**Original Research** 

# Variation of Heavy Metals Accumulation by Malva in Amazonian Ecosystems of Drylands and Floodplains

Lucinete Miranda Gomes<sup>1</sup>, Caroline de Souza Bezerra<sup>1</sup>, Jennifer Souza Tomaz<sup>1</sup>, Carlos Henrique Salvino Gadelha Meneses<sup>2</sup>, Ricardo Lívio Santos Marques<sup>1</sup>, Anderson Mathias Pereira<sup>1</sup>, Ricardo Lopes<sup>3</sup>, Mágno Sávio Ferreira Valente<sup>4</sup>, Santiago Linorio Ferreyra Ramos<sup>5</sup>, Maria Teresa Gomes Lopes<sup>1\*</sup>

<sup>1</sup>Faculdade de Ciências Agrárias, Universidade Federal do Amazonas, Av. Rodrigo Otávio, 3000, Manaus 69060-000, AM, Brasil

<sup>2</sup>Programa de Pós-Graduação em Ciências Agrárias, Departamento de Biologia, Centro de Ciências Biológicas e da Saúde, Universidade Estadual da Paraíba, Campina Grande 58429-500, PB, Brasil
<sup>3</sup>Embrapa Amazônia Ocidental, rod. AM 10, Km 29, s/n, CP 319, Manaus 69010-970, AM, Brasil
<sup>4</sup>Instituto Federal do Amazonas, Av. da Onça-Pintada, S/N-Galo da Serra, Presidente Figueiredo 69735-000, AM, Brasil
<sup>5</sup>Instituto de Ciências Exatas e Tecnologia, Universidade Federal do Amazonas, rua Nossa Senhora do Rosário, 3863, Itacoatiara 69103-128, AM, Brasil

Received: 18 June 2024 Accepted: 16 November 2024

# Abstract

The rapid urbanization, industrialization of cities, agricultural activities, and utilitarian actions of the environment have caused increased pollution on the planet, mainly caused by heavy metals. The advancement of ecosystem contamination has been the subject of studies to prevent or reduce risks to environmental health. The use of plants to remove heavy metals from soil has become a prominent research area in phytoremediation. Considered an herbaceous plant, malva (*Urena lobata* L.) is widely cultivated for its economic value in natural fibers and medicinal applications. The objective of this work was to analyze the concentrations of inorganic components in soils and tissues (roots and leaves) of *U. lobata* in two areas: an anthropized area and a floodplain area, using X-ray fluorescence by total reflection. Eight samples of malva soil and tissues (leaves and roots) were collected in each area, totaling 48 samples. The graphical dispersion was performed by the Principal Component Analysis Method (PCA). The soils of the anthropized area provided a predominance of elements such as Pb, Nb, and Yb, and the floodplain area presented high concentrations of Al, Cr, Ni, Cu, and Hf. The results indicate that malva is a promising species for use as a phytoremediator in areas with heavy metal-contaminated soil. It is characterized as an accumulator of Ti, Ni, Br, and Y in its tissues (roots and leaves)

and as a hyperaccumulator of Hf and Yb. It is suggested that the species be used for environmental biomonitoring, mainly metals Ti, Yb, Hf, Ni, Br, and Y. This is the first report of this species as a bioaccumulator of heavy metals.

Keywords: environmental contamination, bioindicator plant, phytoremediation, x-ray fluorescence, soil contamination

#### Introduction

Heavy metals have contaminated the environment due to the intensification of human activities and economic development, particularly with the rapid urbanization and industrialization of cities [1]. Contamination has also affected the soil and water and accumulated at various levels of the food chain and ecosystems [2, 3]. Various bioavailable and mobile forms of heavy metals and trace elements are also released in activities such as mining, the combustion of fossil fuels, and the use of chemicals in agriculture [2, 4].

In Manaus, Brazil, a city surrounded by the Amazon rainforest, there are significant emissions of vehicle gases and fossil fuels, along with an industrial hub that contributes to the release of solid waste and greenhouse gases [5, 6]. The effects of these pollutants are harmful not only to the population's health but also to local ecosystems, due to the emission of elements that, when accumulated in the environment, cause phytotoxicity in plants, animals, microorganisms, and humans [7]. Knowing the heavy metals or trace elements released into the environment, as well as their respective concentrations, is crucial to monitoring environmental health.

To identify and quantify these chemical elements, it is essential to use reliable analytical techniques. Among them, we highlight the techniques of X-ray fluorescence spectrometry (XRF), a technique that allows for fast results and high reproducibility [8]. TXRF is a versatile microanalytical tool with detection limits at the picogram scale [9]. It presents competitiveness with techniques that analyze metals due to their qualities, including practicality, precision, and reliability. It is a tool used for simultaneous multi-elemental analysis and requires small amounts of samples. In addition, it can detect most chemical elements at concentrations below one part per million (ppm) and uses a known internal standard that considers the sensitivity of the elements [10, 11].

The technique has been applied in environmental pollution control studies [12], tea analyses [9], the determination of phytochelatins in aquatic plants [11], the analysis of plant materials and soils [8, 13, 14], and the analysis of rainwater, clouds, rivers, and drinking water [12, 15]. It is an appropriate technique for the environmental monitoring of anthropogenic areas, being applied in studying different plant species as bioindicators of pollution such as the species

*Stryphnodendron polyphyllum* Mart., *Brassica juncea* (L.) Czern., and *Sorghum bicolor* (L.) [7, 12].

Some plant species demonstrate greater adaptation and an enhanced ability to thrive in diverse environments, effectively removing, degrading, or containing contaminants such as heavy metals and trace elements in soils, sediments, groundwater, and surfaces [16]. These species have attracted attention to phytoremediation and phytostabilization research, techniques considered efficient, less expensive, and environmentally friendly, and are more widely accepted for the removal of contaminants [7, 17].

Phytoremediation has become the most applied technology in remediation projects and research in developed countries due to its economy and ease of implementation [16, 18]. Research is being conducted using this methodology, applied to the monitoring of herbicide residues in soil [19], the phytoremediation of soils contaminated with forest species [7, 20], and the environmental recovery of landfills [21], among other applications. The use of plant species capable of accumulating large amounts of chemicals, heavy metals, and trace elements is one of the most adopted practices for the management of contaminated soils, sediments, and waters [16].

Urena lobata L., belonging to the Malvaceae family and popularly known as malva, is classified as a natural fiber. Its occurrence is restricted to tropical and subtropical regions of the world. This herbaceous species can reach up to 2.5 m in height, with leaves that vary in size and shape [22]. The characteristics of its fiber are similar in strength and appearance to those of jute (*Corchorus capsularis* L.), kenaf (*Hibiscus cannabinus* L.), and roselle (*Hibiscus sabdariffa* L.), being commonly used in the making of nets and fishing lines, bags, mats, and ropes. Its rods are also used as mooring materials [22]. In addition, they can serve as reinforcements in polymer matrices, replacing fiberglass [23].

Malva is considered a medicinal plant with active ingredients that possess antioxidant, antibacterial, and anti-diabetic properties [24]. *U. lobata* has also been studied as an alternative adsorbent material for the removal of Pb<sup>2+</sup>, Ni<sup>2+</sup>, and Cd<sup>2+</sup> from aqueous solutions, demonstrating high efficiency in metal ion adsorption [25]. As a species of medicinal use, it is necessary to know the capacity of absorption of heavy metals.

By analyzing plant tissues and rhizosphere soils of a plant species, it is possible to evaluate their phytoremediation potential or the ability to extract trace elements from cultivation areas. This study aimed to analyze the concentrations of inorganic components in the soils, roots, and leaves of *U. lobata* in two areas: one anthropic and one floodplain, to assess the species' potential as a bioindicator of heavy metal contamination.

#### **Materials and Methods**

One of the study areas is in the municipality of Novo Airão, Amazonas, Brazil, between coordinates 2°37'33''S and 60°56'37''W [26]. This municipality is characterized as an anthropic area with the deposition of municipal solid waste in the soil. The soil of the region is classified as latosol and has a clay texture [26].

The second study area is a high floodplain, located on Ilha do Marrecão, in the community of Paraná do Supiá, in the municipality of Manacapuru, Amazonas, located between the coordinates 3°22'60" S and 60°43'0" W. This area is characterized by a floodable environment during the final stages of flooding, with gleysol-type soil [26]. The areas were selected based on differences in the ecosystem, vegetation types (dryland forest and floodplain forest), level of pollution in the environments, and occurrence of species in both areas.

# Collection and Preparation of Soil Samples and Plant Material of *U. lobate*

Eight individuals of *U. lobata* were randomly selected from each area, and samples of soils, adult healthy leaves, and fine roots were collected, totaling 24 samples per study site. The collection, preparation, storage, and conservation of fine root and soil samples followed the methodology described by Bezerra et al. [14].

## Preparation of Samples to Obtain Dry Mass

The samples of leaves and roots were washed in a sieve with a 1 mm mesh with a solution of water and neutral detergent (1 mL 1 L<sup>-1</sup>), then washed in running water and later in distilled water. After washing, they were placed in perforated paper bags and stored in an oven with forced air circulation. The temperature was maintained at 65-70°C for 48 h until a constant mass was achieved.

Leaf dry mass (LDM), root dry mass (RDM), and total dry mass (TDM) = (LDM + RDM) were determined with the aid of a Mettler PM 30-K balance (Mettler Toledo, Columbus, OH, USA) with an accuracy of 0.001 g. From the dry mass data, we calculated the root dry mass ratio (RDMR) along with the dry mass ratio of the leaves (DMRL) [27].

# Preparation of Samples for Analysis of X-Ray Fluorescence by Total Reflection (TXRF)

The preparation and analysis of the samples were performed at the Plant Breeding Laboratory of the Federal University of Amazonas and the Scientific Technical Laboratory of Chemical Analysis of the Regional Superintendence of the Federal Police of Amazonas.

The preparation of the samples for analysis followed the protocol established by Bezerra et al. [14]. For the analysis, a benchtop spectrometer, model S4 T-STAR/ Bruker (Bruker, Billerica, MA, USA), was used. It is equipped with two X-ray tubes: a Molybdenum (Mo) anode at 17.5 keV, consisting of a metal that may be Rh, Mo, or Cr, and a cathode with a tungsten filament (W). White samples were made to identify any possible source of contamination, and duplicates were prepared for each soil sample and tissue of U. lobata. For the determination of the relative sensitivities of the elements, gallium (Ga) was used as a known internal standard because it is an element that is not present in the analyzed samples and won't interfere with the target elements [28], following the protocol established by Bezerra et al. [14].

#### Statistical Analysis of Data

The results of the analysis of inorganic components in plant tissues were subjected to tests for outlier removal, normality, and homoscedasticity of variances [29]. Data that did not meet the assumptions of ANOVA were analyzed using the Mann-Whitney test [30].

Principal component analysis (PCA) was performed in the R environment (RStudio Team, Boston, USA) and its complement, RStudio Team (RStudio Team, Boston, USA) [31]. The Pearson correlation analysis between the variables analyzed was performed by the software Genes (Viçosa, Brazil) [32].

# **Results and Discussion**

In the floodplain area (FA), the values observed in the soils were higher than those found in the anthropized area (AA) for 10 elements analyzed (Ca, Mg, K, Si, Al, V, Mn, Cr, Ni, and Hf) when compared to the others (P, Cu, Se, Pb, Nb, and Yb) (Table 1). The soils of AA and FA showed pH in  $H_2O$  of 4.0 and 5.0, respectively. The values of this study corroborate works carried out on dryland soils in the Amazon [33]. Most primary and secondary elements are expected to be available at a slightly acidic pH for plants, which is an indicator of soil chemical fertility. The concentration of these elements in the soil solution, their mobility, and the ionic form made available to plants are pH-dependent; however, plants have different response mechanisms to pH change [34].

The leaching of basic cations such as  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $K^+$  is controlled by soil pH, providing the H<sup>+</sup> and Al<sup>3+</sup> ions for exchangeable cations dominant in acidic pH [35]. The soils of AA and FA showed higher concentrations of Al; although high levels of the element have been detected in soils, there was no translocation of the element to the tissues of *U. lobata*. Based on the content of the elements essential to plant growth, the increasing order in AA for macronutrients was Mg>P>Ca>K and for micronutrients Cu>Mn, while in FA the order for macronutrients followed Mg>K>Ca and micronutrients Cu>Mn (Table 1). Most of the macronutrients and micronutrients available to plants were observed in the soils of the floodplain area, which are considered sedimentary soils of high natural fertility due to the processes of removal, transport, and deposition [33].

Some elements, such as Si and Se, are not considered essential but can improve plant growth and stress resistance when present [36]. In the FA, the Si presented a higher concentration in relation to the AA, while for the Se, the inverse occurred, with a higher content in the soil of the AA (Table 1). The concentration of Si in the soil increases the availability of elements Ca, P, S, Mn, Zn, Cu, and Mo [37]. In the present study, it was found that the highest concentration of the element increased the availability of Ca, K, Mg, and Mn in FA.

With the water cycle in FA, it is also possible to observe the deposition of heavy metals in these soils,

Table 1. Contents of inorganic elements in surface layer soils with occurrence of *Urena lobata* in Anthropized Area (AA) in the municipality of Novo Airão and Floodplain Area (FA) in the municipality of Manacapuru, Amazonas, Brazil.

Inorganic components	AA	FA
	$(mg \cdot kg^{-1})$	
Phosphorus (P) *	900.1	0
Calcium (Ca) *	462.35	3,536.0
Magnesium (Mg) *	1,853.5	12,005.0
Potassium (K) *	97.01	9,615.5
Silicon (Si) *	1,686.5	12,565.0
Aluminium (Al) *	1,105.0	5,943.0
Vanadium (V) *	30.15	59.4
Manganese (Mn) *	61.44	79.82
Chromium (Cr) *	17,380.0	20,230.0
Nickel (Ni) *	54.58	2,489.58
Copper (Cu) *	6,132.0	3,028.0
Selenium (Se) *	1,351.07	50.29
Hafnium (Hf) *	0	2,947.1
Lead (Pb) *	26.28	19.06
Niobium (Nb) *	20.27	0
Ytterbium (Yb) *	87.45	0

\* and ns, significant and not significant, respectively, at 5% by the Mann-Whitney nonparametric test. The average pH of  $H_2O$  in soils of the anthropized area in the municipality of Novo Airão and the floodplain area in the municipality of Manacapuru, was 4.0 and 3.0, respectively.

being released through mining or industrial areas which, depending on their concentration and ionic forms, may present a serious environmental risk [38]. Except for the non-essential elements Pb, Nb, and Yb that presented higher contents in AA, the elements (Al, V, Cr, Ni, and Hf) had their highest concentrations in FA. The decreasing order of absorption of the elements in AA followed: Cr>Al>Yb>Ni>V>Pb>Nb, while for FA, Cr>Al>Hf>Ni>V>Pb (Table 1).

The soils of AA and FA showed considerable levels of heavy metals in the soil, as is the case with Pb. This element comes mainly from vehicle emissions, agricultural production, and industrial and thermal plants [39]. *U. lobata* plants located in these areas are exposed to this contaminant in the soil. Notably, they do not assimilate in their tissues. According to Vinogradova et al. [40], plants act as a barrier to the propagation of heavy metals in the soil.

In the PCA, the first two principal components account for 67.2% of the total variation among the samples, indicating a strong representation of the original sample distances. Through visual analysis of the graphical dispersion, two distinct groups were identified among the genotypes of *U. lobata*, with each group corresponding to an area of origin: floodplain (genotypes 1 to 8) and anthropized area (genotypes 9 to 16) (Fig. 1).

For the analysis of the roots of *U. lobata*, 6 inorganic elements were detected. Between them 2 micronutrients (Fe and Mn), a beneficial element (Se), and non-essential elements (Ti, Hf, and Yb). The order of absorption of non-essential elements in AA was Ti>Yb, while in FA it was Hf>Ti (Table 2).

The TXRF technique efficiently analyzes soil and root samples in dryland or floodplain environments. According to Lu et al. [41], conventional methods for detecting chemical elements in soil are complicated due to the difficulty of pre-processing samples. Consequently, the development of techniques such as TXRF has become one of the primary tools for analyzing soil chemical elements because of its fast measurement and ease of operation. Thus, the technology proved effective in determining heavy metals in floodplain soils [42, 43] and dryland soils [14, 44], as well as being applied to studies on soil fertility attributes [45] and the analysis of plant tissues, such as roots and leaves [11, 14, 46, 47].

The method efficiently detected macronutrients (P, K, Ca, and Mg), micronutrients (Cu and Fe), nonessential elements (Si, Al, Mn, and Se), and heavy metals (V, Cr, Ni, Pb, Nb, Yb, Ti, and Hf) in the soil and roots of *U. lobata*. However, some elements found in the soil were not present in the roots. Additionally, not all the chemical elements present in the anthropized area (drylands) were detected in the floodplain area. The elements Ti and Fe showed higher concentrations in the roots of AA plants. Yet, these elements were not present in the soils (Table 2). The majority of Mn, Se, Hf, and Yb were found in the soil and roots of *U. lobata*, with most being concentrated in AA.



Fig. 1. Principal Component Analysis with the graphical distribution of sixteen genotypes of *U. lobata* in the anthropized area (1 to 8) and the floodplain area (9 to 16) for inorganic elements in the soil. The ellipses indicated in the graph represent the regions of confidence for the characterization of the analyzed areas.

In phytoremediation, plants are utilized to remove heavy metals from the soil through mechanisms such as phytoextraction and phytostabilization [48]. Plants of *U. lobata* can tolerate high concentrations of Al, Cr, Ni, Cu, Hf, and Pb in the soil in various environments, showing their adaptive capacity and phenotypic plasticity. Additionally, these plants can absorb certain heavy metals through their roots, reducing the metals' bioavailability.

Plants have three tolerance strategies when exposed to high concentrations of heavy metals in the soil: metal exclusion (metal accumulation in plant tissues is

Table 2. Contents of inorganic elements in roots of *Urena lobata* in Anthropized Area (AA) in the municipality of Novo Airão and the Floodplain Area (FA) in the municipality of Manacapuru, Amazonas, Brazil.

Inorganic components	AA	FA
	$(mg \cdot kg^{-1})$	
Manganese (Mn) *	0	42.61
Selenium (Se) *	0.88	0
Titanium (Ti) *	2,076.5	509.55
Iron (Fe) *	6,140.0	4,996.0
Hafnium (Hf) *	0	4,101.5
Ytterbium (Yb) *	787.55	0

\* and ns, significant and not significant, respectively, at 5% by the Mann-Whitney nonparametric test. The average pH of  $H_2O$  in soils of the anthropized area in the municipality of Novo Airão and the floodplain area in the municipality of Manacapuru, was 4.0 and 3.0, respectively.

limited), metal indication (accumulation in plant tissues is proportional to soil concentrations), and accumulation or hyperaccumulation of metals (metal accumulation in plant tissues is higher than soil concentration) [49]. Based on this, in the present work, *U. lobata* plants can be considered hyperaccumulators of Hf and Yb, as the concentration of Hf in the roots in the FA was higher than in the soil, and the concentration of Yb in the roots of plants in the AA was 700 times higher than in the soil.

The distance between the sampled individuals was adequately represented with low distortion, as the first two principal components of the PCA accounted for 67.23% of the total variation observed in the samples (Fig. 2). Except for individuals 3 and 7, the others in the anthropic area showed greater proximity to each other, separating the two areas differently. In the floodplain area, only genotype 9 showed distancing from the group of individuals analyzed (Fig. 2).

Leaf analysis of *U. lobata* identified ten elements: Si, Cr, Ni, Hf, Yb, Ti, Zn, Br, I, and Y (Table 3). The concentration of heavy metals, trace elements, and metalloids in the environment is expected to increase over the years, as it did in the last century. The list of elements that make up these groups is small and includes elements such as Hf and Ni detected in FA, Cr in AA, and Zn in both areas of this work. Over the years, much research has been developed covering some of these elements that can be detected in both soils and plants. However, the biogeochemistry of most of them is not yet known [50].

Except for the elements Ni, I, and Yb, the other elements were in higher concentrations in FA. The order of absorption of these elements in the same environment followed: Ti>Hf>Ni>Si>Br>Y>Zn>Yb. While in



Fig. 2. Principal Component Analysis with a graphical distribution of sixteen genotypes of *Urena lobata* in the anthropized area (1 to 8) and the floodplain (9 to 16) for inorganic elements present in the roots. The ellipses indicated in the graph represent the regions of confidence for the characterization of the analyzed areas.

AA, the order of absorption of the elements followed: Yb>Si>I>Zn>Ti>Br>Cr (Table 3).

The presence of elements such as Hf and Ni in the FA could be due to the area's proximity to the farmer's residence, leading to sewage deposition and pesticide use from nearby plantations. The global increase in the industrial use of Hf in recent years has resulted in its frequent presence in soil and plant samples [50-52]. There was greater accumulation of the element in the leaves of FA individuals, while it was not detected in the leaves of plants from AA. The FA individuals demonstrated absorption capacity and accumulation of the element, and the data showed a significant negative correlation with the dry mass of these individuals (LDM x L\_Hf: -0.60) and leaf mass ratio (DMRL x L\_Hf: -0.51) as observed in fig. 3 and 4, thus, it can be expected that plants grown in soil

Table 3. Levels of inorganic elements in leaves of *Urena lobata* in Anthropized Area (AA) in the municipality of Novo Airão and the Floodplain Area (FA) in the municipality of Manacapuru, Amazonas, Brazil.

Inorganic components	AA	FA
	(mg·kg <sup>-1</sup> )	
Silicon (Si) *	319.85	2,134.5
Chromium (Cr) *	0.45	0
Nickel (Ni) *	0	2,184.5
Titanium (Ti) *	16.82	3,662.5
Zinc (Zn) *	28.83	61.49
Bromine (US) *	1.08	1,734.5
Iodine (I) *	279.75	0
Yttrium (Y) *	0	1,429
Hafnium (Hf) *	0	3,587.5
Ytterbium (Yb) *	719.25	0

\* and ns, significant and not significant, respectively, at 5% by the Mann-Whitney nonparametric test. The average pH of H2O in soils of the anthropized area in the municipality of Novo Airão and the floodplain area in the municipality of Manacapuru, was 4.0 and 3.0, respectively. such as FA, with Hf accumulation, may experience growth suppression [50], resulting in biomass differences between individuals of AA and FA.

As for Ni, it is an element considered a natural metal and can negatively or positively affect the development of plants depending on its concentration. Activities such as industrialization, the use of fertilizers, chemicals, and sewage sludge increase the concentration of heavy metals to undesirable levels, making them toxic to plants and the environment. High Ni concentrations reduce seed germination, root, shoot, and biomass growth [53]. The obtained results corroborate findings in the literature, as a significant negative correlation was observed (DMRL x L Ni: -0.57), as shown in Fig. 3 and 4. In a study developed by Amadi et al. [25] U. lobata is an effective species in removing Ni from an aqueous solution, which makes it a species with phytostabilizer capacity, and а heavy metal phytoextractor from contaminated environments. Environments contaminated with Ni can pose risks to crop production, making it essential to study species that exhibit tolerance to this element [53].

Cr, along with other heavy metals, is introduced into the environment through anthropic activities, and contamination by this element is considered a problem for both the environment and human health. Phytoremediation is a promising technology for remediating environments contaminated by Cr [48]. In this work, high concentrations of the element were detected in the soils of both areas studied, FA being higher than AA. Nonetheless, in the foliar analyses, the element was identified only in individuals with AA. It is believed that because it is alluvial soil, there was greater leaching of the element and adsorption of it when compared with plants from the AA. More than 400 plant species are known to accumulate Cr and have the potential to remediate soils and water contaminated with it through mechanisms such as phytoextraction [48]. U. lobata has been demonstrated to be a potential species for this activity, with the presence of Cr in its leaves confirming its ability to extract the element from the soil. Further research is needed to optimize



Fig. 3. Principal Component Analysis for *U. lobata* Dry Mass. The ellipses indicated in the graph represent the regions of confidence for the characterization of the analyzed areas.



Fig. 4. Pearson correlation between inorganic components, leaf dry mass (LDM), leaf dry mass ratio (LDMR), root mass ratio (RMR), leaf root reason (LRR), leaf (L) and root (R) of *U. lobata*. The colors refer to negative correlations (red) and positive (blue), respectively, the more intense the color, the greater the correlation. The asterisks indicate the significance level (\* p 0.05 and \*\* p 0.01).

phytoremediation methods for specific environmental conditions, such as drylands or floodplains.

Zn is considered an air pollutant and one of the main pollutants emitted into the atmosphere [54]. In our study, we detected concentrations of zinc only in the leaves of *U. lobata*, both in AA and FA. Since we found no concentrations of zinc in the soil and roots that we analyzed, the presence of zinc in the leaves is likely due to atmospheric deposition. Therefore, the species was able to accumulate this metal in its plant tissues, acting as a biomonitor species by absorbing atmospheric pollutants [54].

Ti, Si, and Br were identified in both areas, but at a much higher concentration in FA. While I was detected only in AA and Yb and Y only in FA, Ti and I are characterized as trace elements that, according to the literature, are of environmental interest. However, at high concentrations, they can be toxic to plants [55]. Br can be derived from fertilizers and can be absorbed, transported, and accumulated in plant parts, which can be ingested by humans [56]. As with *U. lobata*, which is also considered a medicinal species and is consumed by the population in the form of tea and other preparations [24], Br was detected only in the leaves. Furthermore, it is believed that it was presumably caused by the application of fertilizers around the planting.

Y and Yb are considered rare earth elements and are not generally toxic to most plants. At low concentrations, they can benefit plant species, which may still be classified as accumulators or hyperaccumulators.



Fig. 5. Principal Component Analysis with a graphical distribution of sixteen genotypes of U. *lobata* in the anthropized area (1 to 8) and the floodplain (9 to 16) for inorganic elements present in the leaves. The ellipses indicated in the graph represent the regions of confidence for the characterization of the analyzed areas.

The data obtained for *U. lobata* characterize it as an accumulating species since the concentration of the elements was higher than 1000  $\mu$ g g<sup>-1</sup> [57].

Based on the first two principal components generated in the PCA for foliar analysis, approximately 72% of the total variation observed in the samples confirmed the separation of individuals into two distinct groups (anthropized area and floodplain area), except for individuals 6 and 13, which showed distancing from the groups corresponding to their respective areas of origin (Fig. 5).

The first two components of the PCA explained 93.34% of the total variation of the samples, indicating that with these components, the original variation between the samples was well represented. As verified in the dry mass analysis for *U. lobata*, the samples were separated into groups according to their origin, with a separation between AA and FA samples (Fig. 3).

The correlations between the inorganic components were represented by leaf dry mass (LDM), root dry mass (RDM), leaf dry mass ratio (LDMR), and root dry mass ratio (RDMR). Strong (0.7>0.9) and positive significant correlations were observed between LDM x L I (0.86). Moderate positive correlations (0.5>0.7) were observed for LDM x L Yb (0.68), LDM x L I (0.51), RMR x L Ti (0.52), RMR x L\_Ni (0.57), RMR x L\_Y (0.56), RMR x L\_Hf (0.51), LDMR x L\_Ti (0.51), LDMR x L\_Ni (0.60), LDMR x L\_Y (0.58), LDMR x L\_Hf (0.56), LDM x L \_Yb (0.59), LDMR x L\_Ti (0.53), LDMR x L Yb (0.56) and negatives between LDM x L Y (-0.62), LDM x L Hf (-0.60), LDMR x L Ti (-0.52), LDMR x L Ni (-0.57), LDMR x L Y (-0.56), LDMR x L Hf (-0.51), RMR x L I (-0.51), LRR x L I (-0.52), LDM x R\_Mn (-0,53), LDM x R\_ Hf (-0.53), RMR x R\_Ti (-0.53), RMR x R\_Yb (-0.56). Weak correlations (0.3>0.5) were observed between LDMR x L\_Yb (0.50), LDMR x R\_Mn (0.54), and were negative in RMR x L\_Yb (-0.4963) (Fig. 4).

Environmental pollution caused by heavy metals in soils, waters, and the atmosphere has become an urgent issue worldwide. Phytoremediation is the most efficient and ecological way to remedy these contaminated environments [58], and it can be achieved through various means, such as phytoextraction, phytostabilization, and phytodegradation, among others [48]. It should also be noted that it is a technique that requires greater attention since the systematic understanding of this field is still limited [58]. U. lobata is a species that has already demonstrated great potential as a phytoremediator since it was able to remove Pb, Ni, and Cd from a solution [25]. In addition, it was also shown to act as a bioaccumulating species for elements such as Yb, Hf, and Ti, and as a biomonitor, as it was able to accumulate metals in its leaves from the atmosphere deposition.

#### Conclusions

Concentrations of heavy metals present in soils of anthropized areas in Novo Airão (Amazonas, Brazil) and the floodplain area in Manacapuru (Amazonas, Brazil) are responsible for the contamination of *U. lobata* tissues in both ecosystems.

The concentration of heavy metals in the root and leaf tissues of *U. lobata* indicates that the elements are translocated by the plant. The species can act as a phytorromediator or bioindicator of the heavy metals Ti, Yb, Hf, Ni, Br, and Y.

The differences in concentrations of heavy metals detected in the roots and leaves of *U. lobata* in anthropized areas and floodplain areas demonstrate the existence of genetic variability among individuals as bioaccumulating, bioindicators, and phytorromediators.

The use of medicinal malva should be restricted to environments free from heavy metal contamination to prevent harm to human health.

## **Author Contributions**

Conceptualization, L.M.G., S.L.F.R., C.H.S.G.M., R.L., and M.T.G.L.; methodology, L.M.G., J.S.T., C.S.B., S.L.F.R., R.L., R.L.S.M., and M.T.G.L.; software M.S.F.V., S.L.F.R., and R.L.S.M.; validation, R.L., J.S.T., C.S.B., A.M.P., R.L.S.M., and S.L.F.R.; formal analysis, J.S.T., C.S.B., S.L.F.R., and M.T.G.L.; resources L.M.G., M.T.G.L., R.L.S.M., C.H.S.G.M., S.C.S.L., and S.L.F.R.; writing – original draft preparation, L.M.G., J.S.T., C.S.B., S.L.F.R., R.L., M.T.G.L., and C.H.S.G.M.; writing – review and editing, M.T.G.L., R.L., and C.H.S.G.M.; funding acquisition, M.T.G.L., C.H.S.G.M., and A.M.P. All authors have read and agreed to the published version of the manuscript.

## Acknowledgements

Lucinete Miranda Gomes was supported by fellowships from The Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES). Carlos Henrique Salvino Gadelha Meneses (Process  $n^{\circ}$  313075/2021-2), Santiago Linorio Ferreyra Ramos (Process  $n^{\circ}$  305280/2022-8), Ricardo Lopes (Process  $n^{\circ}$  308815/2023-8), and Maria Teresa Gomes Lopes (Process  $n^{\circ}$  310307/2018-0) were supported by fellowships from CNPq.

## Funding

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Brasil – Edital nº 16/2020 - PROCAD-SPCF, Process Number: 88881.516217/2020-01, and. Edital nº 16/2022 - Programa de Desenvolvimento da Pós-Graduação (CAPES–PDPG) Pós-doutorado Estratégico, Process Number: 88887.692247/2022-00.

## **Conflict of Interest**

The authors declare no conflicts of interest.

- BRIFFA J., SINAGRA E., BLUNDELL R. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon. 6, e04691, 2020.
- VAREDA J.P., VALENTE A.J.M., DURÃES L. Assessment of heavy metal pollution from anthropogenic activities and remediation strategies: A review. Journal of Environmental Management. 246, 101, 2019.
- CAMPÓ W.J.C., CUSTODIO M., ORIHUELA C.E.V., FERNÁNDEZ R.P.P. Metal phytosorption potential of *Hydrocotyle ranunculoides* for mitigation of water pollution in high Andean wetlands of Peru. Revista Ambiente & Água. 15 (5), e2535, 2020.
- YADAV K.K., GUPTA N., KUMAR A., REECE L.M., SINGH N., REZANIA S., KHAN, S.A. Mechanistic understanding and holistic approach of phytoremediation: a review on application and future prospects. Ecological Engineering. 120, 274, 2018.
- DUTRA J., RELVAS H., CANALEZ G., PINHEIRO H., WAICHMAN A., LOPES M. Inconsistencies between Estimated Transport Greenhouse Gas Emissions and Urban Mobility Plan of Manaus City. Revista do Departamento de Geografia. 38, 169, 2019.
- FERREIRA M.D.S., FONTES M.P.F., PACHECO A.A., KER J.C., LIMA H.N. Health risks of potentially toxic trace elements in urban soils of Manaus city, Amazon, Brazil. Environmental Geochemistry and Health. 43, 3407, 2021.
- SILVA T.J., HANSTED F., TONELLO P.S., GOVEIA D. Fitorremediação de Solos Contaminados com Metais: Panorama Atual e Perspectivas de uso de Espécies Florestais. Revista Virtual de Química. 11 (1), 18, 2019 [In Portuguese].
- SINGH V.K., SHARMA N., SINGH V.K. Application of X-ray fluorescence spectrometry in plant science: Solutions, threats, and opportunities. X-Ray Spectrometry. 51 (3), 304, 2022.
- MALTSEV A.S., CHUPARINA E.V., PASHKOVA G.V., SOKOL'NIKOVA J.V., ZARUBINA O.V., SHULIUMOVA A.N. Features of sample preparation techniques in the total-reflection X-ray fluorescence analysis of tea leaves. Food Chemistry. 343, 128502, 2021.
- BILO F., BORGESE L., ZACCO A., LAZO P., ZOANI C., ZAPPA G., BONTEMPI E., DEPERO LE. Total Reflection X-Ray Fluorescence Spectroscopy to Evaluate Heavy Metals Accumulation in Legumes. Journal of Analytical & Bioanalytical Techniques. 7 (1), 1000292, 2015.
- LARA-ALMAZÁN N., ZARAZÚA-ORTEGA G., ÁVILA-PÉREZ P., CARREÑO-DE LEÓN C., BARRERA- DÍAZ C.E. Multielemental analysis by total reflection X-ray fluorescence spectrometry and phytochelatins determination in aquatic plants. X-Ray Spectrometry. 50 (5), 414, 2021.
- FERNÁNDEZ-RUIZ R. TXRF spectrometry in the bioanalytical sciences: A brief review. X-ray Spectrometry. 51 (3), 279, 2022.
- ALLEGRETTA I., BILO F., MARGUÍ E., PASHKOVA G.V., TERZANO R. Development and Application of X-rays in Metal Analysis of Soil and Plants. Agronomy. 13 (1), 114, 2022.
- 14. BEZERRA C.D.S., TOMAZ J.S., VALENTE M.S.F., ESPINDULA M.C., MARQUES R.L.S., TADEU H.C., FERREIRA F.M., SILVA G.S., MENESES C.H.S.G., LOPES M.T.G. Phenotypic Diversity and Genetic

Parameters of *Coffea canephora* Clones. Plants. **12** (23), 4052, **2023**.

- FOMBA K.W., DEABJI N., BARCHA S.E.I., OUCHEN I., ELBARAMOUSSI E.M., El MOURSLI R.C., HARNAFI M., EL HAJJAJI S., MELLOUKI A., HERRMANN H. Application of TXRF in monitoring trace metals in particulate matter and cloud water. Atmospheric Measurement Techniques Discussions. 13 (9), 4773, 2020.
- 16. NAZ R., KHAN M.S., HAFEEZ A., FAZIL M., KHAN M.N., ALI B., JAVED M.A., IMRAN M., SHATI A.A., ALFAIFI M.Y., ELBEHAIRI S.E.I., AHMED A.E. Assessment of phytoremediation potential of native plant species naturally growing in a heavy metal-polluted industrial soils. Brazilian Journal of Biology. 84, e264473, 2024.
- FESTIN S.E., SALK C., TIGABU M., SYAMPUNGANI S., ODÉN C.P. Biological traits of tropical trees suitable for restoration of copper-polluted lands. Ecological Engineering. 138, 118, 2019.
- HAUPTVOGL M., KOTRLA M., PRČÍK M., PAUKOVÁ Ž., KOVÁČIK M., LOŠÁK T. Phytoremediation potential of fast-growing energy plants: challenges and perspectives: a review. Polish Journal of Environmental Studies. 29 (1), 505, 2019.
- JONAS B.F.S., JAKELAITIS A., SILVA C.H.L., LEÃO V.C. Seleção de espécies bioindicadoras de resíduos de flumioxazina e saflufenacil no solo. Research, Society and Development. 9, e1379119486, 2020.
- VASCONCELO S.M.A., JAKELAITIS A., COSTA M.L.M., OLIVEIRA R.R.C., SANTOS V.S. Selection of tolerant species for contaminated soil phytemedication using imazapic. Revista de Ciências Agroveterinárias. 19 (2), 149, 2020 [In Portuguese].
- MORITA A.K.M., MORENO F.N. Fitorremediação aplicada a áreas de disposição final de resíduos sólidos urbanos. Engenharia Sanitaria E Ambiental. 27 (2), 377, 2022 [In Portuguese].
- 22. NJOKU C.E., ALANEME K.K., OMOTOYINBO J.A., EKELEME A.C., UGWU E.I., IKELE U.S. Phytochemical, Proximate and Mineral Analyses of *Urena lobata* Stems from Imo State Nigeria. IOP Conference Series: Materials Science and Engineering. **1107**, 012183, **2020**.
- ELANCHEZHIAN C., RAMNATH V.B., RAMAKRISHNAN G., RAJENDRAKUMAR M., NAVEENKUMAR V., SARAVANAKUMAR M.K. Review on Mechanical Properties of Natural Fiber Composites. Materials Today Proceedings. 5 (1), 1785, 2018.
- PURNOMO Y., TILAQZA A. Analgesic and Antiinflammatory activities of *Urena lobata* L. Leaf extracts. Indonesian Journal of Pharmacy. 33 (4), 566, 2022.
- 25. AMADI O.K., OKOYEAGU A., AKOH O.U. Biosorption of Pb (II), Ni (II) and Cd (II) ions from aqueous phase by Ceaser weed (*Urena Lobata*) bark, a low cost biosorbent. Journal of Chemical Society of Nigeria. 45 (4), 580, 2020.
- 26. MONTEIRO D.N.C., LIMA R.H.C., COSTA S.S., SANTOS E.T.V.S. Análise dos parâmetros texturais e mineralógicos do solo por espectroscopia na região do parque nacional de Anavilhanas (AM). Revista Geonorte, 15 (48), 30, 2024.
- DICKSON A., LEAF A., ALBERT L., HOSNER J.F. Quality appraisal of white spruce and white pine seedling stock in nurseries. The Forest Chronicle. 36 (1), 10, 1960.
- 28. DE LA CALLE I., CABALEIRO N., ROMERO V., LAVILLA I., BENDICHO C. Sample pretreatment strategies for total reflection X-ray fluorescence analysis:

A tutorial review. Spectrochimica Acta Part B. 90, 23, 2013.

- SHAPIRO S.S., WILK M.B. An analysis of variance test for normality (complete sample). Biometrika. 52 (3-4), 591, 1965.
- MACFARLAND T.W., YATES J.M. Introduction to Nonparametric Statistics for the Biological Sciences Using R, 1st ed.; Springer Cham: Switzerland AG, pp. 329, 2016.
- R CORE TEAM. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https:// www.R-projet.org/ 2010.
- RSTUDIO TEAM. RStudio: Integrated Development for R; RStudio, PBC: Boston, MA, USA. Available online: http://www.rstudio.com/.2020.
- 33. GASPAR A.B.S., BEZERRA D.P., REIS I.M.S., SILVA R. Chemical and textural properties of floodplain forest soils in the Eastern Amazon, Brazil. African Journal of Agricultural Research. 19 (1), 91, 2023.
- 34. HARTEMINK A.E., BARROW N.J. Soil pH nutrient relationships: the diagram. Plant Soil. **486**, 209, **2023**.
- NEINA D. The role of soil pH in plant nutrition and soil remediation. Applied and Environmental Soil Science. 2019, 1, 2019.
- 36. KAUR H., KAUR H., KAUR H., SRIVASTAVA S. The beneficial roles of trace and ultratrace elements in plants. Plant Growth Regulation. 100, 219, 2023.
- PAVLOVIC J., KOSTIC L., BOSNIC P., KIRKBY E.A., NIKOLIC M. Interactions of silicon with essential and beneficial elements in plants. Frontiers in Plant Science. 12, 697592, 2021.
- 38. KAWAŁKO D., KARCZEWSKA A., LEWIŃSKA K. Environmental risk associated with accumulation of toxic metalloids in soils of the Odra River floodplain – case study of the assessment based on total concentrations, fractionation and geochemical indices. Environmental Geochemistry and Health. 45, 4461, 2023.
- 39. LIU X., JU Y., MANDZHIEVA S., PINSKII D., MINKINA T., RAJPUT V.D., ROANE T., HUANG S., LI Y., MA L.Q., CLEMENS S., RENSING C. Sporadic Pb accumulation by plants: Influence of soil biogeochemistry, microbial community and physiological mechanisms. Journal of Hazardous Materials. 444, 130391, 2023.
- VINOGRADOVA N., GLUKHOV A., CHAPLYGIN V., KUMAR P., MANDZHIEVA S., MINKINA T., RAJPUT V.D. The content of heavy metals in medicinal plants in various environmental conditions: A review. Horticulturae.
   9 (2), 239, 2023.
- 41. LU X., LI F., YANG W., ZHU P., LV S. Quantitative analysis of heavy metals in soil by X-ray fluorescence with improved variable selection strategy and bayesian optimized support vector regression. Chemometrics and Intelligent Laboratory Systems. 238, 104842, 2023.
- RASULOV O., SCHWARZ M., HORVÁTH A., ZOIROV F., FAYZ N. Analysis of soil contamination with heavy metals in (the three) highly contaminated industrial zones. SN Applied Sciences. 2, (2013), 2020.
- DEWEY C., JUILLOT F., FENDORF S., BARGAR J.R. Seasonal Oxygenation of Contaminated Floodplain Soil Releases Zn to Porewater. Environmental Science & Technology. 57 (12), 4841, 2023.
- 44. LUI F., WANG X., DAI S., ZHOU J., LIU D., HU Q., BAI J., ZHAO L., NAZIR N. Impact of different industrial activities on heavy metals in floodplain soil and ecological risk assessment based on bioavailability: A case study

from the Middle Yellow River Basin, northern China. Environmental Research. 235, 116695, 2023.

- KANDPAL L.M., MUNNAF M.A., CRUZ C., MOUAZEN A.M. Spectra Fusion of Mid-Infrared (MIR) and X-ray Fluorescence (XRF) Spectroscopy for Estimation of Selected Soil Fertility Attributes. Sensors. 22 (9), 3459, 2022.
- 46. MAC SWEENEY E., CHIOCCHIO I., MANDRONE M., SANNA C., BILO F., MACCARINELLI G., POPESCU V.S., PUCCI M., MORANDINI S., MEMO M., UBERTI D.L., BORGESE L., TRINCIA S., POLI F., MASTINU A., ABATE G. Exploring the Anti-Inflammatory and Antioxidant Potential, Metabolite Composition and Inorganic Profile of *Cistus monspeliensis* L. Aerial Parts and Roots. Antioxidants. 13 (7), 753, 2024.
- 47. SYAHFITRI W.Y.N., ATMODIO D.P.D., LESTIANI D.D., SANTOSO M., KURNIAWATI S., DAMASTUTI E., PRANAWIDITIA G., SARI D.K. Identification of selected elements in plants collected from industrial sites in Gresik by total x ray fluorescence. AIP Conference proceedings. 2967 (1), 040008, 2024.
- 48. ULLAH S., LIU Q., WANG S., JAN A.U., SHARIF H.M.A., DITTA A., WANG G., CHENG H. Sources, impacts, factors affecting Cr uptake in plants, and mechanisms behind phytoremediation of Cr-contaminated soils. Science of the Total Environment. 899, 165726, 2023.
- RABBANI M., RABBANI M.T., MUTHONI F., SUN Y., VAHIDI E. Advancing phytomining: Harnessing plant potential for sustainable rare earth element extraction. Bioresource Technology. 401, 130751, 2024.
- 50. SHTANGEEVA I. Accumulation of scandium, cerium, europium, hafnium, and tantalum in oats and barley grown in soils that differ in their characteristics and level of contamination. Environmental Science and Pollution Research. **29**, 40839, **2022**.
- 51. ANJUM S., BAZAI Z.A., RIZWAN S., BENINCASA C., MEHMOOD K., SIDDIQUE N., SHAHEEN G.,

MEHMOOD Z., AZAM M., SAJJAD A. Elemental characterization of medicinal plants and soils from Hazarganji Chiltan National Park and nearby unprotected areas of Balochistan. Pakistan Journal Oleo Science. **68** (5), 443, **2019**.

- 52. KUMAR S., ZHAO M., ZHANG H., RAHMAN M.A., LUO C., MOHAMMAD RAHMAN M. Distribution, contamination status and source of trace elements in the soil around brick kilns. Chemosphere. 263, 127882, 2021.
- 53. HASSAN M.U., CHATTA M.U., KHAN I., CHATTHA M.B., AAMER M., NAWAZ M., ALI A., KHAN M.A.U. KHAN, T.A. Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities – a review. Environmental Science and Pollution Research. 26, 12673, 2019.
- 54. ISINKARALAR K., KOC I., ERDEM R., SEVIK H. Atmospheric Cd, Cr, and Zn Deposition in Several Landscape Plants in Mersin, Türkiye. Water, Air, Soil and Pollution. 233, 120, 2022.
- 55. STRACHAN S. Trace elements. Current Anesthesia & Critical Care. 21, 44, 2010.
- 56. COUTO R., FAVERSANI J., CERETTA C.A., FERREIRA P.A.A., MARCHEZAN C., BASSO FACCO D., GARLET L.P., SILVA J.S., COMIN J.J., BIZZI C.A., FLORES E.M.M., BRUNETTO G. Health risk assessment and soil and plant heavy metal and bromine contents in field plots after ten years of organic and mineral fertilization. Ecotoxicology and Environmental Safety. 153, 142, 2018.
- 57. PURWADI I., NKRUMAH P.N., PAUL A.L.D., VAN DER ENT A. Uptake of yttrium, lanthanum and neodymium in *Melastoma malabathricum* and *Dicranopteris linearis* from Malaysia. Chemoecology. **31**, 335, **2021**.
- 58. ZHANG K., LIU F., ZHANG H., DUAN Y., LUO J., SUN X., WANG M., YE D., WANG M., ZHU Z., LI D. Trends in phytoremediation of heavy metals-contaminated soils: A Web of science and CiteSpace bibliometric analysis. Chemosphere. 352, 141293, 2024.