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RESEARCH ARTICLE

Assessment of the phytoremediation potential of *Amaranthus retroflexus* L. grown on ash dumps

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ABSTRACT

A major threat to the environment and human health is the contamination of soils with heavy metals resulting from the mining industry, and phytoextraction is known as one of the main ecological methods for the decontamination of polluted soils. In this work, the determination of the heavy metals Cd, Pb and Ni was performed by atomic absorption spectrometry, from different organs of *Amaranthus retroflexus* L. plants, harvested from ash dumps near the Ișalnița Thermal Power Plant located in Dolj, Romania. In the root, Cd content was 0.44 mg kg $^{-1}$ DW, in the stem 0.59 mg kg $^{-1}$ DW, in leaves 0.55 mg kg $^{-1}$ DW, and in the flowers 0.60 mg kg $^{-1}$ DW. Regarding Pb content, the following values were recorded: In root 5.66 mg kg $^{-1}$ DW, in stem 7.59 mg kg $^{-1}$ DW, in leaves 8.29 mg kg $^{-1}$ DW, in flowers 15.86 mg kg $^{-1}$ DW. The values determined for Ni were: In root 5.37 mg kg $^{-1}$ DW, in stem 2.69 mg kg $^{-1}$ DW, in leaves 2.97 mg kg $^{-1}$ DW and in flowers 3.65 mg kg $^{-1}$ DW. Our findings indicated that the accumulation of the three heavy metals in the plant organs had a different behavior: In root it was lower for Cd and Pb and higher for Ni. In the case of these metals, their concentration in leaves and stems was approximately comparable. In flowers, Pb recorded the maximum value. High values of bioconcentration factor and translocation factors were found for Cd and Pb, indicating the high bioaccumulation potential of these metals in *A. retroflexus* and the successful transfer of these metals to the aerial organs of the plants.

Key words: Cadmium, lead, nickel, redroot pigweed.

INTRODUCTION

Among the current environmental problems, heavy metal pollution occupies an important place. Heavy metals persist in the environment for a long time, some of them forming insoluble combinations, which is why they are not carried into the lower layers of the soil by infiltration waters. Another reason that explains their persistence in the environment is the fact that they are not biodegradable and, above all, they form, with the substances in the composition of plants, stable combinations that disappear only with the respective plant. These metallic elements are frequently designated as “silent killers” when their density exceeds 5 g cm^{-3} (Orji et al., 2018).

The main routes by which humans can be exposed to heavy metals include ingestion, inhalation, and transdermal absorption, each of which has the potential to cause significant adverse health effects. Prolonged or high-concentration exposure can lead to serious conditions, such as acute and chronic kidney damage and nervous system dysfunction (Rehman et al., 2018). The use and ingestion of plants grown in polluted areas, which contain Pb and Cd even in small quantities, can determine chronic and acute toxicity in humans, toxicity which has been associated with the development of tumour disorders (Pazalja et al., 2023).

Beyond the impact on human health, heavy metals negatively influence the balance of terrestrial and aquatic ecosystems, causing a decrease in biodiversity, affecting plant and animal populations, and disrupting the dynamics of food webs (Pujari and Kapoor, 2021).

Plants can serve as effective bioindicators of the presence of harmful concentrations of heavy metals in soil or atmosphere, providing essential information about the behavioural changes of these contaminants and their degree of bioavailability in plant tissues (Świsłowski et al., 2022). Certain plant species have the ability to accumulate toxic trace elements from the environment in their aboveground parts, which gives them considerable utility in pollution monitoring, providing relevant information in an economically efficient way on environmental quality (Vergel et al., 2022).

The phytoextraction process is based on plants with a high capacity to absorb heavy metals (Zalewska and Nogalska, 2014). In this sense, lichens and mosses are frequently used as bioindicators in monitoring heavy metal contamination, due to their high sensitivity to atmospheric pollutants (Saib et al., 2023). Unlike lichens and mosses, vascular plants have protective cuticles and well-developed root systems, characteristics that give them superior adaptability to environmental conditions specific to urban and industrial areas (Baluška and Mancuso, 2021). In addition, herbaceous species with a high growth rate allow for a faster assessment of heavy metal exposure, thus facilitating the implementation of effective biomonitoring strategies (Orji et al., 2018).

Fly ash is a very hazardous waste resulting from the combustion of coal for the production of electricity. It is generally deposited in landfills, the management of which represents a serious environmental problem, due to the leaching of heavy metals and the release of various pollutants into the atmosphere (Kostić et al., 2022b). This ash can be very toxic to living organisms because, in addition to the essential chemical elements (Ca, Mg, Si, Al, Fe and K), it also contains toxic trace elements (Cd, Pb, Ni, B, As, Cr, Cu, Co, Mn, Mo, Se and Zn) whose concentrations can be up to 30 times higher during the combustion of organic materials in coal (Jańczak-Pieniążek et al., 2023).

For the remediation of soils highly contaminated with multiple pollutants, as is the case of ash dumps exposed to deflation, the use of phytostabilizing plant species is very important. Such bioaccumulating plants can immobilize polluting elements in and on the roots and in the rhizosphere, and by effectively covering the plant substrate they prevent wind erosion, particle dispersion and surface water runoff (Kostić et al., 2022a).

Therefore, the identification of plant species suitable for phytoremediation and management of ash dumps subject to the phenomenon of blowing is urgently needed.

Amaranthus retroflexus L. (redroot pigweed, green amaranth or redroot amaranth) of the Amaranthaceae family, is an adventitious species in the flora of Romania, originate in central and southeastern regions of North America, especially between 30 and 60° N lat. It was introduced to Europe in 1750, being cultivated by Linnaeus in Uppsala (Anastasiu et al., 2017). It later spread shortly thereafter, throughout the world, currently being naturalized, both in tropical and subtropical regions, and in temperate ones (South America, Central and Southern Europe, the Mediterranean region), Asia Minor, the Caucasus to Siberia, North and South Africa, Australia (Rai and Singh, 2020). This species is widely distributed in metal-contaminated soils, suggesting a promising opportunity for metal phytoremediation, especially for phytoextraction (Sipos et al., 2023). The stems and leaves of *A. retroflexus* accumulate high concentrations of metals in contaminated sites, the rate of their accumulation varying significantly depending on the plant organs: Ba, Mn, Sr and Zn are found in high concentrations in the leaves, while Al, Cr, Cu, Fe and Pb are found in the roots (Mahar et al., 2016).

Given the above, this study focused on evaluating the possibilities of using *A. retroflexus* for the phytoremediation of ash dumps. In this regard, the content of each heavy metal (Cd, Pb, Ni) in each plant organ (root, stem, leaves and inflorescence) was determined and the translocation factors and bioconcentration factor were calculated for each metal.

MATERIALS AND METHODS

Soil sampling and analysis

This study was conducted on the fly ash dumps resulting from the Ișalnița Thermal Power Plant which is located 15 km from Craiova (44°23′15″ N, 23°43′5″ E), Dolj County, Romania, on the left bank of the Jiu River. This area is characterized by an arid climate with an average monthly temperature of around 11.0 °C. Precipitation is unevenly distributed throughout the year, with an average of around 540 mm yr^{-1}.

Soil samples were collected from 0 to 40 cm depth. In the laboratory, these samples were oven-dried (60 °C) for 72 h. The pH measurement was performed by the potentiometric method. The determination of the soil particle size composition was performed by treating the soil samples to obtain dispersion (Kacinski method), and then sieving for particles < 0.02 mm and pipetting for particles with a diameter ≥ 0.02 mm was performed, in order to separate the particle size fractions.

The total N content (%) was determined by the Kjeldahl method, and the mobile P and K content in the soil by the Egner-Riehm-Domingo method.

Heavy metals were extracted by weighing 5 g dry soil, then mineralizing the sample by adding 20 mL HCl and HNO_3 (3:1, v/v), drying, leaching the residue with 100 mL 5% HNO_3 and subsequently determining the heavy metals using an atomic absorption spectrophotometer.

Plant harvesting and chemical analysis

Amaranthus retroflexus L. plants (Figure 1) were harvested in June for leaves, August for stems and flowers and September for roots, from ash dumps near the Ișalnța Thermal Power Plant, which is the main polluter. Plant material samples were collected from randomly selected specimens from the spontaneous flora, then packed in plastic bags and transported to the laboratory for analysis, being sectioned into organs.

Figure 1. *Amaranthus retroflexus* plants with inflorescences.

To perform the determinations, the plants were cleaned of non-conforming parts (washing with tap water and distilled water), sectioned and dried at room temperature, on plastic nets, in a dark and well-ventilated room. After drying, each part of the plant was ground in a coffee grinder until a uniform powder was obtained.

Three samples of 0.2 g were taken from this powder, weighed with analytical precision. The samples were quantitatively transferred into 25 mL volumetric flasks, by introducing 19-20 mL 70% nitric acid solution and 2 mL 30% hydrogen peroxide. The flask was well shaken, then filled with 5% nitric acid. These were then maintained at room temperature for 1 h, then the determinations were performed using an atomic absorption apparatus (AAS-ML7800, Shanghai YOKE Instrument, Shanghai China), fully automatic and fully PC-controlled, compact and flexible design, allowing automatic switching with the help of software of the flame/furnace/hydride working mode, configured and equipped with graphite furnace, automatic sampler and hydride generator, unique mechanical-optical design, with integrated flame system, also having the convenient functions offered by the workstation.

Phytoremediation factors

To evaluate the potential for metal accumulation in *A. retroflexus*, the following factors were calculated:
Bioconcentration factor (BCF):

$$\mathrm{BCF} = \frac{\mathrm{C\ plant}}{\mathrm{C\ soil}}$$

where, C plant is the metal concentration (mg kg^{-1} DW) in the plant organs; C soil is the metal concentration in the soil.
Translocation factor (TF):

$$\text{TF stems} = \frac{\text{C stems}}{\text{C roots}}$$
$$\text{TF leaves} = \frac{\text{C leaves}}{\text{C roots}}$$
$$\text{TF flowers} = \frac{\text{C flowers}}{\text{C roots}}$$

when BCF > 1.0, it means that the plant has the potential to accumulate heavy metals and can be used for phytoremediation purposes, and if BCF ≤ 1.0, the plant is not applicable for phytoremediation. If TF > 1.0 the plant can be used for phytoextraction purposes, and if TF ≤ 1.0, the plant is not so practical for this purpose.

Statistical analyses

The data were expressed as the mean value of the samples replicated three times. Differences of metal concentrations in the different organs of the plants were analysed by using the Duncan's multiple range test (DMRT) in the one-way ANOVA analysis, to see if there was any significant difference at $p < 0.05$.

RESULTS

Metal concentration in soil. The granulometric results showed that the soil is loamy-sandy, with an alkaline pH, with an average total N content, an excessive P content and a high K content. The concentrations of heavy metals in the soil had the following sequence: Ni > Pb > Cd (Table 1). According to OMWFEP (1997), the values of Pb and Ni concentrations found in the soil are above the normal values mentioned for soils with sensitive use, but maintained below the alert level (Table 2).

Table 1. Soil characterization, main physicochemical properties.

Properties	Values
Sand < 0.2 mm, %	65.4
Sand ≥ 0.2 mm, %	8.1
Dust < 0.2 mm, %	22.0
Clay < 0.002 mm, %	4.8
pH	8.02
N, total, %	0.166
P, mg kg^{-1}	423.0
K, mg kg^{-1}	270.0
Cd, mg kg^{-1}	0.58
Pb, mg kg^{-1}	29.1
Ni, mg kg^{-1}	58.9

Table 2. Reference values for heavy metals in soil (for sensitive soil), according to OMWFEP (1997).

Metal	Normal values	Alert thresholds	Action levels
	mg kg^{-1} DW		
Cd	1	3	5
Pb	20	50	100
Ni	20	75	20

Metal accumulation in plant organs. Based on metal concentrations, plant organs recorded significantly different concentrations of Cd, Pb, and Ni (Figures 2, 3 and 4).

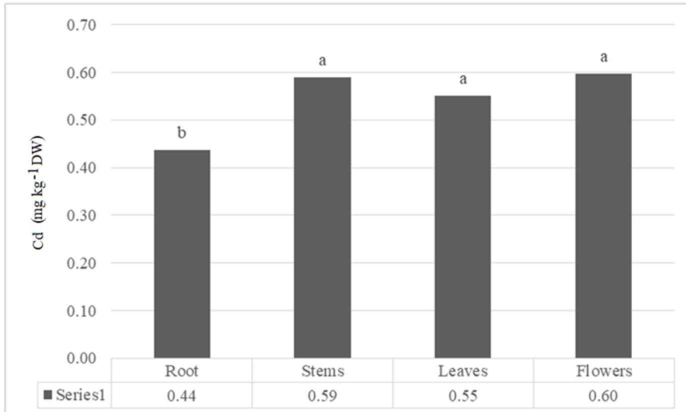

Figure 2. Content of Cd in the organs of *Amaranthus retroflexus*. Different letters indicate significant differences ($p < 0.05$).

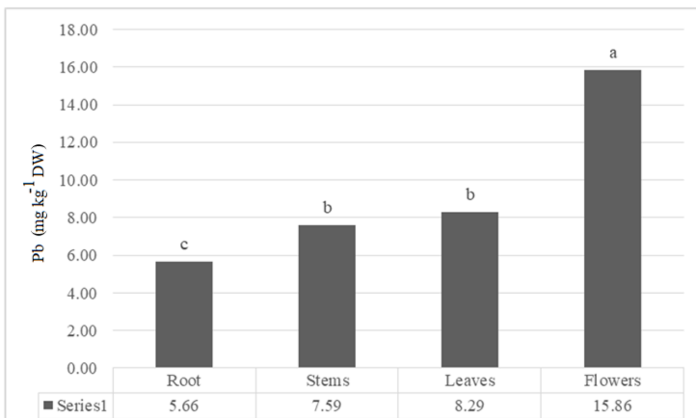

Figure 3. Content of Pb in the organs of *Amaranthus retroflexus*. Different letters indicate significant differences ($p < 0.05$).

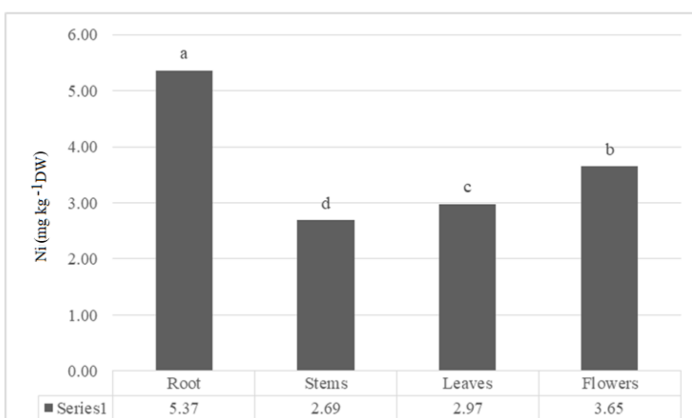

Figure 4. Content of Ni in the organs of *Amaranthus retroflexus*. Different letters indicate significant differences ($p < 0.05$).

BCF and TF values. The BCF values > 1.0 were found only for Cd and Pb. For TF, values > 1 were found for all aerial plant organs, only for Cd and Pb (Table 3).

Table 3. Phytoremediation factors of *Amaranthus retroflexus*.

Factors	Cd	Pb	Ni
Bioconcentration factor	3.75	1.28	0.24
Translocation factor stems	1.34	1.33	0.50
Translocation factor leaves	1.25	1.46	0.55
Translocation factor flowers	1.36	2.80	0.67

DISCUSSION

Thermal power plants used for electricity production, which use coal as the main fuel, generate large amounts of residues, such as fly ash. This contains persistent organic pollutants and many heavy metals, which lead to environmental pollution. Numerous invasive plant species that grow without human intervention, having good adaptability to local conditions, offer a practical and ecological alternative for landscaping and eco-restoration of these polluted lands (Gajić et al., 2018). The species *Amaranthus retroflexus* easily colonizes ash dumps, due to its invasive nature and its toxitolerant characteristics. Previous research has shown that the accumulation of heavy metals in different plant organs, such as roots, stems and leaves, can be an indicator of the level of exposure and the associated ecological risk (Trejo et al., 2016; Alengebawy et al., 2021).

Our results revealed that the level of accumulation of heavy metals was different depending on the plant organs, namely, the highest concentrations of Cd and Pb were in flowers, while the highest concentrations of Ni were in roots.

Cadmium (Cd) is one of the heavy metals, whose toxicity is considered 2-20 times higher than that of other metals (He et al., 2015). The order of Cd concentration in *A. retroflexus* plants, from highest to lowest, was flowers (F) > stems (S) > leaves (L) > roots (R), with values ranging from 0.60 mg kg^{-1} (in flowers) to 0.44 mg kg^{-1} (in roots).

The significant differences between plant organs suggest a greater capacity of *A. retroflexus* to translocate Cd to the aerial parts compared to the underground parts. Chinmayee et al. (2012) also studied Cd uptake in *A. spinosus* in soils treated with heavy metals under laboratory conditions, reporting a much higher Cd accumulation in roots (20-34 mg kg^{-1}) than in other plant organs, which is contradictory to our results. These contradictory results are probably due to the individual characteristics of each *Amaranthus* ssp., respectively their remediation capacity, as well as the influence of external factors.

Lead (Pb) is another toxic metal, with harmful effects on the environment and human health, requiring rigorous monitoring of its presence in plant ecosystems (Ali et al., 2019). In our study, the order of Pb concentration in *A. retroflexus* plants from highest to lowest was F > L > S > R, with values ranging from 15.94 mg kg^{-1} (F) to 5.66 mg kg^{-1} (R), and this finding represents a topic of interest for future research.

Pb uptake by *A. retroflexus* was also investigated by Sipos et al. (2023) in a reclaimed suburban area in Debrecen City (Lovász-zug), which operated as a secondary biological wastewater treatment plant until 2000, where they found concentrations between 1.11 to 3.09 mg kg^{-1} in the plant organs, which are lower values compared to our results. In contrast, in the study by Zhou et al. (2024) with *Amaranthus* ssp. in three mining areas in China, Pb concentrations were closer to the values we found, namely between 1.2-18.6 mg kg^{-1} Pb.

In our study, for Ni, the order of concentration in *A. retroflexus* plants, from highest to lowest was R > F > L > S, with values ranging from 5.37 mg kg^{-1} (R) to 2.68 mg kg^{-1} (S). This suggests the greater capacity of *A. retroflexus* to translocate Ni to the underground parts. Garba et al. (2018) reported contradictory results for *A. hybridus*, identifying the leaves (8.46 mg kg^{-1}) as the part with the highest accumulation.

The phytoremediation factors, bioconcentration factor (BCF) and translocation factor (TF), are generally used to evaluate the phytoremediation capacity of a plant (Aydi et al., 2023). In our study, BCF values were > 1.0 for Cd (3.75) and Pb (1.28) and < 1.0 for Ni (0.24). This indicates that *A. retroflexus* has a good phytoremediation capacity for Cd and Pb, while being a non-accumulating plant for Ni from ash dumps. These BCF results found in our study were supported by the findings of Tőzsér et al. (2023) for *Amaranthus* ssp.

The TF values showed the potential of the plant to transfer the pollutant from the roots to different parts of the plant, thus protecting its roots from toxicity. The minimal accumulation of Ni in the root also reduced the translocation to the aerial parts of the plant. Based on the TF values, the three plant organs (stems, leaves and flowers) had values > 1.0 only for Cd and Pb, while for Ni they had values < 1.0. This suggests that the transfer of heavy metals from roots to aerial parts (stems, leaves, flowers) was efficient only for Cd and Pb. Also, these high values show that *A. retroflexus* is suitable for phytoextraction on ash dumps, due to its ability to translocate metals from roots to aerial parts. Yap et al. (2022) reported that by values of BCF and TF, *A. viridis* indicated a very promising phytoextraction potential for Ni and Cd and less for Pb in Peninsular Malaysia.

CONCLUSIONS

The species *Amaranthus retroflexus* easily colonizes ash dumps, due to its invasive nature and its toxitolerant characteristics. Our findings highlighted the feasibility of natural growth of *A. retroflexus* for phytoremediation and sustainable phytomanagement of ash dumps, due to the high values of bioconcentration factor > 1.0 and translocation factor > 1.0 for Cd and Pb.

The accumulation of the three heavy metals in the plant organs had a different behavior: In the root it was lower for Cd and Pb and higher for Ni. In the case of these metals, their concentration in leaves and stems was approximately comparable. In flowers, Pb recorded the maximum value, and this finding represents a topic of interest for future research.

Author contribution

Conceptualization: A.P.S. Methodology: L.M.E.C. Software and statistical analyses: E.C., D.B., I.A.S. Validation: I.S. Formal analysis: A.P.S., E.C. Investigation: A.P.S., I.A.S. Writing-original draft preparation: E.C., D.B. Writing-review and editing: E.C., D.B. Visualization: I.A.S., I.S. Supervision: A.P.S., L.M.E.C. All co-authors reviewed the final version and approved the manuscript before submission.

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