

Assessment of Some Wetland Plants for Heavy Metal Accumulation, and Their Feasibility in Phytoremediation Technology

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ABSTRACT

The growth of industrialization and overpopulation has resulted in significant heavy metal contamination of freshwater resources and wastewater. In this study, we investigated the efficiency of three native macrophytes (*Helosciadium nodiflorum*, *Phragmites australis* and *Typha domingensis*) in remediating heavy metals: Arsenic (As), Chromium (Cr), Iron (Fe) and Zinc (Zn) in a constructed wastewater treatment plant. As a result, the potential of plants to accumulate metals was ordered as follows: *H. nodiflorum* > *T. domingensis* > *P. australis*. Iron (Fe) was the most accumulated metal in root and shoot parts, followed by Zn, Cr and As. The mean values ranged from 118-5944, 4.2-60.6, 0.3-6.7, and 0.22-2.99 mg/kg respectively, those concentrations were much higher than the threshold limits, excluding Zn. All estimated bioconcentration factors (BCF) were higher than 1, and the maximum BCF for As, Cr, Fe, and Zn were observed in the roots of *H. nodiflorum* plants, with values of 7.2, 48.5, 463, and 542, respectively. Thus, the three plants were signified as hyperaccumulators (BCF>1) to take up the selected metals from the sand. Their translocation factors (TF) were limited to below 1 (TF<1), which confirms the involvement of plants in the phytostabilization mechanism within root tissues and the rhizosphere. In conclusion, the three plants successfully contributed to heavy metal removal, and they are strongly recommended for use as promising biological agents in phytoremediation.

INTRODUCTION

Municipal and industrial wastewaters are well-known environmental threats owing to an increase in urbanization and industrialization (Ukaogo *et al.*, 2020; Wen *et al.*, 2024). Globally, around 80 percent of wastewater is disposed in freshwater bodies without proper treatment. Moreover, developing nations suffer more from untreated wastewaters than developed ones, primarily due to insufficient treatment and disposal systems (Goala *et al.*, 2021). Toxic metals often found in wastewater are emitted from human activities such as industrial effluents, mining operations, excessive usage of fertilizers, pesticides (Anas *et al.*, 2025; Kumar *et al.*, 2025). Phytoremediation is a biological wastewater treatment system that uses potent aquatic plants to mitigate and

remove pollutants via different mechanisms and provides substantial advantages in contrast to other approaches (Bello *et al.*, 2018; Raza *et al.*, 2023)

Generally, the levels of chemicals and particles in water bodies, including primary pollutants, heavy metals, nutrients, and microorganisms, are the primary factors that influence water quality (Ahmed *et al.*, 2021). Heavy metal emissions into aquatic and terrestrial environments occur via natural phenomena (Jaber & Al-Ghanimy, 2023) and anthropogenic activities (Mwakalukwa *et al.*, 2024). Urgent consideration is needed for environmental protection from the adverse impacts of heavy metal toxicity (Kaur *et al.*, 2025). In addition, public health concerns have emerged due to their toxic properties as bio-accumulative, carcinogenic, and mutagenic potential in living organisms (Namuq, 2021; Letey *et al.*, 2025). Consequently, heavy metal identification, quantification, and remediation are crucial approaches to mitigating the toxicity of heavy metals (Cicero-Fernández *et al.*, 2017; Taha, 2023).

Currently, significant water stress and water crises have been observed overseas, and it is necessary to find novel and alternative solutions for the treatment of wastewater effluents (Khan *et al.*, 2022). Water treatment systems can be achieved through biological (Saleh, 2016) and physicochemical processes (Azabo *et al.*, 2025). Phytoremediation is regarded as a green and sustainable process (Nafea, 2019) because of its economic feasibility as a sustainable solution and the utilization of hyperaccumulator plants for the removal of heavy metals with minimal secondary waste production (Huang *et al.*, 2020; Sangeetha *et al.*, 2025). Plants stimulated by microorganisms in the substrate can achieve the natural uptake of contaminants through plant biomass growth, operating as an *in-situ* remediation strategy (Bello *et al.*, 2018).

This study aimed to explore the heavy metal removal efficacy of three wetland plants in a constructed wastewater treatment plant. Both bioaccumulation and translocation factors were investigated to classify the selected plants and to determine their roles in phytoremediation strategies.

MATERIALS AND METHODS

1. Study area, plant collection, and preparation

The study was conducted during the growing season between August and November 2024. The selected study area is in Soran City (N17 400 08.000 E6 220 29.900). The research project was conducted on the main wastewater canal, a part from the Soran Quarters and carwashes on Jundiyan Road, which is directly opened into the river, as shown in the Fig. (1). Two local semi- wetland plants (: *Typha domingensis* and *Phragmites australis*) and one wetland plant (*Helosciadium nodiflorum*) were selected (Fig. 2). These species demonstrated significant abundance and extensive dispersion throughout the region. Plant selection was made based on the basis of their season of

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growth and their abilities to absorb waste materials. Even, healthy and young plants were collected, and the taxonomic identification of mature plant specimens was confirmed.

The availability of the selected plants under a wide range of environmental conditions was confirmed. Prior to direct transfer and growing of collected plants, they were washed with tap water and soil debris was eliminated, and then they were directly grown in the field where young plants were allowed to adapt and acclimatize to their new environmental conditions for 25 days before experiment initiation (Khalid & Ganjo, 2020). At the end of the experiment, the dry biomass of each plant was examined for heavy metal contents in root and shoot tissues.



Fig. 1. Map of a part of Soran Quarters with their municipal wastewater opened in river, and experiment location (Soran City- KRI)

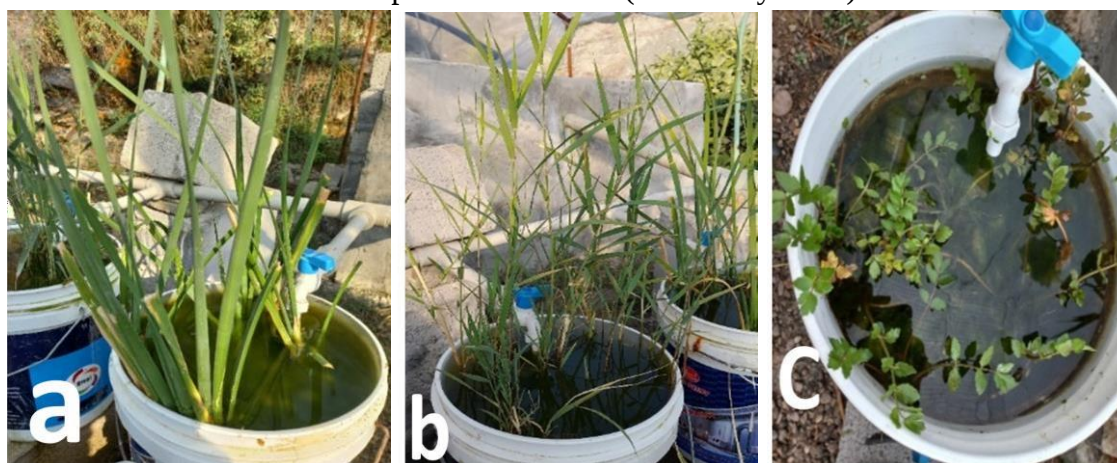


Fig. 2. The plant species used in WWTP. a) *Typha domingensis* (cattail), b) *Phragmites australis* (Common reed) and c) *Helosciadium nodiflorum* (fool's water-cress)

2. Experimental design

A rectangular sedimentation unit with water holding capacity of 3850 liters and ($2.5 \times 1.4 \times 1.1$ m) dimensions was constructed outdoor (*in-situ*) near Soran wastewater sewage channel that provided additional contaminant removal through sieving and primary settler tank operations (Fig. 3). Two steel sieves with different pore sizes (inlet = 8 mm and outlet = 4 mm) were installed through which the water passed, and the sieves were cleaned twice a week.

Polyethylene pots (40 cm $\times$ 36 cm) from top to bottom were filled with a 10 cm layer of wastewater, 10 cm layer of river quartz gravel (20 mm) and 10 cm layer of quartz river's sand (2.0 mm), respectively. The experimental plastic pots were organized into four treatment groups (Fig. 3), each with three replicates (except for the control pot, which was single and not triplicate). The individual plant species were carefully cleaned and planted separately in pots (five individual plants per pot), and then received wastewater from the sedimentation unit through a PVC distribution pipe system. The growth periods for all the plants were the same. The retention time, flow rate of wastewater, and plants appearance were monitored four days per week throughout the 75 days experimental period.

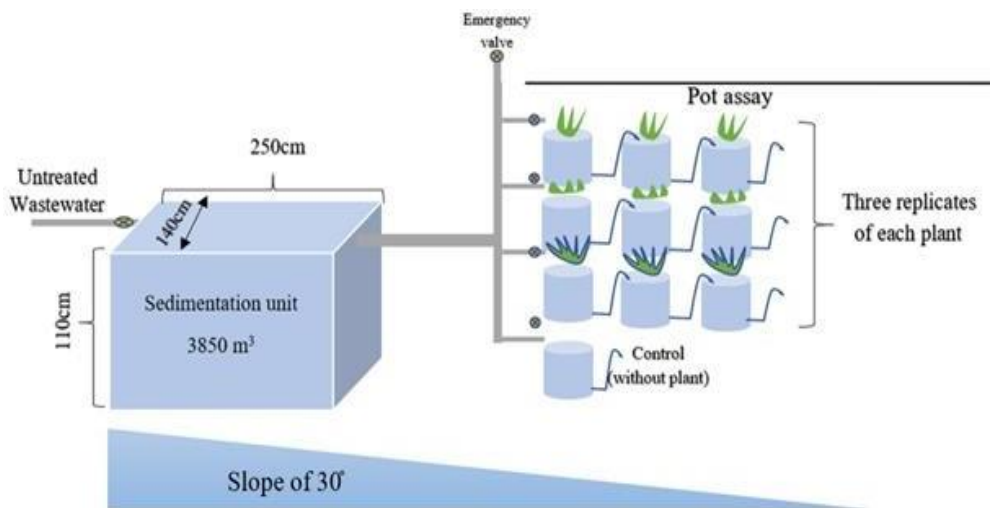


Fig. 3. The experimental design of the prepared WWTP; consisting of sedimentation unit, unplanted control pot (sand and gravel), treated pot (sand and gravel + plant)

3. Heavy metal analyses in plant tissues and sand samples

Different plant tissues and sand samples were analyzed using ICP-MS and ICP-OES instrument models (SHIMADZU ICPE-9820), respectively, for heavy metals arsenic (As), chromium (Cr), iron (Fe), and zinc (Zn), at the Atmosphere laboratory in Erbil-Iraq. Laboratory measurements of metal concentrations in the plant tissues were

performed at the end of the experimental periods. Sample preparation was performed according to the method described by **Khalid and Ganjo (2020)**. The freshly chopped plant samples received a brief heat treatment of 10-minute oven-drying at 105°C then left in the oven for 4 days at 50°C. The separated dried plant parts were ground using a porcelain mortar, and sieved through a 2mm mesh sieve. Plant material weighing 0.2g (dw) was subjected to digestion in 8ml of 65-68% concentrated nitric acid (HNO₃) (TraceMetal Grade), soaked overnight, and then 1ml of H₂O₂ 30% was added to it. The digested samples were then filtered through Whatman No. 42 filter paper to remove the remaining particulates, and a clear solution was obtained then analyzed by ICP-MS instrument, as described by **Velasco-Arroyo et al. (2024)**.

From depths of 10cm to subsurface levels, sand samples were collected from vertical sand pot profiles. Sample preparation involved mixing to obtain a homogenized sample for each experimental pot. Roots and macrofauna were eliminated from the sand samples and placed in plastic zipper bags before laboratory experiments. Sand specimens were air-dried at ambient temperature, then leached to a 1:10 ratio, and subjected to aqua regia digestion using a 3:1 ratio of hydrochloric acid to nitric acid. The solution was evaporated to 2ml prior to filtering into a 20ml volumetric flask, deionized water was added to reach the final volume mark, and the leachates of the sand samples were examined by ICP-OES (**Bonanno & Cirelli, 2017; Mbuyamba et al., 2025**).

Transfer factors (BCF and TF)

The bioconcentration factor (BCF_{shoot} and BCF_{root}) and translocation factor (TF) are essential parameters for assessing the phytoremediation potentials of studied plant species (**Zand & Mühling, 2022**). The Bioconcentration Factor (BCF) indicates the potential of a plant to uptake and sequester metals from the surrounding environment into its tissues. This indicator is essential for assessing the efficacy of a plant in accumulating contaminants, such as metallic elements, from water or soil (**Abid et al., 2025**).

The BCF value for each metal in the selected plant species was determined using the following formula (**Khalid & Ganjo, 2020**):

$$BCF_{shoot/root} = \frac{\text{Metal Concentration in plant}}{\text{Metal Concentration in media}} \quad \text{Eq. (1)}$$

The TF defines the ability of plants to translocate metals from the subaerial (roots) to the aerial parts of a plant (i.e., stems and leaves), and the phytoextraction properties of plant species can be determined for metals (**Khan et al., 2022**). It was calculated according to the formula below:

$$TF = \frac{\text{Metal Concentration in Shoot}}{\text{Metal Concentration in Root}} \quad \text{Eq. (2)}$$

4. Statistical analysis

The study measurements are represented as mean $\pm$ standard deviation. One-way ANOVA was performed to test the significance between means at $P < 0.05$, using IBM SPSS. Tukey's honest significant difference (HSD) test was used for multiple comparisons.

RESULTS

1. Heavy metal accumulation in plant tissues

The selected heavy metals accumulated in different tissues of the three plants. The present study revealed that the highest accumulation of heavy metals (As, Cr, Fe, and Zn) was observed in the shoot and root tissues of *H. nodiflorum*. Iron (Fe) was the most abundant heavy metal in the studied plants, followed by Zn, Cr, and As. Moreover, high levels of the heavy metals were stored and immobilized in the root tissues of the candidate plants, whereas the concentrations transferred to the upper parts were estimated to be less than half of those stored in the roots, as illustrated in Fig. (4).

1.1 Arsenic

Fig. (4a) indicates the feasibility of the candidate plants to uptake arsenic (As), and their efficiency was ordered as: *H. nodiflorum* > *T. domingensis* > *P. australis*, and the mean concentrations in root and shoot systems were 2.99 mg/kg and 1.57 mg/kg, 0.98 mg/kg and 0.87 mg/kg, 0.55 mg/kg and 0.22 mg/kg, respectively. The efficiency of plant parts was significantly different (P value ≤ 0.05), as shown in Fig. (4a). Totally, in each plant part it was much higher than the recommended level (0.1 mg/kg) for plants by FAO/WHO.

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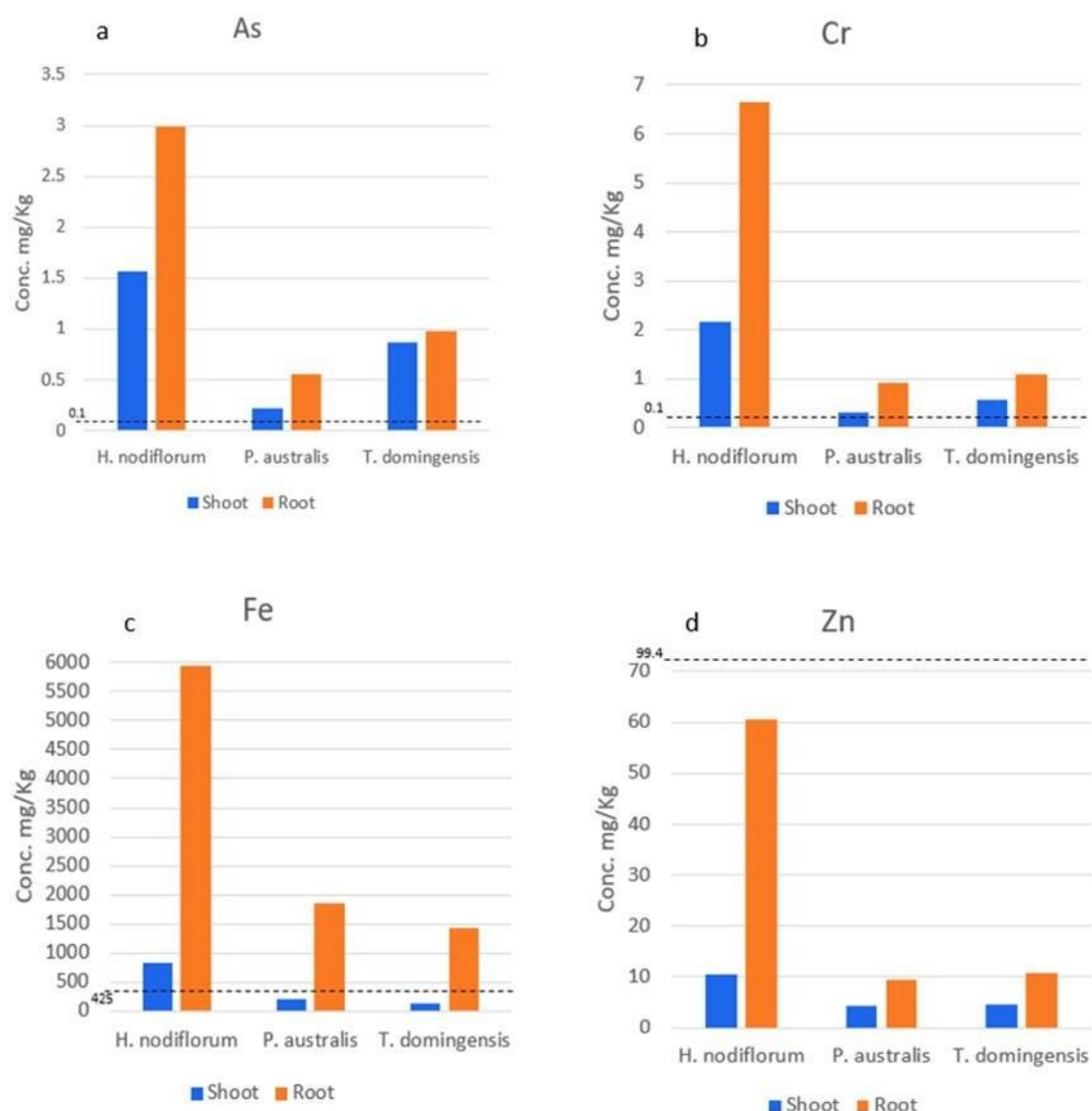


Fig. 4. Mean values of the accumulated heavy metals in root and shoot parts of the studied plants (mg/kg); a) As concentration, b) Cr concentration, c) Fe concentration, d) Zn concentration

1.2 Chromium

The accumulated mean values of Cr within root and shoot tissues of the studied plants were lined up in the following sequences: *H. nodiflorum* (6.65 mg/kg and 2.16 mg/kg) > *T. domingensis* (1.1 mg/kg and 0.58 mg/kg) > *P. australis* (0.93 mg/kg and 0.31 mg/kg), respectively, as depicted in Fig. (4b). The calculated Cr values among the plant parts were significantly different (P -value ≤ 0.05). The concentration of Cr in the root tissues was more than two-fold higher than that in the upper parts.

1.3 Iron

Helosciadium nodiflorum achieved the highest value of Fe uptake, mainly in the underground parts (5944 mg/kg), followed by the roots of *P. australis* and *T. domingensis* (1861 and 1438 mg/kg, respectively). These values are much higher than the permissible level (425 mg/kg); hence, the evidence was confirmed as contamination. However, the plant shoots were in the same order as their roots, but only *H. nodiflorum* accumulated higher than the permissible level, whereas *P. australis* and *T. domingensis* remained below the permissible level (Fig. 4c).

1.4 Zinc

The ability of the plants to uptake and mobilize zinc was ordered as follows: *H. nodiflorum* > *T. domingensis* > *P. australis*. The maximum mean value was 60.6 mg/kg that was accumulated in the roots of *H. nodiflorum*, and for the other plants, it was nearly 10 mg/kg or lower (Fig. 4d). The concentration of Zn in the studied plants was considered to be safe and much lower than the maximum permissible level (99.4 mg/l).

2. Heavy metal accumulation in filtration media

The concentrated metals adsorbed on the surface of the filtration sand from different units of the conducted WWTP units were monitored by ICP-OES, and their mean values are listed in Table (1). Statistically, there were no significant differences between the mean values of each metal in the different plants (P -value > 0.05).

Table 1. Mean $\pm$ SD of Heavy metal accumulation in implanted sand

Implanted pot	Heavy metal in sand (mg/kg)			
	As	Cr	Fe	Zn
<i>H. nodiflorum</i>	0.42b $\pm$ 0.024	0.14c $\pm$ 0.01	12.84a $\pm$ 63.3	0.13c $\pm$ 0.05
<i>P. australis</i>	0.38b $\pm$ 0.028	0.13c $\pm$ 0.01	10.29a $\pm$ 37.1	0.10d $\pm$ 0.03
<i>T. domingensis</i>	0.38b $\pm$ 0.003	0.12c $\pm$ 0.01	9.31a $\pm$ 7.7	0.09d $\pm$ 0.01

The maximum mean values for As, Cr, Fe, and Zn were observed in the homogenized sand samples implanted with *H. nodiflorum* (0.42, 0.14, 12.84 and 0.13 mg/kg). The availability of heavy metals in the substrate is linked to the Bioconcentration factor (BAF) and translocation factor (TF).

3. Bioconcentration factor (BCF) and translocation factor (TF)

3.1 Bioconcentration factor of plants

The results indicated that the three plants achieved shoot and root of BCF >1 over the studied heavy metals (As, Cr, Fe, and Zn), excluding the BCF_{shoot} of *P. australis* (As

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metal was 0.6). Moreover, the BCF_{root} values of the heavy metals were higher than those of BCF_{shoot} (Table 2). The minimum and maximum BCFs for As, Cr, and Zn were achieved by *P. australis* and *H. nodiflorum* and the values ranged from 0.6-7.2, 2.4-48.5, and 41-542, with respect to plants and metals (Table 2). However, this value for Fe ranged from 13-463 for *T. domingensis* and *H. nodiflorum*, respectively.

3.2 Translocation factor

It was observed that the TF values of all the studied plants were < 1 for the selected heavy metals, with the exception of arsenic TF almost 1, which was achieved by *T. domingensis*, as shown in Table (2).

Table 2. Transfer factors (BCF & TF) of heavy metals in plant tissues

Heavy metal	Plant	BCF		TF
		Shoot	Root	
As	<i>H. nodiflorum</i>	3.8	7.2	0.5
	<i>P. australis</i>	0.6	1.4	0.4
	<i>T. domingensis</i>	2.3	2.6	0.9
Cr	<i>H. nodiflorum</i>	15.8	48.5	0.3
	<i>P. australis</i>	2.4	7.3	0.3
	<i>T. domingensis</i>	4.7	8.9	0.5
Fe	<i>H. nodiflorum</i>	65	463	0.1
	<i>P. australis</i>	21	181	0.1
	<i>T. domingensis</i>	13	155	0.1
Zn	<i>H. nodiflorum</i>	81	542	0.1
	<i>P. australis</i>	41	90	0.5
	<i>T. domingensis</i>	50	115	0.4

DISCUSSION

1. Heavy metal accumulation in plant tissues

In the present study, the highest accumulation of heavy metals (As, Cr, Fe, and Zn) was observed in the shoot and root tissues of *H. nodiflorum*. High levels of the heavy metals were stored and immobilized in the root tissues of the candidate plants, whereas the concentrations transferred to the upper parts were estimated to a level less than half of those stored in the roots. The same conclusion was reached by **Pasricha et al. (2021)**. Moreover, plants with fibrous root systems are superior to taproot systems for pollution removal due to their large surface area (**Newete & Byrne, 2016**).

1.1 Arsenic

Arsenic (As) is a non-essential element found in soils, sludge, sediments, and water bodies (**Khalid et al., 2017**). Prolonged or high doses of short-term exposure to As are detrimental to human health (**Liao et al., 2022**). As revealed in Fig. (4a), the potential of candidate plants to uptake arsenic (As) was ordered as: *H. nodiflorum* > *T. domingensis* > *P. australis*. The potency of plant parts to uptake As was significantly different ($P\text{-value} \leq 0.05$), as roots were more potent than shoots. The reason for the higher availability of As in the root parts in compared to the upper ground parts might be associated with As co-precipitation on roots and the presence of iron plaques on roots that lowered As uptake and its translocation to shoots (**Pan et al., 2014; Pardo et al., 2016**). Other factors can be enzymes exuded from plant roots into the rhizosphere that play a critical role in the chemical transformation of metals in soils and the eventual uptake of each chemical state by the plants (**Mallmann et al., 2014**) or some soil microbiotas that transform organic into inorganic arsenic, which influences the bioavailability of elements for plants (**Pasricha et al., 2021**).

1.2 Chromium

Chromium is also considered as a non-essential metal, and its excess amount alters plant growth and leads to toxicity (**Sharma et al., 2020**). It is a well-known disturbance that threatens the environment and human health (**Wang et al., 2023**). The accumulated mean values of Cr within root and shoot tissues of the studied plants were lined up in the following sequences: *H. nodiflorum* > *T. domingensis* > *P. australis*, as depicted in Fig. (4b). The concentration of Cr in the root tissues was more than two-fold higher than that in the upper parts. This may be related to the high affinity of Cr^{3+} for cation exchange sites on the cell wall, and consequently moved in less significant amounts between different plant parts (**Sinha et al., 2018**).

It is noteworthy that high levels of some minerals in the growth medium lowers Cr levels in plants, because of Cr competition with sulphate and phosphate (**Pasricha et al., 2021**). The presence of Fe metal ion metal transport channels permits Cr mobility and

accumulation (**Ao et al., 2022**). The Cr level in plants is also linked to the availability of oxidized forms of Mn, Fe, and organic matter (**Shahid et al., 2020**). Microbial activities in the rhizosphere also affect Cr uptake, since they convert toxic and mobile Cr^{6+} to non-toxic and immobile Cr^3 (**Sharma, 2022**). This study demonstrated that Cr contents in the studied plants were above the phytotoxic threshold according to **Attili and Al-Sa'ed (2022)**, which can occur in plants contaminated by Cr to a value higher than 0.5 mg/kg. Thus, the three candidate plants were faced this phenomenon, and higher Cr values were observed.

1.3 Iron

Unlike most of other metal(loid)s, Fe is considered as the most abundant and beneficial constituent of human health and organisms. However, limited levels of Fe can be hazardous to tissue damage and diseases (**Elango et al., 2022**). *H. nodiflorum* showed the highest capacity for Fe uptake, mainly in the underground parts, followed by the roots of *P. australis* and *T. domingensis*, respectively (Fig. 4c). The high accumulation of Fe in roots is due to the presence of microorganisms in the soil that reduce Fe(III) to Fe(II) (**Lurthy et al., 2021**). The presence of iron hydroxide plaques may lead to high Fe content in the roots, which are then mobilized and deposited as brownish precipitates on the root surface (**Laghlimi et al., 2015**). According to **Alikaj and Brahushi (2017)**, *H. nodiflorum* has bioconcentrated Fe 78 times greater than the Fe concentration found in the water. **Monks et al. (2023)** observed greater Fe accumulation in *Phragmites* biomass than *Typha*. Similarly, *Typha domingensis* absorbed the least amount of Fe.

1.4 Zinc

Zinc (Zn) is an essential micronutrient for the proliferation and metabolism of plants and an important component involved in the biological processes (**Zhang et al., 2023; Abid et al., 2025**). The ability of the plants to uptake and mobilize Zn was ordered as follows: *H. nodiflorum* > *T. domingensis* > *P. australis*. Zinc is abundant in numerous terrestrial and aquatic plant species, possibly because of Zn flexibility in the environment (**Yadav et al., 2018**). **Newete and Byrne (2016)** confirmed that plants with fibrous systems could be used as promising biological agents to mitigate pollutants from terrestrial trials and aquatic ecosystems. Because of this, *H. nodiflorum* as a fibrous root plant was confirmed to be the most potent to uptake Zn from the environment, while *T. domingensis* and *P. australis* were less potent, because both plants are taproot plants according to **Ali et al. (2019)**. However, **Bonanno and Vymazal (2017)** reported that species with a greater biomass size (e.g., *P. australis*) have generally have higher element concentrations.

2. Bioconcentration factor (BCF) and translocation factor (TF)

2.1 Bioconcentration factor of plants

This confirms the potency of plants to uptake and bioaccumulate specific elements in different parts of the plant body (Dan *et al.*, 2017). Plants with $BCF > 1$ were considered hyperaccumulators and were proposed for phytoremediation. Plants with BCF values < 1 are classified as excluders, and do not have them for phytoremediation (Khalid & Ganjo, 2020; Nabuyanda *et al.*, 2022). The type of root system can be considered to have a strong impact on the ability of plants to uptake elements from the substrate and transfer them to the upper parts (Ali *et al.*, 2019).

The results indicated the BCF_{root} values of the heavy metals were higher than those of BCF_{shoot} (Table 2). This is because of the adequate uptake of metals from the rhizosphere zone, storage, and immobilization within root tissues. Previous studies also revealed the same result, and claimed that more metals were concentrated in the roots (Nakamoto *et al.*, 2021; Wdowczyk & Szymańska-Pulikowska, 2023). Bioaccumulation is more frequently observed in root tissues that are directly close to heavy metals (Anjum *et al.*, 2015).

All plants were considered hyperaccumulators because their BCF values were > 1 . Among the candidate plants, *H. nodiflorum* was confirmed to be the most promising hyperaccumulator, and the highest BCF values were obtained in its underground and aerial parts.

2.2 Translocation factor

It represents the transferring of metal(oid)s from the root zone to the upper plant (Abedi & Mojiri, 2019). As a result of analyzing the mobility of elements from roots to shoot tissues, it was observed that the TF values of all the studied plants were < 1 for the selected heavy metals, with the exception of arsenic TF almost 1, which was achieved by *T. domingensis*, as shown in Table (2). According to Muthusaravanan *et al.* (2018) and Velasco-Arroyo *et al.* (2024), plants with $TF > 1$ are suitable for phytoextraction and considered as valuable agents for phytoremediation, whereas $TF < 1$ performs phytostabilization. Poor translocation may be due to myriad metal sequestration within roots, as a programmed response to toxicity and protection of the upper parts (Marchand *et al.*, 2010).

CONCLUSION

This study highlights the considerable potential of the indigenous plant species, *T. domingensis*, *P. australis* and *H. nodiflorum* in phytoremediation. In general, the predominant heavy metal sequestered by the wetland plants is iron, followed by zinc, chromium, and arsenic. *H. nodiflorum* was identified as the most remarkable candidate plant in this study. The studied plants were confirmed as hyperaccumulators with

bioconcentration (BCF>1). More importantly, they achieved a phytostabilization strategy to store and stabilize metals within root tissues and the rhizosphere, as their TF<1 for As, Cr, Fe, and Zn. The results emphasize the flexibility of the plants in immobilizing the hazardous metals, mitigating environmental hazards, and facilitating environmental rehabilitation. In this study, there was evidence of the phytoremediation potential of *H. nodiflorum*, unlike *P. australis* and *T. domingensis*.

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