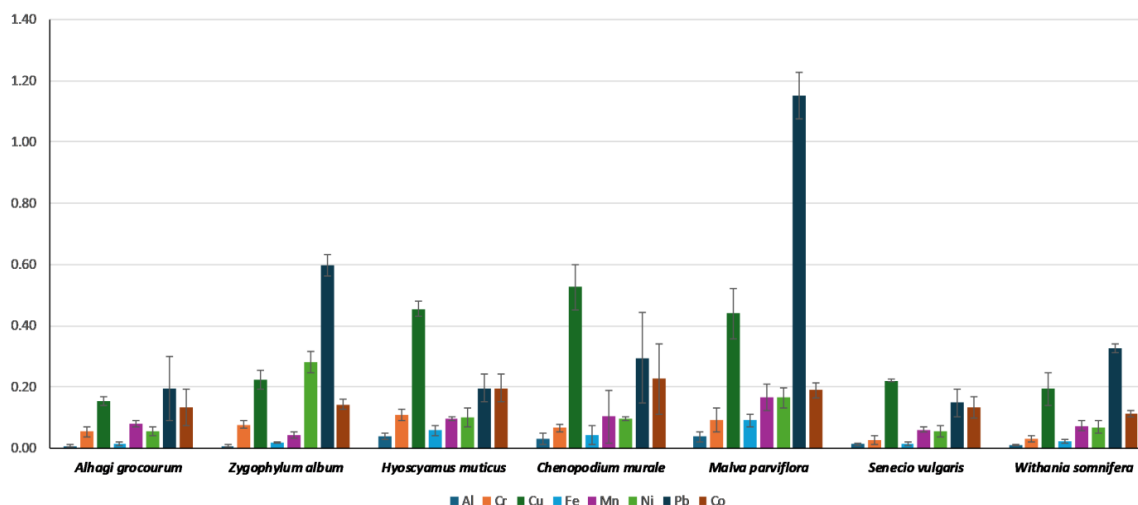
Figure 3 shows shoot enrichment coefficient (TC). The ratio of shoot heavy metal concentration to soil heavy metal concentration was used to compute TC (Branquinho et al., 2007). According to (Djenontin et al., 2012), accumulator plants need  $TC > 1$ . TC greater than 1.0 shows the plant's ability to collect soil metal ions and transmit them to shoot parts, especially central vacuoles (Djenontin et al., 2012). In our study,  $TC < 1$  for HMs (Al, Cr, Fe, Mn, Ni, and Co) in seven native plants was evaluated. Plants can immobilize heavy metal ions by root adsorption, absorption, accumulation, and rhizosphere precipitation (Yan et al., 2020). *Achillea millefolium* has been found to have orders-of-magnitude lower TC levels than ours, even in highly enriched soils (NworieQin & Lin, 2019). TC value of Pb is 1.7 in *Malva parviflora*. This may imply that *Malva parviflora* can mobilize heavy metals in low-enriched alkaline soil. Therefore, *Malva parviflora* can be classified as a Pb-accumulator or non-standard Pb-hyperaccumulator. *Malva parviflora*'s strong BCF and TC values for Pb position as the most effective species for targeted Pb remediation. This aligns with earlier research identifying species within the *Malva* genus as effective hyperaccumulators for heavy metals,

particularly in urban and industrial soils (Mayerová et al. 2017; Elshamy et al. 2019; Shrivastava et al. 2019).

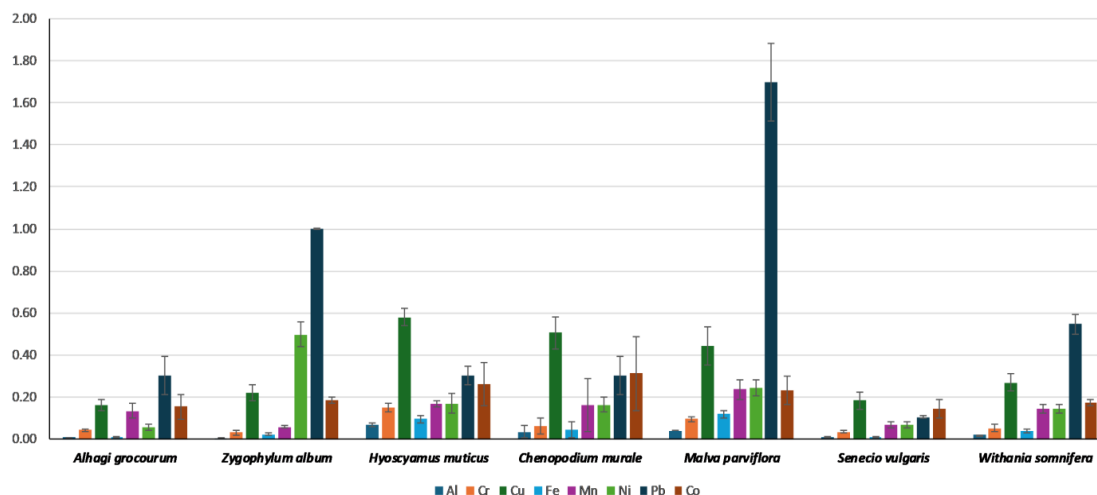
#### Translocation factor (TF)

Figure 4 shows translocation factors (TFs). (TFs) were employed to assess metal ion transport from roots to shoots using mobilization ratio (Gupta et al., 2007, Gupta et al., 2010, Singh & Mohan, 2020, HaneefFaizan & Kausar, 2017). Antoniadis et al. (Antoniadis et al., 2017) expect accumulator plants to have a heavy metal translocation factor (TF) larger than 1. Although the TF (translocation factor) for heavy metals in this study exceeded 1, indicating some level of translocation from roots to shoots, the quantities of heavy metals in both the roots and shoots of plants growing in the soil were relatively low, suggesting only little soil contamination. The current findings demonstrate the impact of TF on indigenous plant species. All the investigated native plants had a transfer factor (TF) higher than 1 for most heavy metals (HMs). The only HMs with a TF less than 1 were Ni (0.66 and 0.37) in *Alhagi grocourum* and *Hyoscyamus muticus* respectively, Cr (0.6, 0.2, and 0.67) in *Alhagi grocourum*, *Zygophyllum album*, and *Senecio vulgaris* respectively, Cu (0.92 and 0.67) in *Chenopodium murale* and *Senecio vulgaris* respectively, Fe (0.8 and 0.44) in *Zygophyllum album* and *Senecio vulgaris* respectively, and Al and Pb (0.5) in *Senecio vulgaris*. Several researchers, such as (Nyman & Lindau, 2016; Sanjosé et al., 2022; Luo et al., 2016; Kong et al., 2022), have documented a higher translocation factor (the ratio of heavy metal content in leaves to roots) for *Metasequoia glyptostroboides*, which other studies have consistently confirmed. The examined heavy metals (HMs) in the seven species have low bioconcentration factors (BCFs) ( $<1$ ) and accumulation coefficients (ACs) or (TC) ( $<1$ ). This indicates that the HMs have safe limits in the aerial sections of the species, resulting in high translocation from roots to shoots and low translocation from the soil.

The effects of alkaline soil on heavy metal immobilization and its limits are also considered. In *Malva parviflora*, lead (Pb) has a BAF of 1.5 and a TC of 1.7. The alkaline pH enables heavy metal release in soil with moderate enrichment. However heavy metal ions can be immobilized in plants by adsorption on roots, absorption and accumulation by roots, or rhizosphere precipitation (Kafle et al., 2022; Yu et al., 2023). *Malva parviflora* accumulates more lead (Pb) than the other plants examined in slightly alkaline soil with low heavy metal contamination.



**Figure 2.** The bioconcentration factor (BCF) of heavy metals in seven investigated native plants, grown at Serapium agroforest, Ismailia City.



**Figure 3.** Transfer Coefficient (TC) of heavy metals in seven investigated native plants grown at Serapium agroforest, Ismailia City.

Plants can be categorized into different groups based on their strategies. Indicators are plants that experience decreased growth and exhibit toxic symptoms in the presence of certain substances. Excluders are plants that prevent the entry of metals into their roots. Accumulators store metals in non-toxic forms within their tissues, using metal phytochelatins/ metallothionein complexes, without showing any toxic symptoms. On the other hand, hyper-accumulators are plants that accumulate metals at levels 100 times higher than what is typically found in non-accumulator plants (Lam et al., 2022).

## CONCLUSION

Seven native plant species from six families grown in slightly alkaline soil with low heavy metal pollution have a pollution load index (PLI) of less than one. Nine heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb & Co) were

studied among the species studied. None of the species had unacceptable concentrations. Statistically, (HMs) showed highly significant differences in each concentration between the studied native plants

The bioconcentration factor (BCF), translocation factor (TF), and shoot enrichment coefficient (TC) assessed phytoremediation in seven species. Except for *Malva parviflora*, which exhibited the greatest BCF and TC for lead (Pb) at 1.15 and 1.7, other species had BCF and TC below one. The transfer factor (TF) study demonstrated that the discovered species had more than one hazardous material (HM). Complete results reveal that all species may live in slightly alkaline soil with low heavy metal pollution. *Malva parviflora* is best for phytoextraction and Pb cleanup. These findings will illuminate heavy metals (HMs) in native plants and soils and their effects on desert plants.

Forest managers, developers, and stakeholders can potentially use our findings to warn about wastewater irrigation dangers. This will allow strict controls against selecting some plants for commercial usage or animal fodder, reducing health concerns.

## RECOMMENDATION

Field research must be expanded and tests on native species (*Malva parviflora*) increased to improve site cleanup. This species must be introduced to a test site and its phytoremediation evaluated annually through harvest cycles. Many plant species will be tested as native phytoremediation plants in a treated wastewater-watered agroforest in this significant study. More research is needed to understand how wastewater irrigation affects plant diversity, distribution, and health. Forest managers, developers, and other concerned parties can educate the public about wastewater irrigation concerns. Wild plant contamination, which can disrupt the food chain and ecology, will also be assessed. These discoveries provide a feasible, non-destructive solution for extremely polluted soils, benefiting the community. Ecosystems benefit from wild plants' soil, water, and carbon sequestration. Long-term ecosystem viability can be tested via treated wastewater irrigation.

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