Metal bioaccumulation, translocation and phytoremediation potential of some woody species at mine tailings

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Abstract

This study aimed to determine the concentrations of ten metals (Mn, Ni, Ca, Mg, Fe, Zn, Cr, Pb, Cd, Cu) in the soil at depths of 10 and 20 cm and selected plant species (*Populus nigra* L., *Fraxinus ornus* L., *Salix alba* L., *Salix caprea* L.), as well as to assess the ability of these plants of metal bioaccumulation, translocation and phytoremediation on the location of the mine pit and flotation tailings of the selected mine (in the center of the Republic of Serbia). The concentrations of Pb and Cu in the investigated soil at a depth of 10 cm were above remediation values stated in the regulations of the Republic of Serbia. At the same time, the concentration of Cd, Cr, Pb and Cu was above the limit values in both of the examined soil depths, while Ni in the soil at a depth of 10 cm was above the limit value determined in the Directives of the European Union. The species *F. ornus* shows the ability to phytoextract Ca, species *P. nigra* Zn, Ca and Cd, while both *Salix* species have the capacity to phytoextract Zn and Ca. The results showed that species *S. alba* is an efficient bioaccumulator of Mn, Fe, Cr, Pb, Zn and Ca, *S. caprea* of Fe, Cu, Cr, Mg and Pb, and *P. nigra* of Mn and Cd. The bioaccumulation and translocation of the investigated elements depend on plant species and their organ. The selection of plant species adequate for remediation should take this into account.

Keywords: bioaccumulation; metals; soil; translocation; woody species

Introduction

So-called 'heavy metals' (metallic elements that have a relatively high density compared to water) (Fergusson, 1990) are naturally occurring elements in the biosphere. This is a group of metals and semimetals (metalloids) that have been associated with contamination and potential toxicity or ecotoxicity. Some of them are essential for life having important roles in plant cell metabolisms (act as cofactors and in oxidation-reduction reactions) but at very low concentrations. However, at high concentrations, are toxic because they

affect plant growth, enzymatic activity, stoma function, photosynthesis, and accumulation of other nutrient elements as well as make damages to the root system (Güne *et al.*, 2004).

Some human activities such as mining and smelting, fossil fuel combustion, electroplating, energy and fuel production, intensive agriculture (Reichman, 2002), the exhaust gases of motor vehicles, corrosion of various vehicle components (Falahi-Ardakani, 1984) and emissions from brake linings and tires (Nriagu, 1979; Hjortenkrans *et al.*, 2007) and military activities lead to environmental pollution with heavy metals. An increasing level of these potentially toxic elements in the environment is an actual global problem. Phytoremediation is one among various methods for its resolution. It is an environmentally friendly cleanup technology that uses plants for removing, transferring, stabilization and degradation organic and inorganic pollutants in soil, water, and air (McGrath *et al.*, 2002). This is an efficient, *in situ* and low-cost technics compared to standard remediation methods, such as soil washing, excavation, incineration and pump-and-treat systems (US EPA, 1997).

Plants are able to uptake various trace elements from the environment and accumulate them in their tissues, acting that way as interceptors of heavy metals (Cobb *et al.*, 2000; Macnair, 2003; Rascioa and Navari-Izzo, 2011). The characteristics of appropriate plants for phytoremediation are fast-growth, high biomass production, tolerance to a specific metal, uptake of its significant amount, as well as a capacity for its translocation and accumulation in their aboveground (harvestable) parts (Krämer, 2010). The selection of a good metal indicator and hyperaccumulator is essential for successful phytoremediation (Cinar and Elik, 2002). There are some reports concerning promising use of trees for this purpose, that is phytoextraction of land polluted by heavy metals (Pulford and Waston, 2003; Placek *et al.*, 2016). Some of them are species belonging to genera Salix (willows) and Populus (poplars), both from the Salicaceae family and Fraxinus from family Oleaceae.

There is particular interest in willows among them (Greger and Landberg, 2006; Tlustoš *et al.*, 2007). Otherwise, it was found that willows and poplars usually accumulate higher amounts of heavy metals in wood, compared to other tree species (Rock, 2003). The species of the genus Salix, which are easily propagated, fast-growing, tolerant to different metals, with great biomass and extensive, spreading roots are attractive as plants of choice for phytoremediation. Besides, a significant accumulation of metal in their roots and shoots was found (Kuzovkina and Quigley, 2005; San-Miguel-Ayanz *et al.*, 2016). In Europe and North America, their use for phytoremediation of numerous pollutants is well established (Branković *et al.*, 2019). In addition, a different uptake and a higher translocation ratio between field plants and that in hydroponics were noticed (Jones, 2005). The reason for this may be a specific microbial activity in the root zone in field conditions.

Salix alba L. (white willow) is a fast-growing dioecious broadleaved tree. It is one of the largest of the willow species and can reach heights of up to 30 m and a diameter of 1 m or more. The goat willow (*Salix caprea* L.) is a deciduous small to medium-size tree or shrub with heights up to 10 m, reaching only exceptionally 15 m. Black poplar (*Populus nigra* L.) is a large, fast-growing deciduous tree, reaching heights of up to 40 m and trunk diameters of up to 2 m. The manna ash (*Fraxinus ornus* L.) is a small to medium-sized deciduous tree, rarely growing up to a height of 25 m and 1 meter in diameter (San-Miguel-Ayanz *et al.*, 2016).
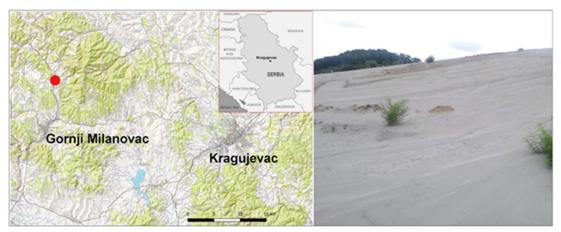
The mine "Rudnik", located in central Serbia, is known for its polymetallic lead-zinc-copper ores. Mining on this mountain that dates back to the Middle Ages, turning intensive after the Second World War, continues to this day. During flotation and processing of ores, flotation products are concentrates and tailings (Company Rudnik and flotacija "Rudnik" d.o.o., 2020), where the last is stored at the tailings pond, which is a source of heavy metal contamination of surface waters, groundwater, soils, and biota (Concas *et al.*, 2006; Liu *et al.*, 2020).

The aim of the study was to determine the metal translocation and accumulation potentials of the already mentioned investigated plant species that naturally grow on the investigated locality and indicate their potential application in the revitalization and remediation of the tailings.

Materials and Methods

Study site

The Rudnik flotation plant and tailing dump of the "Rudnik" d.o.o. are located at 44.10625 north latitude and 20.48484 east longitude. The slope of the tailing dump is next to the Ibar Highway in the village of Majdan, 7 km north of the town Gornji Milanovac, beneath the peak of Mount Rudnik (Figure 1). The entire area is situated in Šumadija, the central part of Republic of Serbia.

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

Soil and plant sampling and analysis

Soil and plant sampling: Sampling was done in the area of the Rudnik tailings pond, whereby the soil was taken from two depths of 10 and 20 cm with appropriate equipment. The sampled material visually differed in color and texture because it belonged to different overflow layers. The plants were collected in the area that covers the tailings, without peripheral parts. Determination of the plant material was done by the keys (Jávorka and Csapody, 1991; Josifović, 1970; Tutin,1964-1980), in the laboratory of the Department of Biology and Ecology, at the Faculty of Science in Kragujevac. The following woody species were analyzed: *Populus nigra* L., *Fraxinus ornus* L., *Salix alba* L. and *Salix caprea* L.

Reagents: Reagents (65% HNO_3, 35% H_2O_2, and 70% $HClO_4$) 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 LGC standards and National Research Council Canada.

Instruments and apparatus: The flame atomic absorption spectrophotometer (FAAS) model Perkin Elmer 3300 with D_2 lamp as a corrector was used for the determination of metals: manganese (λ = 279.8 nm), calcium (λ = 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) mgkg^{-1}, respectively. Detection limits for determining the concentration of metals in the plant material were: 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) mgkg^{-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 hours (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 a 10 to 20 cm depth. Initially 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 24h (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 (Službeni glasnik RS, br. 88/2010, prilog 3). Different extractants, generally "strong acids" such as HNO_3, HF, $HClO_4$ and aqua regia, have been utilized to determine the total or "pseudototal" 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: HNO_3: H_2O_2 = 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 ± 5% of the certified values.

Determination of mobile metals in soil: The extraction was performed with DTPA (diethylene triaminopenta acetic acid), on pH 7.00. The sample of dried soil (3 g), measured to an accuracy of 0.0001 g, was added to 25 ml of DTPA with the magnetically stirred for 1h at room temperature (20 ± 4 °C). After extraction, the solution obtained was transferred to the volumetric flask.

Ten metals (Mn, Ni, Fe, Cu, Zn, Cr, Ca, Mg, Pb, and Cd) were analyzed in the soil and plant material (stems, leaves) of the four woody plants. The five replications of soil and plant material were determined, and also mean values and standard deviation were calculated. Further, different factors as indicators of the ability of the plant species in bioaccumulation, translocation, and phytoremediation of researched metals were calculated in the way presented in the next table (Table 1).

Table 1. Formulas for calculating of bioaccumulation factor (BCF)

Factor	Formulas	Elements of formula
Bioaccumulation factor (BCF) (Ghosh and Sing, 2005)	$BCF_{stem} = C_{stem}/C_{soil}$ $BCF_{leaf} = C_{leaf}/C_{soil}$	C_{soil} - the metal concentration in the soil C_{stem} - the metal concentration in the plant stem C_{leaf} - the metal concentration in the plant leaf

Also, the ratio between metal concentrations of leaf and stem was determined. The content of metals in soil and plant materials was expressed in mg kg^{-1} of dry matter (mg kg^{-1} d.m.).

Statistical analysis

Statistical analysis included determination of the mean (M) and standard deviation (SD) for each of the analyzed chemical elements. Differences between groups: 1) soil at the depths of 10 and 20 cm in terms of the total and available concentrations of chemical elements, 2) stems and 3) leaves of the selected plant species in terms of chemical elements concentrations were determined by analysis of variance (ANOVA) and Fisher's LSD post-hoc test. Statistical analysis was performed by using the package Statistica 10.0.

Results and Discussion

Due to the lack of data related to the possible application of field plants (*in situ*) for heavy metal phytoremediation, this paper aimed to analyze the feasibility of using three species from the Salicaceae family (*Salix alba* L., *Salix caprea* L. and *Populus nigra* L.) and one from Oleaceae (*Fraxinus ornus* L.) on the mine pit and the flotation area of the mine "Rudnik" d.o.o. in the Republic of Serbia.

Metals content in soil and plant samples

The mean concentrations of total and available metals (Mn, Ni, Fe, Cu, Zn, Cr, Ca, Mg, Pb, Cd) (mg kg^{-1} d. m.) in the soil at depths of 10 cm and 20 cm are shown in Table 2.

The results of this research showed the total contents of all tested metals in the soil were higher than their accessible ones, and that their values were greater at a depth of 10 cm compared to a depth of 20 cm of soil (the only exception is the total content of Cr and Cd). In the soil at both tested depths, the total Fe and the accessible Ca contents were the highest in relation to the contents of the other metals tested.

The total content of the tested metals in the soil had a decreasing order: Fe> Ca> Mg> Pb> Mn> Cu> Zn> Cr> Ni> Cd, while the available content decreased in the following order: Ca> Fe> Zn> Mg> Pb> Mn> Ni≥Cu> Cd> Cr.

The results obtained showed that Cd, Pb, Cr and Cu concentrations at both of examined depths in the investigated soil were above the maximum allowable concentration of these substances in the soil, and above limit values, as prescribed in the regulations of the Republic of Serbia (EU Directive 86/278/EEC, 1986; Kabata-Pendias, 2004). Also, according to the same Regulation, Ni concentration was above the maximum allowable and limit value, and Zn above limit value for given metal in the soil, while the concentration of Pb and Cu in the investigated soil at depth of 10 cm was above their remediation values. Furthermore, the limit values for Cu, Zn, Cd, Cr, Ni, Pb, Co, and Hg in European Union soils were reported as 100 mg kg^{-1}, 200 mg kg^{-1}, 1 mg kg^{-1}, 100 mg kg^{-1}, 50 mg kg^{-1}, 60 mg kg^{-1}, 20 mg kg^{-1}, and 0.5 mg kg^{-1}, respectively (Maiz *et al.*, 1997). According to Directives of the European Union (Remon *et al.*, 2013) concerning metals in the soil, concentration of Cd, Cr, Pb and Cu at both of examined depths in the investigated soil were above the limit value, with the exemption of Ni whose concentration in the investigated soil was above the limit value only at the depth of 10 cm. However, the content of the investigated heavy metals considerably exceeded the upper limits according to the threshold of pollution established by Tandy *et al.* (2006), that is 50 (Pb), 70 (Zn), 25 (Ni), Cd (0.5) and 25 (Cu) mg kg^{-1} d.m.

The results showed statistically significantly higher total concentrations of Mn, Ni, Fe, Cu, Zn, Ca, Mg and Pb at a depth of 10 cm compared with 20 cm ($p < 0.001$). However, total concentrations of Cr in soil at a depth of 20 cm were higher compared with 10 cm ($p < 0.001$). There was no statistically significant difference in total Cd content between soils at depth of 10 and 20 cm. The available Mn, Ni, Fe, Cu, Zn, Ca, Mg, Pb and Cd concentrations in soil at a depth of 10 cm were statistically significantly higher than in the soil at a depth of 20 cm ($p < 0.001$). Available concentrations of Cr in the soil at depths of 10 and 20 cm were not detected (ND).

Both, the total and available concentrations of Mn, Ni, Fe, Cu, Zn, Ca, Mg and Pb, as well as the available concentrations of Cd at a depth of 10 cm, were statistically significantly higher compared with those at a depth of 20 cm.

The mean available/total element concentration ratio (%) of Mn, Ni, Fe, Zn, and Cd was higher in the soil at a depth of 10 cm compared with that of 20 cm. However, the mean available/total element concentration ratio (%) of Cu, Ca, Mg and Pb was higher in the soil at a depth of 20 cm compared with the soil at a depth of 10 cm (Table 3).

Table 2. Total and available element concentrations (mg kg^{-1} d. m.) in soil at depths of 10 and 20 cm (the mean value ± standard deviation)

Metal / Soil depth	Total element concentrations	
	10 cm	20 cm
Mn	688.42±2.84 ***	218.62±0.97
Ni	79.78±0.68 ***	15.38±0.36
Fe	66009.78±574.28 ***	49604.42±279.21
Cu	287.5±21.06 ***	164.5±21.10
Zn	192.56±0.77 ***	133.04±1.69
Cr	126.10±1.01	130.82±1.24 ***
Ca	38707.92±95.78 ***	11480.66±129.61
Mg	10536.20±140.94 ***	4077.10±40.67
Pb	861.72±5.64 ***	510.58±1.37
Cd	8.49±0.05 ns	8.62±0.06
Metal / Soil depth	Available element concentrations	
	10 cm	20 cm
Mn	67.24±0.27 ***	1.75±0.05
Ni	3.75±0.04 ***	0.32±0.02
Fe	373.62±2.69 ***	70.82±0.30
Cu	3.75±0.04 ***	2.45±0.05
Zn	167.96±0.73 ***	15.30±0.41
Cr	ND	ND
Ca	13144.30±235.43 ***	9327.56±28.95
Mg	133.22±0.69 ***	77.78±0.59
Pb	120.50±1.20 ***	85.96±0.71
Cd	1.62±0.02 ***	0.64±0.04

ANOVA (Fisher's LSD post-hoc test): data represent the means ± SD (n=5); ***$p<0.001$, ns = not significant, ND = not detected

Table 3. Mean *available / total concentration* ratio (%) of the studied elements in soil at depths of 10 and 20 cm

Metal / Soil depth	Available /total element concentration ratio (%)	
	10 cm	20 cm
Mn	9.77	0.80
Ni	4.70	2.08
Fe	0.57	0.14
Cu	1.30	1.49
Zn	87.22	11.50
Cr	ND	ND
Ca	33.96	81.25
Mg	1.26	1.91
Pb	13.98	16.84
Cd	19.13	7.47

ND = not detected

Otherwise, the concentration of heavy metals in a soil depends on the type and heterogeneity of the soil, the activity of microorganisms, time, vegetation cover, and water regime. However, their bioavailability to plants is different, depending on various factors and soil properties such as the total metal present in the soil, pH and redox potential, mechanical composition, organic matter and clay content, temperature, mineral composition, water regime, cation exchange capacity, as well as the interaction between chemical elements (Liu *et al.*, 2013).

In soil, metals form fractions with different availability. So, in a soil solution, they can be present as free metal ions and soluble metal complexes, but also, they can be adsorbed to inorganic soil constituents at ion exchange sites, bound to soil organic substances, precipitated (in the form of oxides, hydroxides, and carbonates), or embedded in the crystal structure of primary minerals (Lombi *et al.*, 2001). Besides, the characteristics of the plants themselves affect metal absorption and accumulation. There is some observation that speciation of heavy metals, not only their total concentration, is important for estimating the degree of soil contamination and the phytotoxic risk (Elekes *et al.*, 2010).

For the remediation purpose, some extraction methods were developed to evaluate concentrations of heavy metals in soils (the pool of their available forms), using acids, chelating agents, buffered salt solutions and unbuffered salt solutions (Onder *et al.*, 2007). Chelating agents (EDTA, DTPA, TEA) extracting labile forms of metals enhance their availability to plants and thus significantly increase their uptake, decreasing the period needed for remediation (Dipu *et al.*, 2012). Its effectiveness depends on the genetically determined ability of a plant to uptake heavy metals by root and translocate them to aboveground parts.

Therefore, efforts are being made to find the plants that are fast-growing and with high biomass, which are good metal hyperaccumulators. The interest in woody plant species is increasing due to their ability to grow on nutrient-poor and contaminated soil with high biomass productivity, deep root system, fast rate of growth, and economic value (Kacar and Inal, 2008).

Some studies indicate that the application of synthetic chelating agents can lead to an excessive increase in the concentration of available metals in soil, which are toxic to the plant (Sadighara *et al.*, 2012). Further, it induces a reduction in the biomass of the plant and subsequently the total amount of the metal removed (Leštana *et al.*, 2008). Also, their high concentrations can be toxic to plants. Therefore, the chemical enhancement of phytoextraction of heavy metals from contaminated soils by these agents must be carried out carefully.

Generally, the results of our study (Table 4) showed that the total mean concentrations of investigated metals were far higher in the soil samples than in plant samples. It was also shown that the leaves of the investigated species contained higher amounts of tested metals than their stems (stem of *P. nigra* contained more Cu and Ca than leaf, and stem of *F. ornus* more Cr and Cd than leaf). The highest content of Fe, Cu, Cr, Mg and Pb was found in the leaf of *S. caprea*, Zn and Ca in the leaf of *S. alba*, Mn, and Cd in the leaf of *P. nigra*, while the highest content of Ni was shown in the leaf of *F. ornus*.

Statistically significant higher concentrations of Mn, Fe, Cr and Pb were detected in stems of *S. alba* compared with *P. nigra*, *F. ornus* and *S. caprea* ($p < 0.001$). However, the highest concentrations of Cu, Ca and Mg were detected in stem of *P. nigra* compared to *F. ornus*, *S. alba* and *S. caprea* ($p < 0.001$).

Statistically significant higher concentrations of Fe, Cu, Cr were detected in the leaves of *S. caprea* in comparison to *P. nigra*, *F. ornus* and *S. alba* ($p < 0.001$), as well as higher concentrations of Mg and Pb ($p < 0.001$, $p < 0.001$, $p < 0.01$). Higher concentrations of Zn and Ca were detected in *S. alba* compared to *P. nigra*, *F. ornus* and *S. caprea* ($p < 0.001$). Leaves of *P. nigra* had the highest concentrations of Mn and Cd in comparison to *F. ornus*, *S. alba* and *S. caprea* ($p < 0.001$).

Table 4. The chemical element concentrations (mg kg^{-1} d. m.) in leaves and stems of *P. nigra*, *F. ornus*, *S. alba* and *S. caprea* (the mean value ± standard deviation)

Metal / Plant species	Stems			
	P. nigra	*F. ornus*	*S. alba*	*S. caprea*
Mn	40.86±0.48 a*** c***	9.44±0.04	49.58±0.73 d*** b*** f***	17.16±0.38 e***
Ni	2.25±0.05 b***	4.23±0.05 a*** d***	1.25±0.03	2.60±0.17 c*** f***
Fe	151.80±1.02 c***	171.10±2.67 a*** e***	176.38±0.66 b*** d*** f***	88.20±0.69
Cu	20.36±0.36 a*** b*** c***	1.61±0.03	9.06±0.03 d***	13.40±0.37 e*** f***
Zn	152.82±1.76 a*** c***	22.32±0.41	175.32±0.91 b*** d*** f***	64.76±0.77 e***
Cr	1.55±0.03	2.24±0.04 a***	5.31±0.03 b*** d*** f***	2.60±0.04 c*** e***
Ca	14673.80±161.53 a*** b*** c***	4012.58±26.92	11363.10±148.35 d*** f***	7403.44±46.00 e***
Mg	1354.22±28.16 a*** b*** c***	728.76±6.45	975.66±6.38 d***	1149.66±35.46 e*** f***
Pb	1.07±0.02	3.27±0.04 a*** e**	11.44±0.43 b*** d*** f***	2.74±0.03 c***
Cd	5.13±0.04 a*** c***	0.61±0.01	5.34±0.03 b*** d***	2.72±0.03 e*** f***
Metal / Plant species	Leaves			
	P. nigra	*F. ornus*	*S. alba*	*S. caprea*
Mn	173.80±1.77 a*** b*** c***	35.82±0.30	115.50±0.90 d*** f***	34.48±0.52 e^{ns}
Ni	7.11±0.10	10.52±0.23 a*** d^{ns} e***	10.48±0.33 b*** f***	9.46±0.38 c***
Fe	713.38±2.99 b*** c***	291.02±3.29	384.00±3.76 d***	1760.46±36.19 c*** e*** f***
Cu	12.48±0.37 b*** c***	7.82±0.02	10.42±0.33 d***	20.98±0.37 c*** e*** f***
Zn	153.98±2.42 a***	43.88±0.31	215.80±0.94 b*** d*** f***	157.58±2.52 c** e***
Cr	4.18±0.04 a***	1.76±0.04	23.74±0.21 b*** d***	26.58±0.68 c*** e*** f***
Ca	1195.80±20.82	15265.08±101.68 a*** e***	19698.98±37.41 b*** d*** f***	14782.04±98.54 c***
Mg	1544.82±36.10	1897.78±55.78 a***	2578.80±49.55 b*** d***	2702.18±63.81 c*** e*** f*
Pb	17.42±0.36 a***	4.78±0.05	32.36±0.43 b*** d***	37.84±0.42 c*** e*** f*
Cd	10.46±0.38 a*** b*** c***	0.54±0.02	6.79±0.04 d*** f***	4.07±0.04 e***

ANOVA (Fisher's LSD post-hoc test): data represent the means ± SD (n=5); (a) *P. nigra* – *F. ornus*; (b) *P. nigra* – *S. alba*; (c) *P. nigra* – *S. caprea*; (d) *F. ornus* – *S. alba*; (e) *F. ornus* – *S. caprea*; (f) *S. alba* – *S. caprea*); *p<0.05, ***p<0.001, ns=not significant

Literature data indicate that the normal Ni, Pb, and Cu levels in plant tissues are in the range of 0.1-5 mg kg⁻¹, 1-5 mg kg⁻¹, and 3-15 mg kg⁻¹, respectively (Allaway, 1968; Kloke *et al.*, 1984; Markert, 1994; Kastori *et al.*, 1997; Reimann *et al.*, 2001; Djingova *et al.*, 2004; Kabata-Pendias and Pendias, 2001). Zn and Cd levels reported are 50 mg kg⁻¹ and 0.1 mg kg⁻¹, respectively (Wisłocka *et al.*, 2006). According to Olivares *et al.* (2013), the normal range values were reported for Mn and Cr contents in plants, the mean Mn contents in leaves were in the normal range (15-100 mg kg⁻¹), while the mean Cr contents in leaves were generally higher than the normal range (0.2-1.0 mg kg⁻¹). The normal Ni, Pb, and Cu levels reported for plant tissues are in the range of 0.1-5.0 mg kg⁻¹, 1.0-5.0 mgkg⁻¹, and 3.0-15 mg kg⁻¹, respectively (Allen, 1989; Kabata-Pendias and Dudka, 1991; Ross, 1994; Cunningham and Ow, 1996; Wisłocka *et al.*, 2006; Unterbrunner *et al.*, 2007; Olivares *et al.*, 2013; Tőzsér *et al.*, 2017). The results of our study showed that the contents of Cd, Cr and Zn in the leaves and stems of all examined species were higher than the normal values for plant tissues (except Zn for the species *F. ornus*) reported in the literature (Markert and Lieth, 1990; Alloway, 1995; Zayed and Terry, 2003; Chen *et al.*, 2009; Wuana and Okieimen, 2011; Kacálková *et al.*, 2014; Mleczek *et al.*, 2017). Also, the Ni content in the leaves of all species was higher than the values reported above, as well as the content of Pb in the leaves of *S. caprea* and *P. nigra*, and in the leaves and stems of *S. alba*. The Mn content was higher than the normal values in the leaves of *P. nigra*, as well as the Cu content in the stem of *P. nigra* and the leaves of *S. caprea*.

The best bioaccumulation of many metals was shown by the leaves of *S. caprea* (Fe, Cu, Cr, Mg, Pb). The highest content of Mn and Cd was found in the leaves of *P. nigra*, Zn and Ca in the leaves of *S. alba*, and Ni in the leaves of *F. ornus*. The results obtained are in accordance with the research of Salt and Rauser (1995) who studied the content of Cd, Cr, Cu, Ni, and some other metals in leaves of willows and poplars. Their results also confirm higher heavy metal concentrations in leaves of willow than in poplar. However, the metal concentrations that we found exceeded the average values for plants given by some authors (Baker, 1981; Yang *et al.*, 1996; Codex Alimentarius, 2001; Adriano, 2003; Rosselli *et al.*, 2003; Cui *et al.*, 2004; Sharma and Dubey, 2005; Saraswat and Rai, 2009; Malik *et al.*, 2010; Zacchini *et al.*, 2011).On the other hand, our results showed different plant sensitivity to excessive metal levels even within the same genus or plant species, which is in accordance with a similar statement by Zhuang *et al.* (2009a). Also, the results confirmed the characteristic high metal accumulation patterns of *S. caprea* (Baum *et al.*, 2006).

Mn as an essential trace element is important for plant development and growth, but in excessive amounts, it can be toxic to them (Clemens *et al.*, 2002). According to Mleczek *et al.* (2009) phytotoxicity is present when tissue Mn concentrations exceed 300 mg kg⁻¹, compared to 30–300 mg Mn kg⁻¹ in the dry matter, which represents normal values. The results obtained in our study showed that in all investigated species the Mn content was at normal values (except for the stem of the *F. ornus* species) and that its content had higher values in leaves compared to steams. This indicates active transport of Mn, as an essential nutrient, to photosynthetic tissues, which agrees with the opinion of Ernst (1996).

Plant Fe absorption from soil depends on its pH and redox potential, the concentration of macro-nutrients, as well as the proportion of other heavy metals present (Stoltz and Greger, 2002; Dos Santos Utmazian *et al.*, 2007). Although oversized plant accumulation of Fe (that is 50–100 mg kg⁻¹ of dry matter of leaf tissue for most plants) occurs without any harmful effects to them, the so-called critical concentration is 50 mg kg⁻¹ of dry matter (Burd *et al.*, 2000). Concerning that, the total content of Fe in all woody plant species, in this study, was higher than the critical concentration and ranged from 88.20 mg kg⁻¹ in the steam of *S. caprea* up to 1,760.46 mg kg⁻¹ in the leaves of the same species.

The concentration of Cu in plants, an essential element and one of the most mobile, correlates with its concentration in the soil. There are some different values concerning its normal concentration in plant material, such as 2.5-25 mg kg⁻¹, and 8 mg kg⁻¹ in *Salix* species with leaves growing in unpolluted conditions (Khan *et al.*, 2000). According to Chao *et al.* (2007), a phytotoxic level of this metal is 30 mg kg⁻¹, while in polluted plants it is in the range of 20-100 mg kg⁻¹, and the toxic concentrations in the soil are in the range of 60-125 mg kg⁻¹. The obtained results of our study showed that the Cu concentrations varied from1.61 mg kg⁻¹ (steam of species *F. ornus*) to 20.98 mg kg⁻¹ (leaves of *S. caprea*) among investigated plant species. All plant

samples had low contents of Cu, with concentrations close to the normal ones: 20.36 mg kg^{-1} (steam of *P. nigra*) and 20.98 mg kg^{-1} (leaves of *S. caprea*).

Zinc is one of the highest mobile metals in plants and some studies reported its higher accumulation in the leaves than in the steam of the investigated willow species (Chao *et al.*, 2007), suggesting transpiration flow as a driving force for gradual transportation of Cd and Zn from the roots to the leaves during the growing season. According to a report by Borišev *et al.* (2009), concentrations exceeding 150 mg kg^{-1} for Zn could be considered as phytotoxic. The Zn content in our studied species ranged from 22.32 mg kg^{-1} in the stem of *F. ornus* to 215.8 mg kg^{-1} in the leaves of *S. alba*. The obtained results showed that the contents of Zn in the leaves and stems of all examined species (except for the species *F. ornus*) were higher than the normal values for plant tissues reported in the literature (Borišev *et al.*, 2009; Hernandez-Allica *et al.*, 2007; Santos *et al.*, 2006).

Lead is highly insoluble and therefore it is poorly accumulated by plants. Also, it is less mobile than Cd and Zn. A significantly higher accumulation rate of Pb was found in the roots and twigs than in the stems and leaves of willow species (Borišev *et al.*, 2009). This could be explained by restricted translocation of toxic metals between roots and stems and between twigs and leaves, which is necessary for avoidance of damages in photosynthetic processes. According to Borišev *et al.* (2009); Hernandez-Allica *et al.* (2007) and Meers *et al.* (2005), a much lower value than 3 mg kg^{-1} can be considered as a normal one for plants, while Mleczek *et al.* (2017) suggested the value lower than 10 mg kg^{-1}. The average value of the Element Concentration Cadasters in Ecosystems (Markert and Lieth, 1990) is 0.1–5 mg kg^{-1} (EU Directive 86/278/EEC, 1986). Ross (1994) gave the opinion that the concentrations in polluted plants are in the range of 30-300 mg kg^{-1}, while the toxic concentrations in the soil are in the range of 100-400 mg kg^{-1}, where the level of 43 mg kg^{-1} is the threshold followed by the tree death. The results of our study showed that the Pb content in the examined species ranged from 1.07 mg kg^{-1} in the stem of *P. nigra* to 37.84 mg kg^{-1} in the leaves of *S. caprea*. These values revealed that the concentration of Pb in the leaves of *P. nigra* and *S. caprea*, as well as in the stem and leaves of *S. alba* exceeded normal concentrations.

Among plant species, there is a difference in cadmium intake and accumulation. According to some data, its concentrations in plants are in the range of 0.006-18 mg kg^{-1} (Zayed and Terry, 2003), and concentration of 100 mg kg^{-1} is toxic to higher plants (Kacálková *et al.*, 2014). The results of our study showed a higher content of Cr in the leaves of *S. caprea* and *S. alba* (26.58 and 23.77 mg Cr kg^{-1}, respectively).

Factors that influence plants′ uptake of Ni are the concentration of Ni^{2+} in soil solution, plant metabolism, the acidity of the soil, the presence of other metals, and organic matter composition (Kabata-Pendias and Pendias, 2001). Ni values in plant tissues vary between 0.5-5 mg kg^{-1} of dry weight (Djingova *et al.*, 2004). In the leaves of all studied plants (*P. nigra, F. ornus, S. alba* and *S. caprea*) concentrations of Ni were: 7.11 mg Ni kg^{-1}, 10.52 mg Ni kg^{-1}, 10.48 mg Ni kg^{-1} and 9.46 mg Ni kg^{-1}, respectively. These contents were found to be higher than those in the data reported by Kabata-Pendias and Pendias (2001).

Cd is the most abundant heavy metal that plants easily absorb, accumulate (Wuana and Okieimen, 2011) and translocate from root to aboveground parts (Salt and Rauser 1995), depending on its availability in soil, pH, temperature, redox potential, and concentration of other elements. The Cd level of 2 mg kg^{-1} in plants is considered to be normal, while the range of 3-8 mg kg^{-1} to be toxic (Codex Alimentarius, 2001). The results of our study show that the Cd contents in the leaves of *P. nigra* (10.46 mg Cd kg^{-1}) were above the limit of toxicity for plants, while the other studied species contained Cd in normal values. Otherwise, some authors reported a higher Cd content in roots and leaves of poplar than in willow species (Zacchini *et al.*, 2011), where it is placed in the vacuoles of aboveground tissues, to minimize its toxic effect (Yang *et al.*, 1996).

The bioaccumulation factor (BCF) as a measure of plant ability to tolerate and accumulate heavy metals was calculated for assessment of the metal accumulation and translocation efficiency in the plants (Table 5). In this calculation, the metal concentration in the aboveground biomass and its concentration in the soil (Malik *et al.*, 2010) is considered and it represents the ratio of total metal concentration in plant stem (BCF$_{stem}$) and leaves (BCF$_{leaf}$) to the one in the soil (Saraswat and Rai, 2009). 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 shoots (stem and leaf) with BCF >1 are considered the species with phytoextraction potential (Zacchini *et al.*, 2009).

Table 5. Minimal and maximal values of BCF among investigated woody species

Metal	Min value / species	Max value / species
Mn	0.0137 (stem of *F. ornus*)	99.0878 (leaf of *P. nigra*)
Fe	0.0013 (stem of *S. caprea*)	24.8582 (leaf of *S. caprea*)
Cu	0.0056 (stem of *F. ornus*)	8.5493 (leaf of S. caprea)
Zn	0.1159 (stem of *F. ornus*)	14.1046 (leaf of *S. alba*)
Cr	0 (stems and leaves of all species)	0.2108 (leaf of *S. caprea*)
Ca	0.0309 (leaf of *P. nigra*)	2.1119 (leaf of *S. alba*)
Mg	0.0692 (stem of *F. ornus*)	34.7413 (leaf of *S. caprea*)
Pb	0.0012 (stem of *P. nigra*)	0.4402 (leaf of *S. caprea*)
Cd	0.0626 (leaf of *F. ornus*)	16.2422 (leaf of *P. nigra*)

BCF values (Table 6) for *P. nigra* species ranged from 0 (stem and leaf, for bioavailable Cr at both tested soil depths) to 99.0878 (leaf, for bioavailable Mn at 20 cm depth). The results for stem and leaf of *P. nigra* species for the tested metals were higher for bioavailable contents of these metals than the total ones. Also, the leaf showed better accumulation of the tested metals than the stem of this species (except for Cu and Ca) from a soil depth of 20 cm compared to the one of 10 cm.

Our results showed that a stem and a leaf of *P. nigra* had BCF > 1 if they adopted Zn from a soil depth of 20 cm, a stem of the same species if it adopted Ca from a depth of 20 cm and a leaf of this species when it adopted Cd from both examined depths.

BCF > 1 was determined for the leaf of the species *F. ornus*, if it absorbed Ca from a depth of 20 cm. Both tested species of the genus Salix showed BCF > 1 for Zn and Ca if the leaves uptake these metals from a depth of 20 cm. Also, a stem of *S. alba* from a depth of 20 cm and leaves from a depth of 10 cm had BCF > 1 for Zn. All these facts indicate the intensive phytoextraction ability of these plants in the accumulation of mentioned metals.

The obtained results showed that the potential application of DTPA would increase the bioavailability of the tested metals, and because of that, the values of BCF factors calculated for bioavailable metal concentrations differed significantly from the values that should be their total values in the soil. Using DTPA, the stem and leaf of *P. nigra* showed BCF> 1 for almost all tested metals (except Cr, Ca and Pb) from a soil depth of 20 cm. Also, based on the values of BCF factors, we can conclude that this species absorbs metals better if the soil is treated with DTPA from a depth of 20 cm compared to a depth of 10 cm.

The stem and leaf of *P. nigra* species showed a medium accumulative potential (BCF = 1-0.1) for most of the examined metals. It depended on the specific metal, the plant organ, whether the soil was treated with DTPA and the depth from which the metals were adopted. Also, a weak accumulation capacity (BCF= 0.1-0.01) of the stem and leaf of *P. nigra* species was shown during the accumulation of Mn, Ni, Cu and Cr (exception - Mn for leaf and Fe for the stem) and for the leaf of this species in the accumulation of Ca and Pb if they adopted these metals from a depth of 10 cm. The weak accumulation ability of the leaf of this species was also determined for Fe, Cu and Cr (Cr for the stem, too) if they absorbed these metals from a depth of 20 cm. The results showed that the stem does not have the ability (BCF = 0.01-0.001) to accumulate Fe, Pb and Cr if DTPA is applied in the soil treatment.

Table 6. BCF for stem (BCF_stem) and leaf (BCF_leaf) of species *Populus nigra*

Metal	*Populus nigra*							
	BCFstem 10 cm	BCFstem 20 cm	BCFstem 10 cm	BCFstem 20 cm	BCFleaf 10 cm	BCFleaf 20 cm	BCFleaf 10 cm	BCFleaf 20 cm
	Total	Total	Available	Available	Total	Total	Available	Available
Mn	0.0594	0.1869	0.6077	23.2953	0.2525	0.7950	2.5848	99.0878
Ni	0.0282	0.1462	0.5991	7.0250	0.0891	0.4624	1.8955	22.2250
Fe	0.0023	0.0031	0.4063	2.1435	0.0108	0.0144	1.9094	10.0731
Cu	0.0708	0.1238	5.4351	8.2967	0.0434	0.0759	3.3316	5.0856
Zn	0.7936	1.1487	0.9099	9.9882	0.7996	1.1574	0.9168	10.0641
Cr	0.0123	0.0118	0	0	0.0332	0.0320	0	0
Ca	0.3791	1.2781	1.1164	1.5732	0.0309	0.1042	0.0910	0.1282
Mg	0.1285	0.3322	10.1653	17.4109	0.1466	0.3789	11.5960	19.8614
Pb	0.0012	0.0021	0.0089	0.0124	0.0202	0.0341	0.1446	0.2027
Cd	0.6042	0.5950	3.1589	7.9658	1.2320	1.2132	6.4409	16.2422

The BCF values obtained for *F. ornus* species (Table 7) showed that they ranged from 0 (stem and leaf for bioavailable Cr at both tested soil depths) to 32.875 (leaf for bioavailable Ni at 20 cm depth). These values for bioavailable metal contents in the stem and leaf were higher than the total ones for tested metals. Also, the leaf showed a better accumulation of tested metals than the stem of this species (except Cr and Cd) from a soil depth of 20 cm compared to a 10 cm one.

BCF values obtained indicate that the application of DTPA increased bioavailability of the tested metals and more intensive accumulation of almost all tested metals (except Pb) in the leaf than in the stem of this species at a soil depth of 20 cm. Metal accumulation depended on metal type, plant organ, depth from which the element was adopted and whether it is a total or a bioavailable (value obtained by applying the DTPA) metal concentration.

Also, the results showed that the stem and leaf of *F. ornus* species do not have the ability to accumulate (BCF = 0.01-0.001) Fe and Pb (and Cu for the stem) and that the application of DTPA would increase the bioavailability of these metals to the level of weak, medium, and for some metals intensive accumulation (stem and leaf for Fe, leaf for Cu).

Table 7. BCF for stem (BCF_stem) and leaf (BCF_leaf) of species *Fraxinus ornus*

Metal	*Fraxinus ornus*							
	BCFstem 10 cm	BCFstemf 20 cm	BCF stem 10 cm	BCFstem 20 cm	BCFleaf 10 cm	BCFleaf 20 cm	BCFleaf 10 cm	BCFleaf 20 cm
	Total	Total	Available	Available	Total	Total	Available	Available
Mn	0.0137	0.0432	0.1405	5.3843	0.0520	0.1638	0.5327	20.4219
Ni	0.0530	0.2750	1.1274	13.2188	0.1319	0.6840	2.8038	32.8750
Fe	0.0026	0.0034	0.4580	2.4160	0.0044	0.0059	0.7789	4.1093
Cu	0.0056	0.0098	0.4287	0.6544	0.0272	0.0476	2.0886	3.1883
Zn	0.1159	0.1678	0.1329	1.4588	0.2279	0.3298	0.2613	2.8680
Cr	0.0178	0.0171	0	0	0.0140	0.0135	0	0
Ca	0.1037	0.3495	0.3053	0.4302	0.3944	1.3296	1.1613	1.6366
Mg	0.0692	0.1787	5.4703	9.3695	0.1801	0.4655	14.2455	24.3993
Pb	0.0038	0.0064	0.0271	0.0380	0.0055	0.0094	0.0397	0.0556
Cd	0.0721	0.0710	0.3768	0.9503	0.0636	0.0626	0.3325	0.8385

Results obtained for *S. alba* species showed that BCF values (Table 8) ranged from 0 (stem and leaf for bioavailable Cr at both tested soil depths) to 65.8495 (leaf for bioavailable Mn at 20 cm depth). Values of BCF for stem and leaf of this species for the tested metals were higher for bioavailable than the total metal contents.

Also, the leaf showed better accumulation of the tested metals than the stem from a depth of 20 cm compared to a 10 cm soil depth.

Table 8. BCF for stem (BCF_stem) and leaf (BCF_leaf) of species *Salix alba*

Metal	*Salix alba*							
	BCFstem 10 cm	BCFstem 20 cm	BCF stem 10 cm	BCFstem 20 cm	BCFleaf 10 cm	BCFleaf 20 cm	BCFleaf 10 cm	BCFleaf 20 cm
	Total	Total	Available	Available	Total	Total	Available	Available
Mn	0.0720	0.2268	0.7374	28.2668	0.1678	0.5283	1.7177	65.8495
Ni	0.0156	0.0810	0.3321	3.8938	0.1314	0.6814	2.7932	32.7500
Fe	0.0027	0.0036	0.4721	2.4905	0.0058	0.0077	1.0278	5.4222
Cu	0.0315	0.0551	2.4186	3.6919	0.0362	0.0633	2.7816	4.2461
Zn	0.9105	1.3178	1.0438	11.4588	1.1207	1.6221	1.2848	14.1046
Cr	0.0421	0.0406	0	0	0.1883	0.1815	0	0
Ca	0.2936	0.9898	0.8645	1.2182	0.5089	1.7158	1.4987	2.1119
Mg	0.0926	0.2393	7.3237	12.5438	0.2448	0.6325	19.3575	33.1551
Pb	0.0133	0.0224	0.0949	0.1331	0.0376	0.0634	0.2685	0.3765
Cd	0.6285	0.6189	3.2857	8.2857	0.7998	0.7875	4.1810	10.5435

Values of BCF (Table 9) obtained for *S. caprea* species had a range from 0 (stem and leaf for bioavailable Cr at both tested soil depths) up to 34.7413 (leaf for bioavailable Mg at 20 cm depth). The results also showed that the BCF values for the stem and leaf of the *S. caprea* species for the tested metals were higher for bioavailable than the total metal contents. Further, the leaf showed better accumulation of the tested metals than the stem from a depth of 20 cm compared to a 10 cm depth.

Table 9. BCF for stem (BCF_stem) and leaf (BCF_leaf) of species *Salix caprea*

Metal	*Salix caprea*							
	BCFstem 10 cm	BCFstemf 20 cm	BCF stem 10 cm	BCFstem 20 cm	BCFleaf 10 cm	BCFleaf 20 cm	BCFleaf 10 cm	BCFleaf 20 cm
	Total	Total	Available	Available	Total	Total	Available	Available
Mn	0.0249	0.0785	0.2552	9.7834	0.0501	0.1577	0.5128	19.6579
Ni	0.0326	0.1692	0.6935	8.1313	0.1186	0.6151	2.5213	29.5625
Fe	0.0013	0.0018	0.2361	1.2454	0.0267	0.0355	4.7119	24.8582
Cu	0.0466	0.0814	3.5771	5.4605	0.0730	0.1275	5.6006	8.5493
Zn	0.3363	0.4868	0.3856	4.2327	0.8183	1.1845	0.9382	10.2993
Cr	0.0206	0.0199	0	0	0.2108	0.2032	0	0
Ca	0.1913	0.6449	0.5632	0.7937	0.3819	1.2876	1.1246	1.5848
Mg	0.1091	0.2820	8.6298	14.7809	0.2565	0.6628	20.2836	34.7413
Pb	0.0032	0.0054	0.0228	0.0319	0.0439	0.0741	0.3140	0.4402
Cd	0.3201	0.3152	1.6736	4.2205	0.4796	0.4723	2.5074	6.3230

Based on the values of BCF, that is BCF> 1, for both studied species of the genus Salix, it was shown that Fe was intensively accumulated in their leaves from both examined depths and Ca from a depth of 20 cm of the soil. Also, the application of DTPA would increase the ability of the leaves of these plants to absorb almost all tested metals (except Pb), primarily from a greater depth of soil (20 cm). This low BCF for Pb could be due to its low translocation within plants, being a toxic and non-essential element.

The results obtained (Table 10) indicated that the leaves of both studied species of the genus Salix showed a better accumulation of all tested metals in comparison to the stem (*S. caprea* species - about 20 times more Fe than the stem). Also, the stem of the *F. ornus* accumulated more Cr and Cd, and the stem of *P. nigra* more Cu and Ca than the leaves of the species mentioned.

Table 10. Ratios of the investigated metals content (leaf/stem) of species *P. nigra* and *F. ornus*, *S. alba* and *S. caprea*

Metal	*P. nigra*	*F. ornus*	*S. alba*	*S. caprea*
	Leaf/Stem	Leaf/Stem	Leaf/Stem	Leaf/Stem
Mn	4.25	3.79	2.33	2.01
Ni	3.16	2.49	8.41	3.64
Fe	4.70	1.70	2.18	19.96
Cu	0.61	4.87	1.15	1.57
Zn	1.01	1.97	1.23	2.43
Cr	2.70	0.79	4.47	10.23
Ca	0.08	3.80	1.73	2.00
Mg	1.14	2.60	2.64	2.35
Pb	16.31	1.46	2.83	13.80
Cd	2.04	0.88	1.27	1.50

Many studies recorded a significant difference between the BCF values and the transfer factor among a variety of investigated genera and species (Cui *et al.*, 2004; Zhuang *et al.*, 2009b; Yan *et al.*, 2020). Generally, these values depend on a plant organ, type of metal ion and its concentration in the soil, as well as the depth from which the investigated metal is uptaken. A translocation of absorbed metal ions through a plant from the root to aboveground organs occurs by cell sap in the xylem, through membrane pump or channel (Baum *et al.*, 2006; Clemens *et al.*, 2002) depending on a metal form and concentration, accumulation ability of a storage organ as well as interactions between metals (Mleczek *et al.*, 2009).

There are some reports concerning different uptake and translocation ratios in field plants comparing to those in hydroponics, such as a case with *Salix* species that has significantly higher metal translocation ratio *in situ*, than in hydroponics. The cause of this may be the activity in a root zone in field conditions, such as root-bacteria and/or root-mycorrhizae, as well as interactions between root and soil particles that influence mentioned processes (Cui *et al.*, 2004; Burd *et al.*, 2000). Some researchers indicate a benefit of the proper chemical agent application that increases phytoavailability and uptake of metals, together with a reduction of their phytotoxicity (Salt and Rauser, 1995; Adriano, 2003). However, this application is associated with several negative consequences such as their toxicity to plants and soil microorganisms, formation of high stability complexes with other heavy metals in soil, high solubility and increasing of environmental pollution (Elekes *et al.*, 2010; Mleczek *et al.*, 2017). The problem concerning an insoluble form of metal in the soil, plants solve through the acidification of the rhizosphere by the action of plasma membrane proton pumps and secretion of ligands for metal chelation. Our results confirmed high levels of accumulated heavy metals (Fe, Cu, Zn, Mn, Cr and Pb) – mainly in species of genus *Salix*, which appear as good metal accumulators from contaminated areas. This agrees with data introduced by other authors showing that species of the genus Salix appear to be good heavy metal accumulators from polluted soil (Košnář *et al.*, 2020; Wani *et al.*, 2020; Nedjimi, 2021). Some tree species possess appropriate characteristics for good phytoremediation efficiency and represent a good choice for phytoextraction of heavy metals from a contaminated area.

The obtained results show the different abilities of the investigated species in phytoextraction of Zn, Ca, Fe and Cd from the tailings of lead, zinc, and copper ore mines, depending on species and organ. Thereby, species *S. alba* is an efficient bioaccumulator of Mn, Fe, Cr, Pb, Zn and Ca, while *S. caprea* of Fe, Cu, Cr, Mg and Pb. Also, it was found that species *P. nigra* efficiently accumulates Mn and Cd. That opens the possibility of further research in applying various plant species and chemical agents for increasing of bioavailability of heavy metals and consequently phytoremediation efficiency.

Conclusions

The concentrations of some investigated elements in the tailings of lead, zinc, and copper ore mine were higher than those prescribed in the regulations of the Republic of Serbia. Pb and Cu in the soil at a depth of 10 cm were also above the remediation values. The same applies to the Directive of the European Union: Cd, Cr, Pb and Cu in both of the examined depths of the soil were above the limit value, as was Ni in the soil at a depth of 10 cm. These data indicate the need for revitalization and remediation of this soil. Both the total and available concentrations of Mn, Ni, Fe, Cu, Zn, Ca, Mg and Pb, as well as available concentrations of Cd at a depth of 10 cm, were statistically higher than at a depth of 20 cm. The contents of Zn, Pb and Cr in the examined species were higher than normal values, while the content of Fe was at the critical level, and the content of Cd only in the leaves of *P. nigra,* exceeded the toxicity limit for plant tissues. The results related to BCF values showed that the species *F. ornus* has the ability of Ca phytoextraction, *P. nigra* of Zn, Ca and Cd, and the species of the genus Salix of Zn and Ca. It was found that species *S. alba* is an efficient bioaccumulator of Mn, Fe, Cr, Pb, Zn and Ca, *S. caprea* of Fe, Cu, Cr, Mg and Pb, and species *P. nigra* of Mn and Cd. The statistical analysis revealed that the bioaccumulation and translocation of the investigated elements depend on plant species and organ, and the selection of adequate plant species for remediation should be related to these and chemical composition of a polluted soil.

Authors' Contributions

Conceptualization: SRB; Investigation: DLB, RMG, SRB; Methodology: ZBS, SRB; Writing - original draft: RMG, SRB; Writing - review and editing: DLB, LSBR, JDM, ZBS, RMG, FJG, SRB; Statistic analysis: FJG.

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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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