

Metal bioaccumulation and translocation potential of three herbal plant species at mine tailings

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Abstract

Phytoremediation as a technique for cleaning soil contaminated with potentially toxic metals uses plants that can absorb them, and then translocate and accumulate them in above-ground parts. Its effectiveness depends on the selection of adequate plants. Therefore, this study aimed to investigate the potential of three wild herbaceous plant species (*Holcus lanatus* L., *Agrostis alba* L., and *Eupatorium cannabinum* L.) growing on mine tailings for phytoremediation applications. The content of selected metals in soil and plant samples was determined by flame atomic absorption spectrophotometer. The research results indicate that the content of Pb and Cu in the soil of the rhizosphere of the studied species was higher than their remediation values, after the exploitation of polymetallic ore (lead-zinc-copper), which made the tailings an ecological hazard. The species *H. lanatus* proved to be a good candidate for the phytoaccumulation of Cr, and the species *A. alba* of Ca, while the species *E. cannabinum* proved to be phytoaccumulator of all tested metals in the researched mine tailings. Potential for phytoextraction applications were shown by the species *H. lanatus* for Cd, Cu, Mg and Ni, and *A. alba* for Cd and Zn.

Keywords: metals; phytoaccumulation; phytoextraction; soil; tailings; translocation

Abbreviations: BCF- Bioaccumulation factor; TCF- Translocation factor

Introduction

Mining activities disturb natural landscape, but also many physico-chemical properties of soil, such as pH, electrical conductivity, and cation exchange capacity (Saleem *et al.*, 2020). Except that, mines are sources of environmental pollution, because during the processing of ore, tailings are formed that contain increased

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amounts of metals, especially “heavy metals” (Concas *et al.*, 2006). Only plants that have a tolerance for metals grow on such inhospitable soil.

Vascular plant adaptation to excessive concentrations of metals in the soil that are toxic for most other higher plant species is a phenomenon that has been first described in the second half of the 20th century (Rune, 1953; Bradshaw *et al.*, 1965; Antonovics *et al.*, 1971). First reports of the heavy metal tolerance of some grasses species that colonize mine tailings initiated researches related to the genetic background of this ability (Wilkins, 1960; McNeilly and Bradshaw, 1968; Urquhart, 1971). All these studies concluded that the metal tolerance of the examined higher plants is a hereditary characteristic. The second direction of investigations was to determine mechanisms included in this tolerance. The physiological mechanisms involved in metal hyperaccumulation are enhanced metal uptake, increased xylem loading, and increased detoxification in the shoot (Memon and Schröder, 2009; Zhao *et al.*, 2016).

Tolerant plants are often excluders, which limit the entry of metals and their translocation from root to aboveground organs. However, there are plants called hyperaccumulators, which can accumulate large concentrations of metals in their aboveground organs without visible symptoms of toxicity and harmful effects. This feature makes them interesting because of their potential use in phytoremediation, which is one form of bioremediation.

Phytoremediation is an effective, low-cost, sustainable, and environmentally friendly technology that use higher plants and their associated microbes to clean up contaminated soil, water, and air from organic and inorganic pollutants, and to revegetate devastated sites (Chaney *et al.*, 1997; Pilon-Smits, 2005; Ali *et al.*, 2013; Dixit *et al.*, 2015). This technique is important due to the increasing pollution of the environment worldwide and the existence of numerous more expensive, safety hazard, and risky to ecosystems methods for removing pollutants from it (Ali *et al.*, 2013). Plants, on the other hand, adopt metals through their roots, and in their bodies, they accumulate and reduce their toxicity from soil, sludge and water resources through various mechanisms that include phytoextraction, phytodegradation, phytostabilization, rhizo-degradation or phytovolatilization (Ojuederie and Babalola, 2017).

The effectiveness of phytoremediation depends on the selection of suitable hyperaccumulator species of plants. It is desirable that those plants have a high metal accumulation capacity, a large biomass and that they are easy to grow (Adesodun *et al.*, 2010; Sakakibara *et al.*, 2011). Therefore, it is necessary to examine different plant species, to discover the possibility of their application in phytoremediation. Of particular interest are self-growing, pioneer species that inhabit such inhospitable soils as mine tailings.

Mine “Rudnik” processes polymetallic ore (lead-zinc-copper), and certain herbaceous plants grow spontaneously on its tailings, which are the subject of this study. Some of them are *Holcus lanatus* L., *Agrostis alba* L., and *Eupatorium cannabinum* L. In phytoremediation, plant root zone is particularly important, due to mutual interactions between soil and plants, which affect the metabolism of the plant, so we sampled and investigated rhizosphere soil at mentioned plant species.

This study aimed to investigate the ability of the mentioned species to absorb selected metals from the soil, translocate them from roots to aboveground parts and accumulate in them. The obtained results would indicate a potential metal hyperaccumulator species that could be used in some of the phytoremediation techniques.

Materials and Methods

Study site

The mine “Rudnik” is in the central part of Republic of Serbia (44.10625 north latitude and 20.48484 east longitude), 7 km north of the town Gornji Milanovac, with its tailing in the village Majdan (Figure 1).

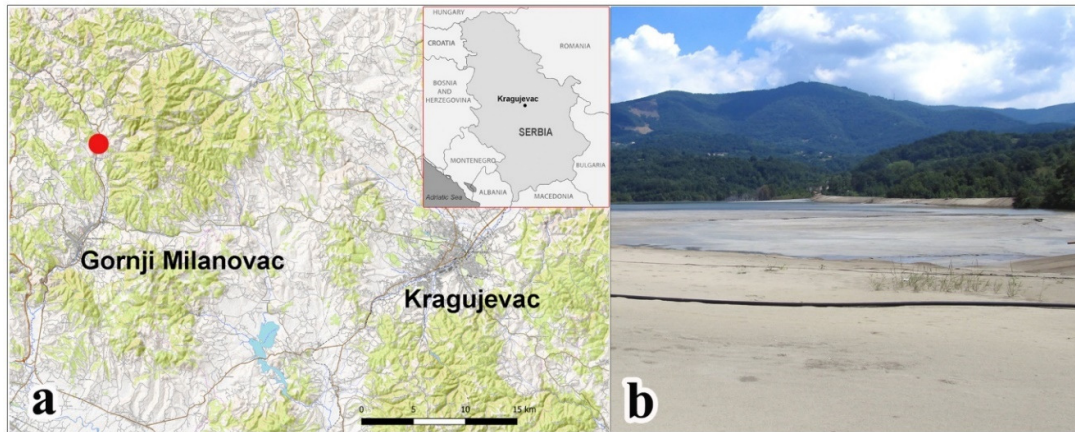


Figure 1. (a) Geographical position according to QGIS 3.16.0 and (b) appearance of the investigated mine tailings (red dot on the map indicate sampling site)

Investigated species

Among herbaceous plant species that were found on the tailing of mine “Rudnik” are *Holcus lanatus* L., *Agrostis alba* L., and *Eupatorium cannabinum* L. (Figure 2), whose general characteristics are presented below (Josifović, 1970).

Species *H. lanatus* (common velvet grass) is a hairy, tufted, fibrous-rooted perennial grass, with soft grey-green velvety leaves and round stems. It grows between 50-100 cm tall. Inflorescences are silvery to purplish, paniculate, contracted, sometimes almost spike-like, 8-15 cm long, occasionally enclosed within the uppermost sheath.



Figure 2. Investigated plant species: a) *H. lanatus*, b) *A. alba* and c) *E. cannabinum*

Species *A. alba* (Redtop) is perennial with a creeping rootstock that generally sends out runners. The stems vary in different varieties, from only a couple of inches high to four feet or more. The leaves vary in size, number, and color. Their ligule is always long and generally acute. The flowers are arranged in a panicle with numerous branches. When in bloom, the top of the panicle has the shape of a pyramid with a broad base. Numerous spikelets are often reddish-brown - hence the name Redtop.

Species *E. cannabinum* (Hemp-agrimony) is a vigorous herbaceous dioecious upright perennial with reddish stems, leaves palmately-divided into narrow leaflets, and dense terminal domed clusters of tiny light pink or purple flowers. It grows to 1.5 m tall or more and 1.2 m wide. The fruit is an achene about 2 or 3 mm long, borne by a pappus with hairs 3 to 5 mm long, which is distributed by the wind.

Soil and plant sampling and analysis

Soil samples were taken for analysis. Five soil samples were collected from 1 to 10 cm depth, near roots of researched plants. This depth corresponds to the major rooting zone of the herbs and small shrubs (Reeves *et al.*, 2007). Determination of the plant species was performed using the standard keys (Josifović, 1970; Jávorka and Csapody, 1979; Tutin, 1964-1980) in the laboratory of the Department of Biology and Ecology, at the Faculty of Science in Kragujevac.

Reagents: Reagents (65% HNO₃, 35% H₂O₂, and 70% HClO₄) used, with a p.a. purity, are produced by the "Sigma-Aldrich Company". Standard solutions of the "Acros Organics Standard - USA", 1000 µg ml⁻¹ concentration, were used to determine metal calibration diagrams as well as for the determination of metals from the samples. Standard reference materials are produced by "LGCstandards" and "National Research Council Canada".

Instruments and apparatus: The flame atomic absorption spectrophotometer (FAAS) model Perkin Elmer 3300 with D2 lamp as a corrector was used for the determination of metals: manganese ($\lambda = 279.8$ nm), calcium ($\lambda = 422.7$ nm), magnesium (285.2 nm), iron (248.3 nm), zinc (213.9 nm), and copper (324.8 nm). Standard solutions of the appropriate concentrations were used to prepare the calibration. The range of standard solutions was 0.5-2.0 mg dm⁻³ for Cu, Zn, Mg and 1.0-5.0 mg dm⁻³ for Mn, Fe, Ca. All samples were analyzed by the FAAS using acetylene flame (2.0 : 10.0) for Cu, Zn, Mg, Mn, Fe and (3.8 : 10.0) for Ca. Detection limits for determining the concentration of metals in the soil were: Ca (1.00), Mg (0.20), K (0.50), Na (0.20), Fe (1.00), Mn (1.00), Cu (0.50), Zn (0.50), Ni (1.00), Pb (2.00), Cd (0.50), Co (1.00), Cr (1.00) mg kg⁻¹, respectively. Detection limits for determining the concentration of metals in the plant material are given here: Ca (1.50), Mg (0.50), K (0.50), Na (0.50), Fe (1.00), Mn (1.00), Cu (0.50), Zn (0.50), Ni (1.50), Pb (2.10), Cd (0.50), Co (1.00), Cr (1.00) mg kg⁻¹, respectively. The measuring of metal concentrations in samples was done at the Institute of Chemistry, Faculty of Science in Kragujevac.

Preparation of samples: The plant samples (stems, leaves) were washed with distilled water to remove all contaminants, dried in a shade at room temperature (20-25 °C) and after that at 105 °C, 24 h (Binder/Ed15053). Dried parts of the plant were self-contained and homogenized, and samples were stored in polyethylene bottles. For determining dry matter 2 g of plant material were used. The soil samples were collected from 1 to 10 cm depth, near roots of researched plants. Initially, they were air dried and stone pieces were removed, then sieved to 2 mm, and stored at 4 °C until analysis. Sub-samples of 3 g were ground to pass a 70-mesh sieve (<215 µm) and then oven-dried at 105 °C for 24 h (Binder/Ed15053).

Digestion of samples: Metals were frequently measured, and regulatory decisions were made for toxicity based on the total metal concentration in a growth substrate (Official Gazette of the RS, No. 88/2010, Annex 3). Different extractants, generally "strong acids" such as HNO₃, HF, HClO₄ and aqua regia, have been utilized to determine the total metal in soil. To determine the total metal content in the soil, the samples were prepared by digestion with nitric acid and hydrogen peroxide according to EPA 3050 b (Tóth *et al.*, 2016). The ratio was: HNO₃: H₂O₂ = 5: 1; relationship soil pattern/digestion mixture was 1: 12. To check the accuracy of the applied method, blank tests and standard reference materials were used: MEES-3 (trace

elements in sediments) and LGC7173, for plant material. The values obtained ranged in the range of $\pm 5\%$ of the certified values.

Bioaccumulation factor (BCF) and Translocation factor (TCF)

Ten metals (Mn, Ni, Fe, Cu, Zn, Cr, Ca, Mg, Pb, and Cd) were analyzed in the soil and plant material (roots, stems, leaves) of the tree herbaceous plants (in the five replications). The content of metals in soil and plant materials was expressed in mg kg^{-1} of dry matter (mg kg^{-1} d.m.). The mean values, standard deviation, Bioaccumulation factor (BCF) and Translocation factor (TCF) were calculated (Table 1). BCF is indicators of the plant ability in bioaccumulation, translocation and phytoremediation of researched metals. BCFs are considered a simple tool to estimate the bioaccumulation of pollutants in a particular organism or organ in a specific medium. BCF for each plant organ were calculated ($\text{BCF}_{\text{organ}} = C_{\text{organ}}/C_{\text{soil}}$; C_{soil} - the metal concentration in the soil, C_{organ} - the metal concentration in the particular plant organ). The translocation factor (TCF) is used to evaluate the translocation of metals from plant root to shoot and leaves. It is defined as the ratio of the total concentration of elements in the aerial parts of the plant ($C_{\text{stem}}, C_{\text{leaf}}$) to the concentration in the root (C_{root}) (Ghosh and Singh, 2005).

Table 1. Formulas for calculating of bioaccumulation (BCF) and translocation (TCF) factors

Factor	Formulas	Elements of formula
Bioaccumulation factor (BCF)	$\text{BCF}_{\text{root}} = C_{\text{root}}/C_{\text{soil}}$ $\text{BCF}_{\text{stem}} = C_{\text{stem}}/C_{\text{soil}}$ $\text{BCF}_{\text{leaf}} = C_{\text{leaf}}/C_{\text{soil}}$	C_{soil} - the metal concentration in the soil C_{root} - the metal concentration in the plant root C_{stem} - the metal concentration in the plant stem C_{leaf} - the metal concentration in the plant leaf
Translocation factor (TCF)	$\text{TCF}_{\text{stem}} = C_{\text{stem}}/C_{\text{root}}$ $\text{TCF}_{\text{leaf}} = C_{\text{leaf}}/C_{\text{root}}$	

Statistical analysis

Statistical analyses included determination of the mean (M) and standard deviation (SD) for each of the analyzed parameters. Differences between groups in terms of the total concentrations of chemical elements in soil, roots, stems, and leaves of investigated species were determined by factorial ANOVA and Scheffé's post-hoc test. Statistical analysis was performed by using the package Statistica 10.0.

Results

This study aimed to indicate the potential metal hyperaccumulator species and their capability for phytoremediation, by investigating their ability to absorb selected metals from the soil, to translocate them from roots to aboveground parts and accumulate in them.

The results of the analysis of the total content of chemical elements (Ca, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in the rhizosphere soil of *H. lanatus*, *A. alba*, and *E. cannabinum* are shown in Table 2. The obtained results show that the rhizosphere soil sampled in the zone of the species *E. cannabinum* contained almost all the examined metals the most. The content of Fe was the highest in the rhizosphere zone of *H. lanatus* species. The soil sampled at all three investigated plant species contained metals in a decreasing sequence: $\text{Fe} > \text{Ca} > \text{Mg} > \text{Pb} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Cr} > \text{Ni} > \text{Cd}$ (only the rhizosphere soil of *E. cannabinum* contained more Zn than Cu).

Statistically significantly higher concentrations of Ca, Cd, Cr, Mg, Mn, Ni, Pb, and Zn were recorded in the rhizosphere substrate of *E. cannabinum* compared to *H. lanatus* and *A. alba* ($p < 0.001$). Also, the content of Cr, Cu, Mg, Mn, Ni, and Pb was statistically significantly higher in the rhizosphere substrate of *A. alba* compared to *H. lanatus*, while the content of Fe was statistically significantly higher in the rhizosphere

substrate of *H. lanatus* compared to *A. alba* and *E. cannabinum* ($p < 0.001$). Also, statistically significantly higher concentrations of Cd were recorded in the rhizosphere substrate of *H. lanatus* compared to *A. alba*.

Table 2. The content of total metal concentrations [mg kg^{-1}] in the rhizosphere soil of the examined plant species

Parameter/ Species	<i>H. lanatus</i>	<i>A. alba</i>	<i>E. cannabinum</i>
	M (SD)	M (SD)	M (SD)
Total concentrations of chemical elements			
Ca	10,507.68 (175.84)	16,386.60 (108.58) a ^{***}	47,625.24 (127.01) b ^{***} c ^{***}
Cd	3.94 (0.04) a ^{***}	1.93 (0.03)	10.26 (0.21) b ^{***} c ^{***}
Cr	103.80 (0.88)	116.78 (0.61) a ^{***}	143.30 (0.89) b ^{***} c ^{***}
Cu	163.90 (0.27)	252.80 (1.11) a ^{***}	338.14 (0.68) b ^{***} c ^{***}
Fe	68,346.92 (292.65) a ^{***} b ^{***}	54,629.02 (408.67)	55,842.76 (90.30) c ^{***}
Mg	4,472.96 (96.82)	4,899.84 (101.96) a ^{***}	11,556.14 (233.79) b ^{***} c ^{***}
Mn	321.66 (5.82)	359.00 (1.56) a ^{***}	843.50 (1.98) b ^{***} c ^{***}
Ni	21.54 (0.86)	24.30 (0.35) a ^{***}	102.52 (1.08) b ^{***} c ^{***}
Pb	808.10 (1.91)	872.84 (5.16) a ^{***}	1,183.04 (11.91) b ^{***} c ^{***}
Zn	193.62 (0.73)	194.50 (1.37) a ^{ns}	203.12 (0.76) b ^{***} c ^{***}

ANOVA (Scheffe's post-hoc test): data represented as mean (M) \pm standard deviation (SD) (n=5); a) *H. lanatus* – *A. alba*, b) *H. lanatus* – *E. cannabinum*, c) *A. alba* – *E. cannabinum*; *** $p < 0.001$, ns = not statistically significant, ND = not detected

The results of the comparative analysis of the content of chemical elements in the root, stem and leaves of *H. lanatus*, *A. alba* and *E. cannabinum* are shown in Table 3. The results of this study showed that the root of the species *H. lanatus* accumulated almost all the examined metals more than the its other examined organs. The highest content of Cr ($54.8 \text{ mg kg}^{-1} \text{ d.m.}$) was determined in the leaf of this species (Table 3). Another examined species, *A. alba* from the Poaceae family, showed a similar metal accumulation pattern to *H. lanatus* species. The root of this species accumulated the most Mn, Ni, Fe, Cu, Zn, Cr, Mg, Pb, and Cd, while the leaf contained the most Ca ($9372.9 \text{ mg kg}^{-1} \text{ d.m.}$). The species *E. cannabinum* belonging to the Asteraceae family showed a different pattern of accumulation of the tested metals in its organs. This species accumulated all the examined metals in the highest content in its leaves.

The content of Cd, Cu, Fe, Mg, and Ni was statistically significantly higher in the roots of *H. lanatus* than in the roots of *A. alba* and *E. cannabinum* ($p < 0.001$), while the content of Cr, Mn, Pb and Zn was significantly higher in *A. alba* root compared to *H. lanatus* and *E. cannabinum* ($p < 0.001$). The roots of *H. lanatus* had statistically significantly higher concentrations of Pb and Zn compared to *E. cannabinum* ($p < 0.001$), while the values of Ca and Cr were significantly higher in the roots of *E. cannabinum* than those of *H. lanatus* ($p < 0.001$). Also, statistically significantly higher concentrations of Cd, Cu, Cr, Fe, Mg, Mn, Ni, Pb, and Zn were found in the roots of *A. alba* compared to *E. cannabinum* ($p < 0.001$).

The concentrations of Ca, Cd, Fe, Ni, Pb, and Zn were statistically significantly higher in the *E. cannabinum* stem than in the *H. lanatus* and *A. alba* stem ($p < 0.001$), while the values of Cu, Mg and Mn were statistically significantly higher in the stem of *A. alba* compared to *H. lanatus* and *E. cannabinum* ($p < 0.001$). The content of Ca and Mn in the stem was statistically significantly higher in *H. lanatus* than in *E. cannabinum* ($p < 0.001$), while Mg values were significantly higher in the stem of *E. cannabinum* than in *H. lanatus* ($p < 0.001$). Differences in the concentration of Cr in the stem of the examined plant species were not statistically significant (ns).

The species *E. cannabinum* had statistically significantly higher concentrations of Ca, Cd, Cu, Fe, Mg, Ni, Pb, and Zn in leaves compared to *H. lanatus* and *A. alba* ($p < 0.001$), while the content of Cr and Mn was

statistically significantly higher in the leaves of *H. lanatus* compared to *A. alba* and *E. cannabinum* ($p < 0.001$). The content of Ca, Mg, Ni, and Zn was statistically significantly higher in the leaves of *A. alba* than *H. lanatus* ($p < 0.001$) and Mn compared to *E. cannabinum* ($p < 0.001$), while the values of Cd, Cu and Fe were significantly higher in *H. lanatus* compared to *A. alba* ($p < 0.01$, $p < 0.001$, $p < 0.001$). The content of Cr was statistically significantly higher in the leaves of *E. cannabinum* compared to the leaves of *A. alba* ($p < 0.001$).

Table 3. The content of chemical elements in the root, stem and leaves of the examined plant species [mg kg⁻¹]

Parameter/ Species	<i>H. lanatus</i>	<i>A. alba</i>	<i>E. cannabinum</i>
	M (SD)	M (SD)	M (SD)
Root			
Ca	4,569.80 (33.18)	5,545.64 (84.98) a ^{***} c ^{ns}	5,381.48 (75.95) b ^{***}
Cd	11.44 (0.33) a ^{***} b ^{***}	3.87 (0.08) c ^{***}	1.82 (0.04)
Cr	1.06 (0.03)	21.72 (0.54) a ^{***} c ^{***}	4.19 (0.03) b ^{***}
Cu	163.14 (0.57) a ^{***} b ^{***}	28.50 (0.39) c ^{***}	14.44 (0.34)
Fe	2,602.48 (19.99) a ^{***} b ^{***}	2,265.42 (35.49) c ^{***}	654.94 (3.23)
Mg	5,892.26 (47.53) a ^{***} b ^{***}	2,335.72 (23.51) c ^{***}	1,087.92 (36.57)
Mn	293.70 (3.20) a ^{***}	322.16 (3.11) b ^{***} c ^{***}	34.00 (0.65)
Ni	29.08 (0.27) a ^{***} b ^{***}	15.14 (0.55) c ^{***}	5.74 (0.04)
Pb	74.90 (0.46) b ^{***}	151.60 (1.34) a ^{***} c ^{***}	22.46 (0.36)
Zn	184.52 (3.00) b ^{***}	194.52 (4.42) a ^{***} c ^{***}	79.78 (0.57)
Stem			
Stem			
Ca	2,347.12 (35.91) a ^{***}	1,623.18 (37.21)	17,518.76 (52.32) b ^{***} c ^{***}
Cd	1.29 (0.05) a ^{ns}	1.48 (0.04)	2.91 (0.04) b ^{***} c ^{***}
Cr	0.36 (0.02) a ^{ns} b ^{ns}	0.83 (0.03) c ^{ns}	24.38 (4.25)
Cu	7.72 (0.07)	14.30 (0.32) a ^{***} b ^{***}	11.30 (0.28) c ^{***}
Fe	40.68 (0.71) a ^{ns}	67.38 (0.67)	236.90 (2.05) b ^{***} c ^{***}
Mg	273.94 (6.51)	1,539.78 (37.54) a ^{***} c ^{***}	1,089.40 (25.65) b ^{***}
Mn	160.30 (0.99) b ^{***}	242.80 (2.82) a ^{***} c ^{***}	30.30 (0.94)
Ni	1.35 (0.03) a ^{ns}	1.45 (0.03)	4.60 (0.04) b ^{***} c ^{***}
Pb	3.54 (0.26)	20.54 (0.31) a ^{***}	34.14 (0.50) b ^{***} c ^{***}
Zn	30.88 (0.26)	69.68 (0.47) a ^{***}	111.14 (0.88) b ^{***} c ^{***}
Leaves			
Leaves			
Ca	1,489.52 (20.02)	9,372.90 (48.16) a ^{***}	27,267.38 (142.67) b ^{***} c ^{***}
Cd	2.27 (0.11) a ^{***}	1.83 (0.03)	3.74 (0.07) b ^{***} c ^{***}
Cr	54.80 (0.28) a ^{***} b ^{***}	21.14 (0.41)	37.42 (0.31) c ^{***}
Cu	15.36 (0.34) a ^{***}	13.40 (0.30)	33.68 (0.31) b ^{***} c ^{***}
Fe	812.68 (9.26) a ^{***}	698.84 (7.62)	2,019.48 (35.54) b ^{***} c ^{***}
Mg	1,250.88 (46.70)	1,747.02 (31.40) a ^{***}	5,104.50 (53.78) b ^{***} c ^{***}
Mn	240.88 (0.54) a ^{***} b ^{***}	163.86 (2.96) c ^{***}	102.22 (0.91)
Ni	4.33 (0.08)	7.29 (0.06) a ^{***}	16.42 (0.31) b ^{***} c ^{***}
Pb	36.80 (0.34) a ^{ns}	37.02 (0.45)	61.26 (0.27) b ^{***} c ^{***}
Zn	56.02 (0.46)	105.30 (1.75) a ^{***}	172.48 (1.15) b ^{***} c ^{***}

ANOVA (Scheffe's post-hoc test): data represented as mean (M) ± standard deviation (SD) (n=5); a) *H. lanatus* – *A. alba*, b) *H. lanatus* – *E. cannabinum*, c) *A. alba* – *E. cannabinum*; *** $p < 0.001$, ns = not statistically significant, ND = not detected

The results of the two-factor analysis of variance (factorial ANOVA) indicated that the factor “*Plant species*” has a statistically significantly greater influence on the content of Ca and Mn ($p < 0.001$), while the influence of the factor “*Plant organ*” is significantly greater on the content of Cd, Cr, Cu, Fe, Mg, Ni, Pb, and Zn ($p < 0.001$) (Table 4).

Table 4. Influence of the factors “Plant organ” (root, stem, leaf) and “Plant species” on the variability of the chemical elements content in the examined plant species

Parameter	Plant species		Plant organ		Plant species * Plant organ	
	F	p	F	p	F	p
Ca	141,112.09	***	39,554.82	***	39,652.93	***
Cd	1,535.33	***	3,233.06	***	2,826.91	***
Cr	81.52	***	1,627.35	***	537.85	***
Cu	61,374.91	***	95,479.61	***	84,433.33	***
Fe	321.00	***	26,840.82	***	9,453.78	***
Mg	991.67	***	11,633.84	***	16,052.31	***
Mn	31,784.11	***	3,882.89	***	4,046.06	***
Ni	776.79	***	11,075.97	***	6,249.00	***
Pb	11,735.81	***	37,728.02	***	22,711.40	***
Zn	1,052.14	***	5,327.38	***	4,220.26	***

two-factor ANOVA (factorial ANOVA), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The value of the BCF reflects the ability of the plant to adopt a certain metal and to translocate it from the roots to its other organs. Phytoextraction potential can be estimated by calculation of bioaccumulation (bioconcentration factor or biological absorption coefficient) and translocation factor and they were calculated for assessment of the metal accumulation and translocation efficiency in the plants. The bioconcentration factor (BCF) is defined as the ratio of the total concentration of element in the harvested plant tissue to its concentration in the soil in which the plant was growing.

The value of BCF in *H. lanatus* ranged from 0.001 (for Fe in the stem) to 2.9 (for Cd in the root) (Table 5). The root of *H. lanatus* showed BCF values greater than or equal to 1 for Ni (1.35), Cu (1.0), Mg (1.32) and Cd (2.9). The highest value of BCF in the species *A. alba* was recorded for Cd in the root (2.01), and the lowest as in the species *H. lanatus* for Fe in the stem. In the species *E. cannabinum*, the lowest value of BCF was for Fe in the stem (0.004), and the highest value did not exceed the value of 1 and was recorded for Zn in the leaf (0.85) of this species.

Translocation factor (TCF), defined as the ratio of the total concentration of elements in the aerial parts of the plant (stem, leaf) to the concentration in the root. The results of this study indicate that the lowest TCF value was for Fe in the stem of both species from the Poaceae family, and extremely high for Cr (51.6) in the leaf of *H. lanatus* and Ca (1.69) in the leaf of *A. alba*. The species *E. cannabinum* from the Asteraceae family showed a different pattern of translocation of the investigated metals. The TCF values for the stem of this species indicate that it has a lower degree of translocation than the leaf. The lowest value of TCF for Fe in the leaf was also determined in this examined species. The highest TCF value is shown for Cr (8.92) in the leaves of *E. cannabinum*. For all examined metals, the TCF values for the leaf of *E. cannabinum* species were greater than 1, while for the stem this value was greater than or equal to 1 for Ca, Cd, Cr, Mg, and Pb.

Table 5. The bioaccumulation (BCF) and translocation (TCF) factor

	<i>H. lanatus</i>				
	<i>BCF_{root}</i>	<i>BCF_{stem}</i>	<i>BCF_{leaf}</i>	<i>TCF_{stem}</i>	<i>TCF_{leaf}</i>
<i>Ca</i>	0.43	0.22	0.14	0.51	0.33
<i>Cd</i>	2.90	0.33	0.58	0.11	0.20
<i>Cr</i>	0.01	0.003	0.53	0.34	51.60
<i>Cu</i>	1.00	0.05	0.09	0.05	0.09
<i>Fe</i>	0.04	0.001	0.01	0.02	0.31
<i>Mg</i>	1.32	0.06	0.28	0.05	0.21
<i>Mn</i>	0.91	0.50	0.75	0.55	0.82
<i>Ni</i>	1.35	0.06	0.20	0.05	0.15
<i>Pb</i>	0.10	0.004	0.05	0.05	0.49
<i>Zn</i>	0.95	0.16	0.29	0.17	0.30
	<i>A. alba</i>				
	<i>BCF_{root}</i>	<i>BCF_{stem}</i>	<i>BCF_{leaf}</i>	<i>TCF_{stem}</i>	<i>TCF_{leaf}</i>
<i>Ca</i>	0.34	0.10	0.57	0.29	1.69
<i>Cd</i>	2.01	0.77	0.95	0.38	0.47
<i>Cr</i>	0.19	0.01	0.18	0.04	0.97
<i>Cu</i>	0.11	0.06	0.05	0.50	0.47
<i>Fe</i>	0.04	0.001	0.01	0.03	0.31
<i>Mg</i>	0.48	0.31	0.36	0.66	0.75
<i>Mn</i>	0.90	0.68	0.46	0.75	0.51
<i>Ni</i>	0.62	0.06	0.30	0.10	0.48
<i>Pb</i>	0.17	0.02	0.04	0.14	0.24
<i>Zn</i>	1.00	0.36	0.54	0.36	0.54
	<i>E. cannabinum</i>				
	<i>BCF_{root}</i>	<i>BCF_{stem}</i>	<i>BCF_{leaf}</i>	<i>TCF_{stem}</i>	<i>TCF_{leaf}</i>
<i>Ca</i>	0.11	0.37	0.57	3.26	5.07
<i>Cd</i>	0.18	0.28	0.37	1.59	2.05
<i>Cr</i>	0.03	0.17	0.26	5.81	8.92
<i>Cu</i>	0.04	0.03	0.10	0.78	2.33
<i>Fe</i>	0.01	0.004	0.04	0.36	3.08
<i>Mg</i>	0.10	0.09	0.44	1.00	4.69
<i>Mn</i>	0.04	0.04	0.12	0.89	3.01
<i>Ni</i>	0.06	0.04	0.16	0.80	2.86
<i>Pb</i>	0.02	0.03	0.05	1.52	2.73
<i>Zn</i>	0.40	0.55	0.85	1.39	2.16
Bioaccumulation factor (BCF): BCF _{root} - Bioaccumulation factor for root BCF _{stem} - Bioaccumulation factor for stem BCF _{leaf} - Bioaccumulation factor for leaf				Translocation factor (TCF): TCF _{stem} - Translocation factor for stem TCF _{leaf} - Translocation factor for leaf	

Discussion

Human activities such as coal production and surface mining activities, in addition to the disposal of ore and fly ash, have a detrimental effect on the landscape and natural ecosystems. Over time, soil degradation occurs, as well as the deterioration of water and air quality in given areas, which negatively affects the living world and humans. There is often an almost complete loss of vegetation. Harmful components of fly ash, mine waste, and tailings (metals, metalloids, organic compounds) can reach the soil, water, and air and cause pollution (Gajić *et al.*, 2018).

There are numerous conditions on fly ash and mine tailings deposits that are not suitable for plant growth, such as inadequate mechanical composition and pH, poorly developed soil structure throughout the profile, coarse texture with more sand fractions and lower clay content, high concentrations of soluble salts, reduced number of nitrogen-fixing microorganisms and reduced presence of mycorrhizae.

Limiting factors for plant growth on mine tailings are its characteristics, such as poor content of essential nutrients (N, P), multi-element pollution and toxic concentrations of metals and metalloids (As, B, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, and Se), above the locally approved values and the maximum allowed values proposed by the regulations of any country (Gajić *et al.*, 2018). The results of this study support these claims.

The results of the analysis of the total content of the examined metals in the rhizosphere soil of the studied species showed that the content of Cu and Pb in the rhizosphere soil of all three species was higher than the limit values and maximum allowed concentrations according to the regulations of the Republic of Serbia (Official Gazette of the RS, No. 88/2010, Annex 3) as well as from the threshold value for given metals according to the European Union Directive (1986). Also, the content of Ni in the rhizosphere soil of *E. cannabinum* had higher values than the limit value and the maximum allowed concentration according to the regulation of the Republic of Serbia, as well as the limit value for the given metal according to the European Union Directive. The content of Pb in the rhizosphere soil of all three studied species, as well as the content of Cu in the rhizosphere soil of the species *A. alba* and *E. cannabinum* were higher than the remediation values according to the Rulebook (Official Gazette of the RS, No. 88/2010, Annex 3). This is despite the fact that the concentration of Cu decreases from about 2% in the mined ore to 0.18% in the deposited tailings.

The content of Zn, Cr, and Cd in the rhizosphere soil of all three tested species had a value higher than the limit value, as well as the content of Cr in the rhizosphere soil of all three tested species, which had a value higher than the maximum allowed concentration in the soil. The content of Cd in the rhizosphere soil of all species of *H. lanatus* and *E. cannabinum* had a value higher than the maximum allowed concentration in the soil of the Republic of Serbia, as well as the limit values for the given metal following EU legislation. The results of this study show that the total content of the examined macro and microelements is within the limits of some literature data (Kabata-Pendias and Pendias, 2001; Kasowska *et al.*, 2018; Wu *et al.*, 2021).

According to some literature data (Kabata-Pendias and Pendias, 2001; Soriano *et al.*, 2012), the analyzed rhizosphere soil in relation to the content of a certain metal present in it, in this study can be classified as:

- according to Pb - soil of the species *H. lanatus* and *A. alba* is in the category of lightly polluted, and the species *E. cannabinum* in the category of moderately polluted soils;
- according to Mg - the soil of all three studied species is in the category of heavily polluted soils
- according to Ni - the soil of the *H. lanatus* and *A. alba* species is in the slightly polluted category, and the *E. cannabinum* species is in the moderately polluted soil category;
- according to Cr - the soil of all three studied species is in the category of lightly polluted soils
- according to Cu – the soil of the *H. lanatus* species in the slightly polluted soil category, and for the *A. alba* and *E. cannabinum* species in the moderately polluted soil category;
- according to Cd - the soil of the *A. alba* species belongs to the lightly polluted soil, and of the *H. lanatus* and *E. cannabinum* species to the moderately polluted soil;

- according to Zn - the soil of the species *H. lanatus* and *A. alba* is in the category of moderately polluted, and that of the species *E. cannabinum* is in the category of highly polluted soils;
- according to Fe, Mn, Ca - the soil of all three studied species is outside the category of unpolluted soils.

The results of this study indicate that the content of Pb and Cu in the soil sampled in the root zone of the studied species was higher than their remediation values (for Pb and twice as high) and indicate that even after the exploitation and processing procedures of polymetallic ore (copper-zinc-lead ore), a large part remains (especially Pb, Cu, Zn, and Mg) in tailings and represents a danger for the growth and development of vegetation, as indicated by the small number and rare populations of wild, pioneer species on the tailings.

The establishment of vegetation on abandoned spoil heaps and metal-bearing wastes is usually difficult because these artificial habitats create unfavorable conditions for plant growth. They are characterized by poor physical properties of the substrate with unsuitable air-water conditions, high levels of toxic metals, salinity, low fertility, and microbial activity (Kasowska *et al.*, 2018). As a result, these wastelands often have no or sparse vegetation and are unable to create sustainable and healthy ecosystems. This is also evident at the studied spoil heap site, where only 10 plant taxa, 4 woody and 6 herbaceous plants, grew even in rare populations. In particular, soils with high or contrasting metal contents exert strong selection pressure on the colonising plants, which can survive in such habitats due to a variety of factors. The lack of nutrients or their low availability is a similarly important selection factor that hardly limits the development of vegetation (Kasowska *et al.*, 2018).

The heavy metal accumulation depends on the total metal concentrations, as well as their bioavailability. Powers for transporting metals through plant, from roots to shoots are root pressure and leaf transpiration. In two investigated grass species, the metal content in roots was much higher than shoots. The reason for that might be ability of the roots to immobilize heavy metals by adsorbing them or forming precipitation in rhizosphere soil (Wu *et al.*, 2021).

The results of our study showed that the normal range values for Mn contents in leaves in investigated plants were in the normal range (15-100 mg kg⁻¹) according to Olivares *et al.* (2013). The iron levels for the examined grasses were above the range of critical deficiency concentration (50–150 mg Fe kg⁻¹), according to Marschner (2012). Also, the metal contents in investigated plants were above the normal levels of general plants except which were 1–160 mg·kg⁻¹ for Zn, 0.4–45.8 mg·kg⁻¹ for Cu, 0.1–10 mg·kg⁻¹ for Ni, 0.2–0.8 mg·kg⁻¹ for Cr, 0.1–41.7 mg·kg⁻¹ for Pb, 0.2–0.8 mg·kg⁻¹ for Cd, respectively (Wu *et al.*, 2021; Zhu *et al.*, 2018).

The further assess the phytoremediation potential of the investigated plants, BCF and TCF are useful indices. According to Antoniadis *et al.* (2017) accumulator plants are often expected to have TCF > 1 for a certain metal. With regard to TCF, it should be as high as possible and close to values of 1, but according to Malinowska *et al.* (2015) it should be BCF > 1 for accumulator plants. Phytoextraction potential can be estimated by calculation of bioconcentration factor. According to Kabata-Pendias and Dudka (1991), depending on the BCF values, the accumulation efficiency can be: intensive, BCF > 1; medium, BCF = 1–0.1; weak, BCF = 0.1–0.01; and no accumulation, BCF = 0.01–0.001. Plants exhibiting organs with BCF > 1 are considered the species with phytoextraction potential (Zacchini *et al.*, 2009).

The obtained results showed that the root of *H. lanatus* intensively accumulated Cd, Cu, Mg, and Ni, while the root of *A. alba* intensively accumulated Cd and Zn. This is supported by the fact that the root of *H. lanatus* contained statistically significantly higher amounts of Cd, Cu, Fe, Mg, and Ni, and the root of *A. alba* Cr, Mn, Pb, and Zn than the roots of the other two studied species.

The mean accumulative capacity of the species *H. lanatus* based on BCF values was determined for the root (Ca, Mn, Pb, Zn); stem (Ca, Cd, Mn, Zn) and leaf (Ca, Cd, Cr, Mg, Mn, Ni, Zn). Weak accumulation of this species was shown for Cr and Fe in roots; Cu, Mg and Ni in the stem and Cu, Mg, and Pb in the leaf. The *H. lanatus* tree did not show the ability to accumulate Cr, Fe, and Pb.

The mean accumulative capacity of roots, stems and leaves of *A. alba* was determined for Ca, Mg, and Mn. Also, the average accumulation of this species was shown by the root for Cr, Cu, Ni and Pb, the stem for

Cd and Zn and the leaf for Cr, Ni, and Zn. A weak accumulative capacity of the species *A. alba* was determined for the root (Fe), stem (Cr, Cu, Ni, and Pb) and leaf (Cu, Fe, and Pb). It is also indicative that the tree of this species does not have the ability to accumulate Fe.

Based on the BCF value, the species *E. cannabinum* has the lowest accumulation efficiency. The mean accumulative capacity of the *E. cannabinum* species was determined for the root (Ca, Cd, Mg, Zn), stem (Ca, Cd, Cr, Zn) and for the leaf for almost all the examined metals (except Fe and Pb for which it has a weak accumulation). Weak accumulation of this species was shown for Cr, Cu, Fe, Mn, Ni and Pb in roots; Cu, Mg, Mn, Ni and Pb in the stem. The tree of this species does not have the ability to accumulate Fe.

Using of TCF allows following the transfer and redistribution of heavy metals among different parts of plants (Zhang *et al.*, 2020; Dai *et al.*, 2013). The strong transportability of accumulated metals possessed by some plants has a protective role, as it allows plants to transfer most of the metals present in the rhizosphere from the roots to their shoots and thus protect the roots from toxicity (Pandey *et al.*, 2016; Rodriguez *et al.*, 2012).

The results of this research indicate a good translocation of Cr from the roots to the leaves of *H. lanatus*, where this metal accumulates, as well as the intense translocation of Ca and its accumulation in the leaves of *A. alba*. Both studied species from the Poaceae family showed a similar pattern of translocation of other investigated metals. Species *E. cannabinum* from the Asteraceae family had a different pattern of translocation and accumulation of the investigated metals. This species adopted and accumulated Ca, Cd, Cr, Mg, Pb, and Zn in the stem and leaves, while it translocated Cu, Fe, Mn, and Ni from the roots to the leaves where they were accumulated.

These results are supported by the results of the statistical analysis of the data, which indicate that the content of Ca in plants is statistically significantly influenced by the plant taxon, while for the content of Cd, Cr, Cu, Fe, Mg, Ni, Pb, and Zn, the type of organ where the metals accumulate is a statistically significant factor.

Different plant species have different absorption and tolerance strategies for different heavy metals and that is a reason for varied values of the BCFs (Rascio and Navari-Izzo, 2011), what the results of this study point to. BCF values show that the roots of grass species had the highest accumulation efficiency, and the stem the lowest, as well as that it depended on the plant species and the type of accumulated metal. In the process of phytoremediation, gradual reduction of heavy metal pollution in the soil could be done by phytoextraction with strong accumulation and transfer capacity.

This study indicates that *H. lanatus* and *A. alba* are accumulator species and that *H. lanatus* species can be applied in the phytoextraction technique for Cd, Cu, Mg and Ni, and *A. alba* species for Cd and Zn. Otherwise, grass have extensive root system (which allows contact with large soil volumes), fast growth rates, most species have high biomass yields, shoot regrowth after harvest and adaptation to soil infertility that make them suitable for phytostabilization (Rabêlo *et al.*, 2021). The phytoremediation potential of each species depends on element bioavailability, the uptake mechanisms, transport, accumulation, toxicity, tolerance to each element and cultivation system (Rabêlo *et al.*, 2021). Phytoremediation by phytostabilization could be done with low TCF, high BCF and well-developed root. Also, values of $BCF < 1$, $TCF < 1$ and $BCF < 1$, $TCF > 1$ indicated that some plant retained more elements in root than in leaves or in spite of efficient transport of some elements from root to leaves, this species possesses mechanism that prevent the uptake of high content of metal (Gajić *et al.*, 2018).

Some research has shown that species of the genus *Agrostis* can be used in phytoextraction and phytoaccumulation of Cd, As, Pb, and Zn (Gajić *et al.*, 2018; Rabêlo *et al.*, 2021). Also, it was shown that the species *H. lanatus* is an accumulator of Pb, Cr, Zn and As (Meharg and Macnair, 1991; Rengel, 2000; Gajić *et al.*, 2018). Results of BCF and TCF values of the studied species indicate that *H. lanatus* species can be applied as a phyto-accumulator species for Cr, and *A. alba* species for Ca. There are not many literature data about the use of *E. cannabinum* in phytoremediation. According to Gonzalez *et al.* (2019), species of *E. cannabinum* can

be used for phytostabilization of As. The results of this study indicate that in contrast to these two species from the Poaceae family, species *E. cannabinum* is a good candidate as a phytoaccumulator of all investigated metals on the investigated tailings of the lead-copper-zinc mine. This study also indicates that the response of plants to heavy metals in the soil depends on the plant species, the total concentration of metals in the soil, the bioavailability of the metals themselves, and the physical and chemical properties of the soil, that is in agreement with other studies (Bonanno, 2011; Ghazaryan *et al.*, 2019).

Conclusions

After the exploitation and processing procedures of polymetallic ore (copper-zinc lead), large part of Pb, Cu, Zn and Mg remains in tailings and represents a danger for the growth and development of vegetation, as indicated by the small number and rare populations of wild, pioneer species on the tailings. Nevertheless, the efficiency of accumulation of investigated plants depended on the plant species and the type of accumulated metal. The roots of grass showed the highest and the stem the lowest metal accumulation. The *E. cannabinum* species showed the lowest accumulation efficiency among investigated species. Intensive accumulation and potential for application in phytoextraction were shown by the species *H. lanatus* for Cd, Cu, Mg, and Ni and *A. alba* for Cd and Zn. Also, the species *E. cannabinum* is a good candidate for phytoaccumulation of all tested metals in the researched mine tailings.

Authors' Contributions

Conceptualization, RG, and SB; methodology, RG, and SB; software, FG; validation, RG, DB and SB; formal analysis, FG; investigation, SB, DB and RG; resources, DB; data curation, JM; writing—original draft preparation, RG, and SB; writing—review and editing, RG, MM, VR and SB; visualization, RG, and SB; supervision, RG, MM, RV and SB; project administration, DB and SB; funding acquisition, SB and DB.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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