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Exploring the Phytoremediation Capability of *Athyrium filix-femina*, *Ludwigia peruviana* and *Sphagneticola trilobata* for Heavy Metal Contamination

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ABSTRACT

Heavy metals are one of the leading environmental pollutants that are hazardous to the health of humans, soils, plants, and aquatic life. This study investigated the potential of *Athyrium filix-femina*, *Ludwigia peruviana*, and *Sphagneticola trilobata* for phytoextraction of Al, Ag, Cd, Cr, Ga, and Sr. To evaluate the heavy metal uptake by the plants, a pot experiment was conducted using uncontaminated soil mixed with a heavy metal solution. At the end of thirty days of planting, the bioconcentration and translocation factors were calculated. Cd accumulated to a greater degree in the shoots of *A. filix-femina* and *L. peruviana* than in their roots (8% and 12% respectively). Conversely, *S. trilobata* accumulated 27% more Cd in its roots than in its shoots. In all three plant species, roots had significantly higher heavy metal concentrations than shoots. These findings demonstrate that *L. peruviana*, *A. filix-femina*, and *S. trilobata* have high potential for phytoextraction and bioaccumulation of Cd, Sr, Ag, and Ga. The herbaceous nature of these plants, coupled with their deep roots and rapid growth rates, make them promising candidates for phytoremediation in heavy metal-contaminated soils.

Keywords: phytoremediation; bioaccumulation; herbaceous plants; metal uptake; green technology.

INTRODUCTION

Industrial, agricultural, and mining activities are the primary anthropogenic contributors of heavy metal soil pollution (Gholizadeh and Hu 2021; Kong et al. 2021; Njoku and Nwani 2022; Papazoglou and Fernando 2017; Su et al. 2014; Xiang et al. 2021; Zhao et al. 2022). Heavy metals are currently considered to be major pollutants because of their persistence, toxicity, and inability to be degraded by biological processes; furthermore, the accumulation of heavy metals in soil presents a risk to agricultural production on a worldwide scale (Long et al. 2021; Prabagar et al. 2021; Zunaidi et al. 2021). Due to the fact that metals are chemically similar to essential micronutrients, plants are able to absorb them through the cortical tissues of their roots (Kafle et al. 2022; Nirola et al. 2015; Yan et al. 2020). Since they pose a significant danger of contamination, they are classified as contaminants in food products (Charvalas et al. 2021; de Oliveira Morais et al. 2021; Solomou et al. 2022). Excessive amounts of elements such as Cd, Pb, Cr, and As have been found in agricultural soils all over the world, which in some cases can have a substantial impact on the population's health and standard of life in some cases (Durante-Yánez et al. 2022; González Henao and Ghneim-Herrera 2021; Li et al. 2019). For example, the soils employed for rice cultivation were shown to contain excessively high levels of As (Atiaga et al. 2021).

Phytoremediation is thought to have various advantages over other approaches for lowering the concentration of contaminants in soil. Some of these advantages include a low cost, *in-situ* remediation, respect for the environment, and improvement of the landscape (Alaboudi et al. 2018; Román-Ponce et al. 2017; Shehata et al. 2019). Phytoremediation, which comprises phytostabilization and phytoextraction, has achieved excellent results and is a potential approach based on plants' intrinsic ability to remove heavy metals from soil (Bhat et al. 2022; D.-M. Xu et al. 2021). For example, native species like *Erato polymnioides* and *Miconia* sp. are preferred since they are adapted to local climatic conditions and have already demonstrated tolerance to Hg, Cd and Zn by thriving in polluted environments (Chamba-Eras et al. 2022). Likewise, metal absorption, transport, and phytoaccumulation from soil are all dependent on plant type (Alves et al. 2022; Lee et al. 2021; Pacwa-Płociniczak et al. 2023; Yakovyshyna 2021). Species like *Tagetes erecta* L., *Calendula officialis* L. and *Sphagneticola trilobata* are capable of accumulating Cd, Pb, and Zn (Tabrizi et al. 2015; Pernía et al. 2019; Madanan et al. 2021; Staroń et al. 2021).

The distribution of heavy metals in the shoots and roots of herbaceous species such as *A. filix-femina*, *L. peruviana*, and *S. trilobata* has not been entirely clarified; therefore, it is unclear which of these species or tissue organs is a bioaccumulator, phytoextractor, or phytostabilizer. Hence, the main contribution of this research is centered on the assessment of the concentration of Al, Ag, Cd, Cr, Ga, and Sr concentration in shoots and roots of three herbaceous plant species. According to the translocation and bioconcentration factors of the examined heavy metals, the evaluation of phytoextraction and bioaccumulation capabilities was presented further.

MATERIALS AND METHODS

Sampling

The sampling site of the three native dominant plant species *A. filix-femina*, *L. peruviana*, and *S. trilobata* was located on the banks of the Carrizal River (Bolívar– Ecuador) (Figure 1). According to the bioclimatic map of Ecuador, this area has characteristics of tropical climate

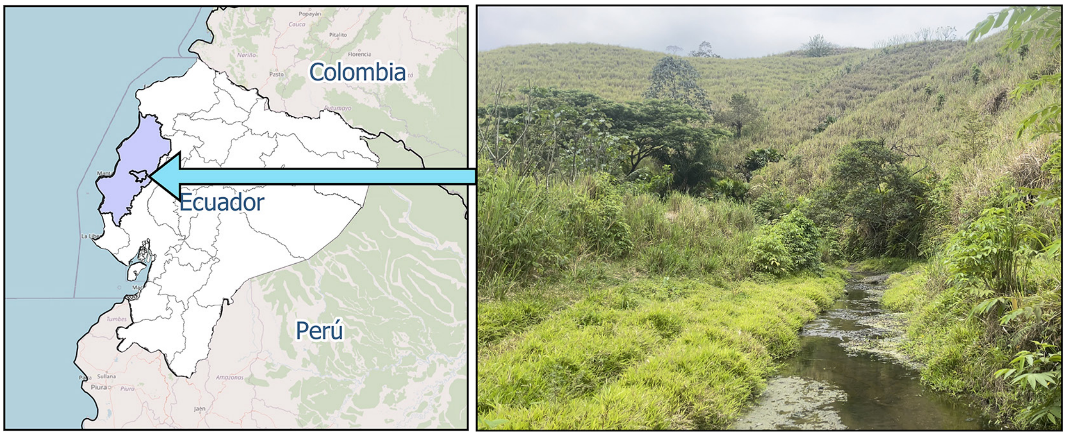

Figure 1. Geographical location of the study area

Figure 2. Plant species: (a) *A. filix-femina,* (b) *L. peruviana*, and (c) *S. trilobata*

and belongs to the tropical dry forest type ecological region according to the Holdrigde classification, where the changes that occur in the Pacific Ocean and by the movement of the intertropical convergence zone are influential (Aveiga-Ortiz et al. 2022).

Pot experiment

In a plastic flowerpot, the *A. filix-femina*, *L. peruviana*, and *S. trilobata* species (Fig. 2) were propagated (diameter: 30 cm; height: 40 cm). The pots were filled with 2 kg of loamy soil (pH 7.6). A mix of a heavy metal solution (Merck, USA) was added, containing: 12 mg/kg Ag, 15000 mg/kg Al, 1 mg/kg Cd, 30 mg/kg Cr, 8 mg/kg Ga, and 90 mg/kg Sr. The content of heavy metals was measured thirty days after plant species were sown. Each measurement was replicated three times.

Elements analysis

The plants were harvested carefully for element analysis after one month of planting in the pots. The harvested plants were separated gently into roots and shoots. These parts were treated by high-temperature desiccation below 105 °C for 30 minutes and dried in an oven at 65 °C to obtain a constant weight; the dry tissues were ground to a fine powder and placed in polyethylene bags for further analysis (Alves et al., 2022). Soil samples were air-dried, ground to a particle size of less than 0.147 mm and stored in polyethylene bags until analysis. The content of heavy metals, micro-and macronutrients was determined by inductively coupled plasma mass spectrometry (ICP-MS/MS ion trap, Perkin Elmer, USA).

Analysis of variance (ANOVA) and Tukey range tests ($p<0.05$) were conducted using R-project and R-studio with ggplot2 package (R Core Team 2022; Wickham 2016).

Bioconcentration and translocation factors

The translocation factor (TF) establishes the relationship of the concentrations of elements in the shoot and root parts of the plant; whereas, in bioconcentration factor (BCF) it is the proportion of concentrations of elements in plant tissues and soil, which represents the ability of plants to accumulate soil elements (Ding et al., 2021; Takarina and Pin, 2017). The plants that have BCF and TF > 1.00 are used as bioaccumulators. Plants are used as phytostabilizers if they have BCF >1.00 and TF <1.00, and as phytoextractors if they have BCF<1.00 and TF>1.00 (Anyinkeng et al. 2020). The BCF and TF were calculated according to the following equations:

$$\mathrm{BCF} = \frac{C_p}{C_s} \tag{1}$$

where: C_p – concentration of the element in the plant;
C_s – concentration of the element in the soil.

$$\mathrm{TF} = \frac{C_{za}}{C_r} \tag{2}$$

where: C_{za} – concentration of the element in the shoot zone of the plant;
C_r – concentration of the metal en the root of the plant.

RESULTS AND DISCUSSION

Effect on micro-and macronutrients

The concentration of micro-and macronutrients varied depending on the plant species. According to Figure 3a, in the root system, *A. filix-femina* accumulated K as the highest concentration (6656.30 mg/kg). In shoots, for *A. filix-femina*, the lowest concentration was for Co (0.00 mg/kg) and Ni (0.02 mg/Kg), while the highest concentration was for Va (923.50 mg/kg) and Ca (4041.62 mg/kg). In general, greater accumulation of Co and Ni occurred in rhizomes than in fronds, suggesting limited mobility and translocation of these minerals once absorbed by ferns (Samecka-Cymerman et al. 2011; Drăghiceanu et al. 2019). The mostly transported minerals from rhizomes to fronds were Ca, Fe, Mg, P, Va, Si, Mn, B and Cu (Fig. 3a).

L. peruviana accumulated high concentrations of Fe (571.13 mg/kg), Ca (868.82 mg/kg), and K (5345.87 mg/kg) in the root system. In the shoots, *L. peruviana* accumulated the lowest concentration of Co (0.00 mg/kg), Ni (0.01 mg/Kg), Va (0.10 mg/Kg) and Cu (1.31 mg/Kg); while the highest concentration was for K (8259.96 mg/kg), Ca (4581.15 mg/kg), P (949.41 mg/kg) and Mg (823.67 mg/kg). Greater accumulation of V and

Co occurred in the root system than shoots. The mostly transported minerals from roots to shoots were K, Ca, Mg, Fe, P, S, Na, Si, Mn, B, and Cu (Fig. 3b).

S. trilobata accumulated high concentrations of K (6431.59 mg/kg), Ca (5618.16 mg/kg), Fe (3012.40 mg/kg), and Mg (1067.56 mg/kg) in the root system (Fig. 3c). In the shoot system, the highest concentration of transported minerals was for P (6747.82 mg/kg), Ca (5402.49 mg/kg), Fe (516.96 mg/kg), Mg (561.55 mg/kg), and S (498.06 mg/kg).

Mg is an important macronutrient for plants because it is part of the chlorophyll structure and protects the molecular structure of ribosomes (Farhangi-Abriz and Ghassemi-Golezani 2021). A sufficient level of Mg is vital for plant salt tolerance. K, being a primary macronutrient, is the most abundant inorganic cation and is important to ensure optimal plant growth; it is an activator of enzymes for protein synthesis, sugar transport, N and C metabolism as well as photosynthesis (Xu et al., 2020). In the complex interactions that occur in soil, Na replaces K which can lead to nutrient deficiencies (Artiola et al. 2019).

Plants have developed highly specific and highly efficient mechanisms for obtaining essential micronutrients from the environment with the help of the root´s chelating agents whereas the pH changes induced by redox reactions, can solubilize and absorb micronutrients of very low levels in the soil, even from almost insoluble precipitates (Usman et al. 2020). Plants have also developed highly specific mechanisms for moving and storing micronutrients, which are involved in the uptake, translocation and storage of toxic elements, the chemical properties of which simulate those of essential elements (Steliga and Kluk 2021). In this regard, phytoremediation is rather interested in micronutrient absorption pathways.

Figure 4 shows the overall count concentration of different elements concentrations (mg/kg) as stack bars of *L. peruviana*, *A. filix-femina* and *S. trilobata* on soil, shoot and root system. Bioavailability of micronutrients, macronutrients, and heavy metals varies amongst plant systems. According to the findings, the rate of absorption and bioaccumulation is higher in roots compared to shoots. The three plant species accumulate Ba, Cd, Cr, Sr, Al, Li, Ag, Ga, In, Te, As, and Sr in shoot and root systems. Elements like Cr are heavy metals that are considered micronutrients but become toxic when consumed in large amounts (Bhat et al. 2022). On the other hand, Cd, Hg, Al, Pb, Sr, Te are non-essential heavy meals that are lethal to living organisms (Ali and Khan 2019). Heavy metals inhibit physiological processes, such as respiration, photosynthesis, cell elongation, the plant-to-water relationship, and metabolism. It has been reported that soil infertility, associated with its acidification, is mainly caused by aluminum, an element capable of negatively affecting plants by inhibiting root elongation and, under such conditions, important nutrients like Mg, Ca, K, P, N become deficient (Ndiate et al. 2022).

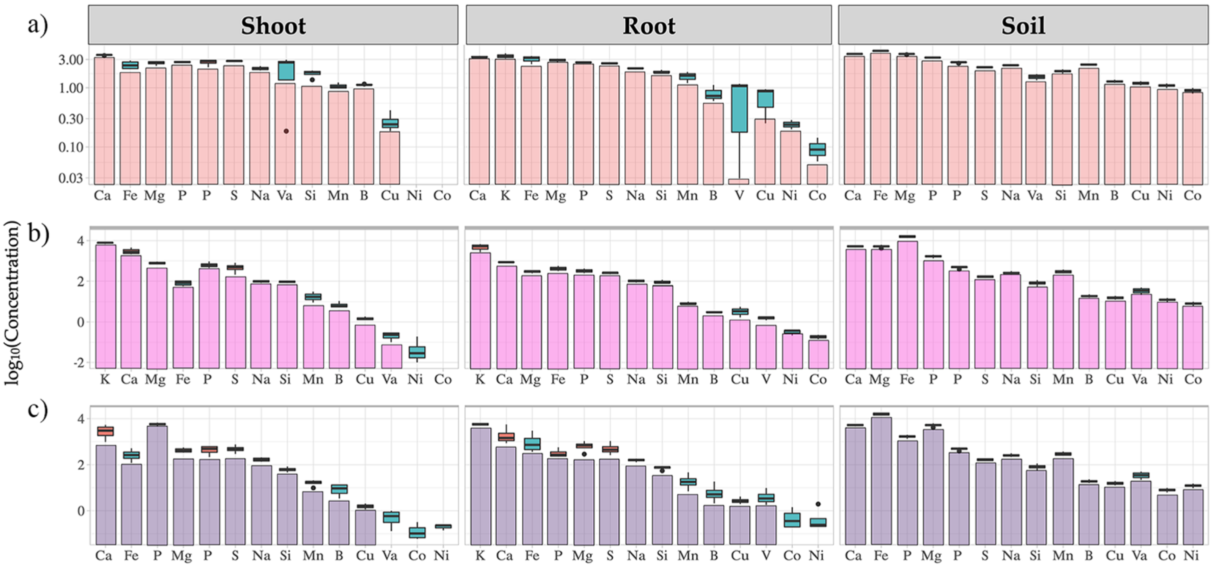

Figure 3. All micro-and macronutrients: (a) *A. filix-femina,* (b) *L. peruviana*, *and* (c) *S. trilobata*

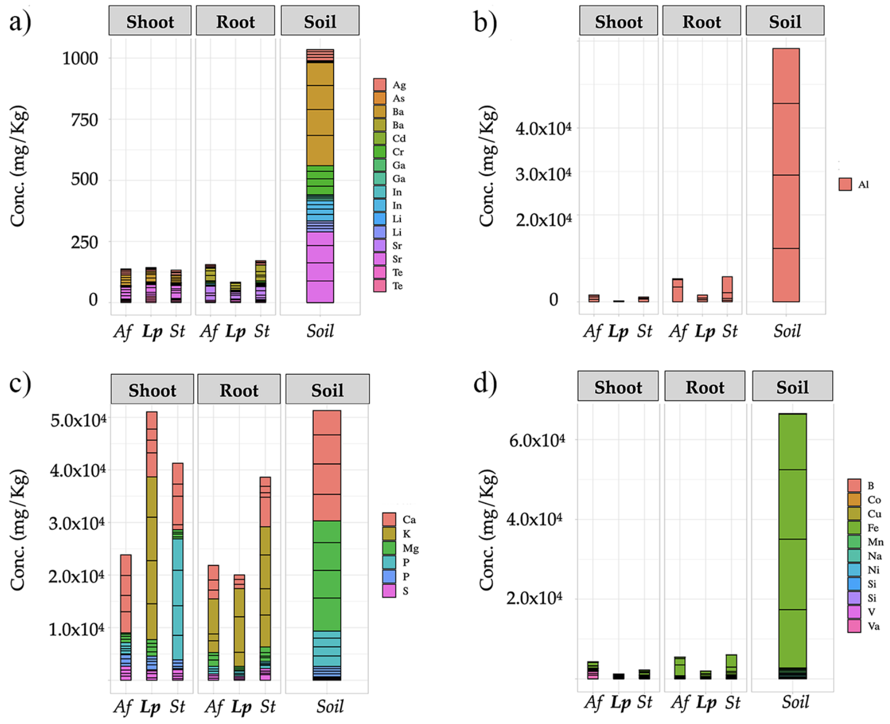

Figure 4. Concentration of (a) heavy metals, (b) aluminum (c) macro-and, (b) micronutrients on *Athyrium filix-femina* (*Af*), *Ludwigia peruviana* (*Lp*), and *Sphagneticola trilobata* (*St*), and soil

EFFECT ON HEAVY METALS

Figure 5 presents the concentrations of the six heavy metals that were transferred from the soil by three different plant species. Aluminium accumulation was up to five times higher in roots than in shoots in the order *A. filix-femina* > *S. trilobata* > *L. peruviana*. The highest percents of aluminum accumulation were found in the roots of the species *A. filix-femina* (12%) and *S. trilobata* (10%). Several plant species are susceptible to aluminium and the root growth inhibition is certainly the most easily recognizable trait of Al toxicity (Mossor-Pietraszewska 2001; Singh et al. 2017). According to results, no inhibition of root and shoot growth was a visible symptom of Al toxicity in all three species.

A silver initial concentration of 12 mg/kg was taken up by *A. filix-femina* shoots in 52% and roots in 40%. Ag accumulation was up to 1.3 times higher in shoots than in roots in the order *A. filix-femina* > *S. trilobata* > *L. peruviana.* High levels of Co, Cu, Fe, Mn, Ni, and Pb in the leaves have been linked to negative effects on the health of *A. filix-femina*, suggesting that this species may utilise components collected in its tissues as a defense against infections (Musilova et al. 2016; Kazienko et al. 2020). The negative impacts of silver on plants' morphology and physiology, especially in the area of growth regulation, are well-documented (Wang et al. 2017; Yan and Chen 2019).

Cadmium accumulated to a greater degree in the shoots (14 and 19%) of *A. filix-femina* and *L. peruviana*, respectively, compared to their roots (8% and 12%). The opposite was observed for *S. trilobata*. Its roots took up in the most Cd, with 27%, but its shoots only accumulated 14%. It is considered that *L. peruviana* is a good accumulator of heavy metals such as Cu, Zn, Pb, and Cd, and it is one of the most important genera of wetland plants (Chowdhury et al. 2013; Anyinkeng et al. 2020). Furthermore, *S. trilobata* accumulates more than 100 mg/kg of Cd and has the tolerance mechanisms that make it a viable candidate for phytoremediation of this element (Pernía et al. 2019).

Chromium had an initial concentration in soil of 30 mg/kg, although the permissible limits for Cr in soil is 3.8 mg/kg (Vodyanitskii 2016). In all plant species, it was found that the root system accumulated the highest concentrations of Cr in the following order *S. trilobata* > *A. filix-femina* > *L. peruviana.* According to results, *A. filix-femina* and *S. trilobata* uptaked up to 19% of Cr in their roots, and up to 2% in their shoots. The toxic effects of Cr on plants result in

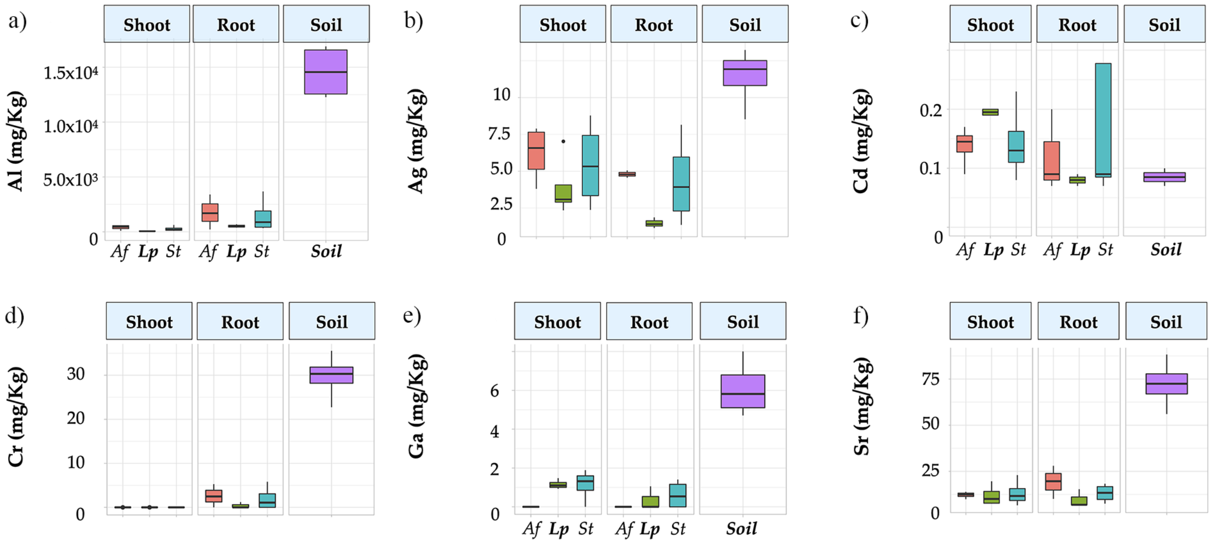

Figure 5. Heavy metals on *A. filix-femina* (*Af*), *L. peruviana* (*Lp*), *S. trilobata* (*St*), and soil

a delay in seed germination, damaged roots and reduced root development, decreased biomass, decreased plant height, impaired photosynthetic function, and ultimately plant mortality (Amin et al. 2013).

Gallium is an emerging contaminant that is used in advanced industries and is considered as toxic to humans and produces an inhibition effect on crops (Chen et al. 2022; Syu et al. 2021). Shoots of *S. trilobata* accumulated up to 24%, whereas roots accumulated up to 18%. From an initial Ga concentration of 8 mg/kg, *S. trilobata* was shown to accumulate the most Ga of any plant species in this investigation.

Strontium was accumulated by *A. filix-femina* in a concentration of 15% in shoots and 31% in roots. The *L. peruviana* and *S. trilobata* shoots took up 26% of Sr, whereas the roots 20%. Strontium limits plant calcium uptake and is extremely toxic, producing metabolic imbalances in the tissues (Mikhailovskaya and Pozolotina 2020).

According to Table 1, there were statistical differences in the heavy metal accumulation in the shoots and roots (Factor A). However, the accumulation response to heavy metals did not depend on the three plant species (Factor B).

Tukey´s test (Table 2) shows that a significant difference exists among soil and plant organs in the accumulation of all tested heavy metals. In this case, the heavy metal content in roots was much higher than shoots for all plant species. Heavy metals are considered to be transferred from roots to shoots via root pressure and transpiration (Wu et al. 2021). Membrane transporters, which are proteins enclosed in the membrane phospholipid bilayer, enable higher plants to efficiently absorb accessible metal ions from the soil (DalCorso et al. 2013).

Table 1. Summary of p-values from analysis of variance (ANOVA) for factors (A) plant system, and (B) specie for Al, Ag, Cd, Cr, Ga, and Sr

Source of variation	Df	Al	Ag	Cd	Cr	Ga	Sr
Plant system	2	0.000 (***)	0.000 (***)	0.000 (***)	0.000 (***)	0.000 (***)	0.000 (***)
Specie	2	0.547 (ns)	0.067 (.)	0.657 (ns)	0.772 (ns)	0.067 (.)	0.567 (ns)
Part:Specie	2	0.786 (ns)	0.841 (ns)	0.351 (ns)	0.713 (ns)	0.618 (ns)	0.497 (ns)

Note: Df – degrees of freedom. Significance codes: 0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 (-) no significance (ns).

Table 2. Summary of p-values from Tukey HSD test for plant parts root and shoot, and soil

Plant system	Al	Ag	Cd	Cr	Ga	Sr
Soil	14572.36 (a)	11.40 (a)	105.29 (a)	29.73 (a)	6083.7 (a)	72311.5 (a)
Root	1264.51 (b)	5.17 (b)	14.40 (b)	2.01 (b)	761.6 (b)	13919.1 (b)
Shoot	241.30 (b)	3.61 (b)	9.57 (b)	0.20 (b)	354.1 (b)	12397.9 (b)

Table 3. Bioconcentration (BCF) and translocation factors (TF)

Heavy metal	*L. peruviana*			*A. filix-femina*			*S. trilobata*		
	BFC_{Root}	BFC_{Shoot}	TF	BFC_{Root}	BFC_{Shoot}	TF	BFC_{Root}	BFC_{Shoot}	TF
Cd	0.95	2.26	2.38	1.42	1.52	1.07	1.01	0.95	1.68
Cr	0.03	0.00	0.11	0.10	0.02	0.17	0.08	0.03	0.21
Sr	0.10	0.14	1.44	0.22	0.21	0.96	0.23	0.10	0.89
Al	0.14	0.03	0.11	0.11	0.01	0.13	0.09	0.03	0.19
Ag	0.13	0.38	2.83	0.44	0.52	1.19	0.40	0.13	1.26
Ga	0.23	0.27	1.18	0.05	0.00	0.00	0.27	0.23	1.01

Bioconcentration factor (BCF) and translocation factor (TF)

Table 3 reveals that the plant species *L. peruviana, A. filix-femina,* and *S. trilobata* have high phytoextraction and bioaccumulation capacities with respect to Cd by itself. Herbaceous plants like *L. peruviana* adapt and synthesize phytochelatins, metallothioneins, stress proteins, and phenolic compounds to tolerate Cd and other heavy metals (Mongkhonsin et al. 2019). An excessive Cd concentration affecting growth overrules some nutrients, such as Ca, Mg, P, N, Fe, and K, leading to growth inhibition, a decrease in biomass, and death of the plant (Mongkhonsin et al. 2019; Raza et al. 2020).

According to Table 3, *L. peruviana* shows a potential as phytoextractor of Sr, Ag and Ga. The process of phytoextraction is distinguished by the rapid production of a significant amount of biomass in a short amount of time, as well as a high rate of water intake and transpiration, and a robust root system. Accordingly, *A. filix-femina* shows a potential as phytoextractor of Ag, while *S. trilobata* shows a potential as phytoextractor of Ag and Ga. Several variables, including soil characteristics, pH, agroclimatic conditions, cultivation techniques, and soil microbial populations, and the total concentration of metals in the soil, affect the process of heavy metal transport to plant tissues (Mikhailovskaya and Pozolotina 2020; Wu et al. 2021; Aveiga-Ortiz et al. 2022).

CONCLUSIONS

The key findings of the study showed that *L. peruviana*, *A. filix-femina*, and *S. trilobata* have the ability to transport Cd, as well as strong phytoextraction and bioaccumulation potentials for this element. Additionally, the plant species have the potential for Sr, Ag, and Ga phytoextraction. There was a clear difference between the heavy metal concentration of roots and shoots across all plant species. The plants that accumulated Cd showed no signs of diminished growth or yield. The rhizosphere of these plants is capable of extracting and accumulating heavy metals. Furthermore, these species are common perennial herbs that may establish, grow, and reproduce rapidly, resulting in a significant biomass yield. The contribution of this work was to use phytoextraction to safely remove metals from shallow contaminated soil and translocate them into plant tissues as soluble compounds. The roots and fast growth rates of the herbaceous plants tested here indicated a potential for survival in the soils contaminated with heavy metals.

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