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Bioaccumulation of heavy metals in common reed (*Phragmites australis*) growing spontaneously on highly contaminated mine tailing ponds in Serbia and potential use of this species in phytoremediation

Milijana Prica[1], Gordana Andrejić[2], Jasmina Šinžar-Sekulić[1], Tamara Rakić[1]* and Željko Dželetović[2]

1 Department of Plant Ecology and Phytogeography, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

2 Institute for the Application of Nuclear Energy, University of Belgrade, Banatska, 31b, 11080 Zemun, Serbia

ABSTRACT: Heavy metal contamination of aquatic ecosystems directly threatens the health, production and biodiversity of aquatic and surrounding terrestrial ecosystems, and it represents a serious global problem. Metal extraction during ore processing produces large amounts of wastes that remain in tailings at the mining site. Fine waste particles represent a long-term source of potentially toxic metals that can be released into the ground and surface water as a result of their progressive chemical weathering. Aquatic macrophythes have a major role in absorption and accumulation of heavy metals and thereby in natural water purification. The presence of naturally growing plants on mine tailing ponds indicates their tolerance of heavy metal pollution and suggests a possible role for them in phytoremediation. In the present study, we analysed the concentrations of heavy metals (Fe, Mn, Ni, Zn, Pb, Cd, Co, Cu) in *Phragmites australis* plants growing spontaneously in shallow water of several mine tailing ponds. The aims of the study were to define chemical properties of the mine spoils, determine the concentrations of heavy metals in different plant organs and assess the phytoremediation potential of common reed. The investigated sediments were notably rich in both total and available forms of Fe, Pb, Zn and Cu, with their upper concentrations close to phytotoxic levels. The greatest amounts of almost all of the investigated metals in plants from all three mine tailing ponds were found in the roots, with their concentrations positively correlated with the amounts of their available forms in the corresponding sediment. The far higher metal concentrations in the roots in comparison with other plant organs clearly indicate that the metals were strongly sequestrated within root cortical tissues and were not transferred across the endodermis. Taken altogether, the presence of the greatest amounts of metals in roots, high bioaccumulation factor and low translocation factor show that *P. australis* is an excluder plant species with a good phytostabilisation potential. As such, it might be efficiently used in rhizofiltration of wastewaters.

Key words: toxic metals, common reed, phytostabilisation, rhizofiltration

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*correspondence: tamaraz@bio.bg.ac.rs

INTRODUCTION

Advanced industrialisation has led to the vast release of anthropogenic contaminants such as hydrocarbons, pesticides and heavy metals into the environment (Kamran *et al.* 2014). Heavy metals cannot be degraded by any biological and chemical process, and for this reason they tend to accumulate in water, soil, sediments and living organisms over time (Ali *et al.* 2013; Sun *et al.* 2013). The problem of heavy metal pollution is not solely restricted to environments with high metal content, but is also present in those with lower levels of contamination (Ali *et al.* 2004). Today, the contamination of aquatic and terrestrial environments with heavy metals represents a global problem that threatens aquatic ecosystems, agriculture and human health (Eid *et al.* 2012). Heavy metal contamination may originate from both natural processes (weathering of ultramafic rocks) and anthropogenic ones (mining, electroplating, agricultural use of pesticides and fertilisers, sludge disposal, industrial discharge and atmospheric deposition) (Ali *et al.* 2013).

Metal extraction and ore processing produce large amounts of wastes which usually remain in tailings at the mining site (Hudson-Edwards *et al.* 2011). Fine waste particles are subject to progressive chemical weathering and therefore represent a long-term source of potentially toxic metals in the environment. Furthermore, due to their dispersion by wind and water erosion, they lead to pollution of waters and soils in the wider surroundings.

Diverse technological techniques have been developed to reduce concentrations of heavy metals in the environment (e.g., chemical precipitation, membrane filtration), but most of them, although effective, have proved to be expensive and not eco-friendly (Olguín & Sánchez- Galván 2012; Martín- Lara *et al.* 2014). The increasing need for remediation of contaminated sites has led to the development of cost-effective and eco-friendly biotechnologies like phytoremediation, which relies on the potential of naturally occurring plant species to extract, sequester and detoxify metal pollutants (Ali *et al.* 2013; Pandey *et al.* 2015; Rezania *et al.* 2016). Data relating to the heavy metal absorption capacity of plants are fundamental not only for phytoremediation purposes, but also for implementation of appropriate actions of ecological restoration and management, thereby rendering phytoremediation more sustainable (Tack & Vandecasteele 2008; Bonanno 2013).

Aquatic macrophytes are indispensable in the natural water purification process owing to assimilation of heavy metals by plant organs, increase in biological diversity in the rhizosphere area and promotion of a variety of chemical and biological reactions (Morari *et al.* 2015). They show significant diversity in their capacity to take up heavy metals and transfer them to aboveground organs (Baldantoni *et al.* 2009). Their ability to take up and accumulate heavy metals in plant organs depends on plant species and age, but also on environmental factors such as temperature, salinity and pH (Bonanno & Giudice 2010).

The wetland macrophyte *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae) is a helophytic perennial plant species typical of different wetland ecosystems. It is a robust and highly productive grass, with shoots up to 4 m tall and an extensive system of rhizomes and stolons involved in its vegetative propagation. *Phragmites australis* (common reed) is highly tolerant in relation to most abiotic factors, such as temperature and salinity. It also demonstrates high tolerance of heavy metal pollution and inhabits very clean to highly polluted sediments and waters (Duman *et al.* 2007; Quan *et al.* 2007). Due to its fibrous roots and their large contact areas, as well as to its production of large amounts of aboveground biomass, common reed was found to be very efficient in accumulation of heavy metals.

In our study we analysed the concentrations of several heavy metals (Fe, Mn, Ni, Zn, Pb, Cd, Co, Pb) in roots, rhizomes, stems and leaves of *P. australis* plants growing spontaneously in shallow water of mine tailing ponds highly contaminated with heavy metals. The aims of the study were: (i) to define chemical characteristics of the mine spoils: (ii) to determine the distribution of heavy metals within the plant: and (iii) to assess the phytoremediation potential of *P. australis* based on bioaccumulation and translocation factors.

MATERIALS AND METHODS

Sampling design. Sediments and plants were sampled from three different mine tailing ponds: at the "Rudnik" mine (central Serbia, 44°07′53″ N, 20°32′25″ E), the "RTB Bor" mine (eastern Serbia, 44°05′ N, 22°06′ E) and the "Lece" mine (southern Serbia, 42°54′15″ N, 21°31′26″ E) (Fig. 1). At each sampling point, 4–6 samples of *P. australis* were collected within a plot measuring 5 m×2 m. The sediment was sampled up to 30 cm in depth, which corresponds to the rhizosphere zone. Sampling was performed during the summer period in 2017.

Chemical characterisation of the sediment. Analyses of chemical properties of the mine tailings were performed on sediment sampled at a depth of 0-30 cm, which corresponds to the zone of plant roots. Samples were dried at 65°C to a constant weight, pulverized using a mortar and pestle, and sifted through a sieve with pore diameter of 0.5 mm. The values of pH of the sediment, both actual (pH_{H2O}) and exchangeable (pH_{KCl}), were determined potentiometrically with a glass electrode (Iskra MA 5730) using a mixture of sediment and deionised water or 1 M KCl (1:2.5, w/v), respectively. Total organic carbon content in the sediment was determined by potassium dichromate oxidation using Simakov's modification of the

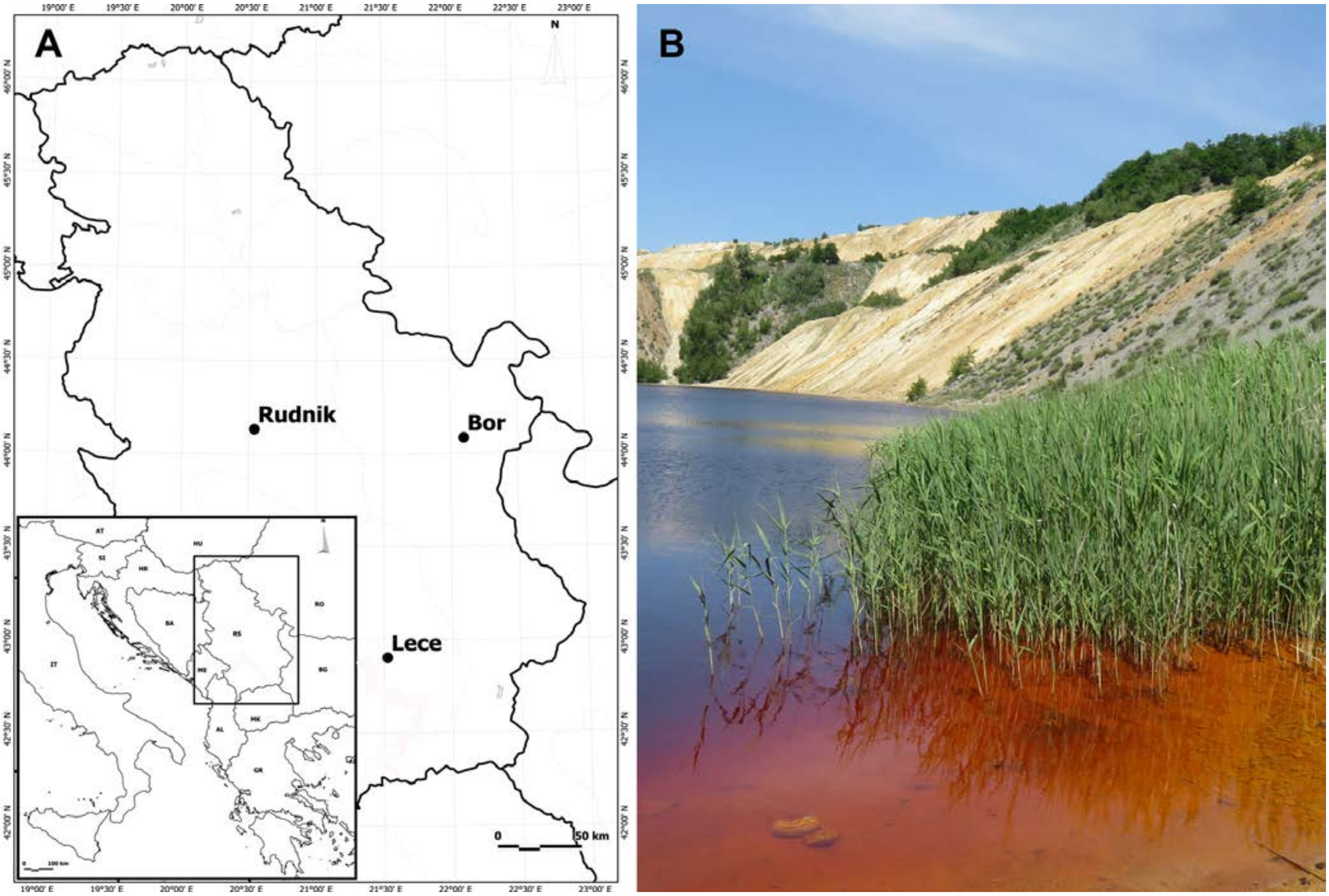

Fig. 1. Location of the study areas on the map of Serbia (A) and *Phragmites australis* on the old mine tailing pond at the "RTB Bor" site (B).

Turin method (Simakov 1957). Total nitrogen content was determined by the Kjeldahl semimicro-method. Available phosphorus (P_2O_5) and potassium (K_2O) were extracted according to the Egner-Riehm method (Egner *et al.* 1960) and determined with a colourimeter (Iskra MA 9507) and an atomic absorption spectrophotometer (Shimadzu AA - 7000), respectively. The total content of elements (Fe, Mn, Ni, Zn, Pb, Cd, Co, Cu) in the sediment was determined after digestion of sieved samples in a mixture of conc. HNO_3 and 30% H_2O_2 (3:1, v/v) according to the method of Jones & Case (1990). Their available contents were analysed according to Pansu & Gautheyroy (2007). Dried and sieved soil samples were continuously stirred for 2 h in a mixture of 1 M ammonium acetate and 0.01 M EDTA (pH 7). The absorbance of metals was detected by atomic absorption spectrophotometry (using a Shimadzu AA – 7000 instrument), and their concentrations in samples were determined by comparison of their absorption values with those of known standards.

Analysis of plant material. The plants were preliminarily separated into roots, rhizomes, stems and leaves after being thoroughly washed in tap and deionised water. Air-dried plant material was ground with a ceramic mortar and pestle and then dried at 105°C to a constant weight. The concentrations of metals (Fe, Mn, Ni, Zn, Pb, Cd, Co, Cu) in plant samples were determined by atomic absorption spectrophotometry (using a Shimadzu AA – 7000 instrument), and their absorption values were compared with those of known standards (Jones & Case 1990).

Determination of the phytoremediation potential of *P. australis*. The phytoremediation potential was estimated by calculating the bioaccumulation factor (BAF) and translocation factor (TF). Representing the ratio between the concentration of a metal in roots (C_{root}) and its available form in the substrate ($C_{subsrate}$), the BAF indicates the rate of the metal´s accumulation within plant roots. The TF represents the ratio between the concentration of a metal in leaves (C_{leaf}) and its concentration in roots (C_{root}). As such, it is an indication of the metal´s translocation from underground to aboveground organs. Both factors were calculated according to Baker (1981):

$$BAF = C_{root} / C_{substrate}$$
$$TF = C_{leaf} / C_{root}$$

Table 1. Chemical properties of the sediment on mine tailings (n = 5) (mean ± SD).

	pH_{H2O}	pH_{KCl}	C (%)	N (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)
"Rudnik"	6.91	6.64	2.21±0.14	0.006±0.0	7.59±0.05	9.57±1.16
"RTB Bor"	3.00	2.92	0.29±0.01	0.004±0.0	0.30±0.12	1.84±0.18
"Lece"	4.67	4.18	0.31±0.08	0.001±0.0	3.36±0.01	1.66±0.32

Table 2. Concentrations of heavy metals in the sediment (mg/kg) from *Phragmites australis* stands (n = 5) (mean ± SD).

	"Rudnik"			"RTB Bor"			"Lece"		
	Total	Available	a/t	Total	Available	a/t	Total	Available	a/t
Fe (%)	6.79±0.33	0.01±0.0	0.001	5.1±0.07	0.09±0.0	0.018	2.3±0.04	0.01±0.0	0.004
Pb	1949±185	812±1	0.417	31.2±0.8	LDL	-	1304±1	27.7±0.4	0.021
Zn	1432±225	194±3	0.135	75.4±1.7	10.5±0.3	0.139	842±25	121±2	0.144
Cu	761±60	45.3±0.4	0.06	232±6	37.7±0.5	0.162	78.7±1.8	4.5±0.08	0.057
Mn	1940±146	272±12	0.140	254±5	62.2±0.5	0.245	373±16	81.5±0.7	0.218
Ni	85.7±0.8	6.4±0.0	0.075	5.64±0.02	0.54±0.1	0.096	3.33±0.07	0.23±0.01	0.068
Cd	11.3±0.3	4.00±0.01	0.354	0.02±0.0	0.01±0.0	0.561	3.50±0.01	0.90±0.00	0.250
Co	11.7±0.5	1.76±0.08	0.151	8.98±0.18	1.13±0.16	0.126	10.9±0.1	0.79±0.11	0.073

note: LDL - lower than the detection limit; a/t - ratio between available and total amount of the element.

Statistical analysis. All data are expressed as the mean ± the standard deviation (M ± SD) of at least five replicates per sampling site. Statistically significant differences between sampling sites with respect to the concentrations of heavy metals were calculated using the Kruskal–Wallis test. Principal component analyses (PCA) based on correlation matrices of the concentrations of eight heavy metals (Fe, Mn, Ni, Zn, Pb, Cd, Co, Pb) in roots and rhizomes, and in stems and leaves, respectively, were done in order to explore relationships of the analysed variables, as well as to reveal groups of samples with similar patterns of metal concentrations. All statistical analyses were performed in R, ver. 3.5.1 (R Core Team 2018).

RESULTS

The sediment of the investigated mine tailings from "RTB Bor" and "Lece" had pH reactions that were ultra and extremely acidic, respectively, whereas that from the "Rudnik" mine showed a neutral pH reaction (Soil Survey Division Staff 1993). Very small amounts of total organic C, total N and available P and K were detected in the "RTB Bor" and "Lece" sediment samples (Table 1). Slightly higher concentrations of the above-mentioned elements were found in sediments from the "Rudnik" mine.

Table 2 shows the ranges of concentrations of heavy metals, both their total and available forms, in sediments from the examined mine tailing ponds. The mean concentrations of metals differed significantly between sampling points (Kruskal-Wallis test, $p = 0.02732$). The samples of sediment from the "Rudnik" mine were characterised by far greater amounts of Fe, Pb Zn, Cu Mn, Ni and Cd in comparison with those detected in sediments from the other two localities. Sediments from the "RTB Bor" tailing pond were especially rich in Fe and Cu, whereas those from the "Lece" mine were rich in Pb and Zn.

The greatest amounts of Fe, Pb, Zn, Cu, Mn and Cd in plants from each mine were detected in roots, with their concentrations positively correlated to the amounts of their available forms in the corresponding sediment (Table 3, Fig. 2). This resulted in the following descending orders of dominant metals in roots of plants from "Rudnik", "RTB Bor" and "Lece": Fe>Pb>Zn>Mn>Cu, Fe>Cu>Mn>Zn, and Fe>Zn>Pb>Mn>Cu, respectively. Besides in the roots, significant amounts of Mn were also found in other plant organs, especially in leaves.

A bioaccumulation factor (BAF) higher than 1 was detected for Fe, Zn, Cu and Cd in plants from all three mine tailing ponds (Table 4). Moreover, BAF > 1 was also detected for Mn and Ni in plants from the "Rud-

Table 3. Heavy metal concentrations (mg/kg) in different organs of *Phragmites australis* plants (n = 5) (mean ± SD).

		Fe (%)	Pb	Zn	Cu	Mn	Ni	Cd	Co
"Rudnik"	Root	1.12±0.02	675.3±15.3	442.7±12.5	298.6±2.1	365.6±0.2	39.9±0.3	6.03±0.09	22.39±0.15
	Rhizome	0.09±0.03	18.1±3.9	60.8±11	24.9±3.8	53.1±5.3	2.2±1.3	LDL	6.54±1.54
	Stem	0.04±0.01	LDL	126.6±5.9	12.6±0.15	35.8±3.9	LDL	LDL	7.76±0.97
	Leaf	0.02±0.002	5.4±5.6	37.5±2.1	11.3±1.05	189.6±7.9	LDL	LDL	9.13±0.48
"RTB Bor"	Root	1.61±0.03	LDL	48.9±2.6	329.6±44.9	191.4±13.4	4.2±0.5	0.13±0.4	LDL
	Rhizome	0.18±0.005	LDL	45.0±9.9	53.1±3.2	51.9±0.6	4.1±0.5	0.39±0	LDL
	Stem	0.02±0.001	LDL	12.4±1.0	17.2±0.96	39.3±1.8	2.7±0.1	LDL	LDL
	Leaf	0.04±0.001	LDL	10.2±0.04	22.3±0.0	112.0±1.1	1.7±0.8	LDL	LDL
"Lece"	Root	0.19±0.02	117.3±11.7	425.2±50.0	55.1±1.2	76.2±3.6	LDL	5.64±5.64	LDL
	Rhizome	0.05±0.02	17.5±2.1	186.8±8.0	23.7±0.8	81.8±5.9	4.1±0	0.54±0.09	0.99±2.0
	Stem	0.02±0.003	11.2±2.0	141.9±46.7	18.9±1.9	69.7±3.3	3.1±0.5	0.0±0	3.65±1.42
	Leaf	0.01±0.0	5.8±0.9	210.4±18.23	8.4±0.7	101.2±3.3	2.1±0.5	0.05±0.02	2.28±1.01

note: LDL - lower than the detection limit.

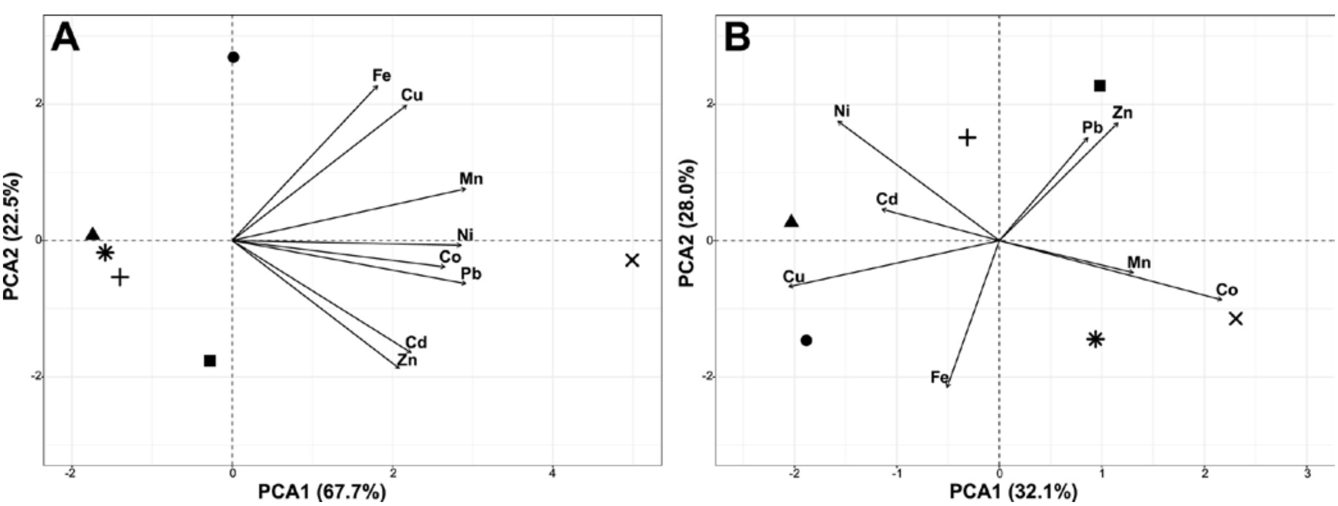

Fig. 2. Ordination plots of roots and rhizomes (A) and stems and leaves (B) of *Phragmites australis* based on concentrations of eight heavy metals (Fe, Mn, Ni, Zn, Pb, Cd, Co, Pb). (A) Roots: times sign – "Rudnik", dot – "RTB Bor", square – "Lece". Rhizomes: asterisk – "Rudnik", triangle – "RTB Bor", plus sign - "Lece". (B) Stems: asterisk – "Rudnik", triangle – "RTB Bor", plus sign – "Lece". Leaves: times sign – "Rudnik", dot – "RTB Bor", square - "Lece".

nik" mine and "RTB Bor", and for Pb in plants from the "Lece" mine. Heavy metal distribution between underground and aboveground plant organs is described by the translocation factor (TF). We found TF< 1 for all metals in plants from the "Rudnik" mine and "RTB Bor", as well as for all metals except Mn (TF = 1.33) in plants from the "Lece" mine.

It should be noted that PCA ordination of the content of elements in belowground organs revealed that PC1—which showed a significant positive correlation with Mn, Ni, Pb and Co (r > 0.75)-- discriminated between roots of *P. australis*, with positive scores for the "Rudnik" mine and "RTB Bor", and rhizomes, with all negative scores (Fig. 2A). Moreover, the marked separation

Table 4. Mean bioaccumulation factor (BAF) and translocation factor (TF) in *Phragmites australis* from mine tailing ponds.

	"Rudnik"		"RTB Bor"		"Lece"	
	BAF	TF	BAF	TF	BAF	TF
Fe	143	0.02	17.40	0.02	19.6	0.05
Pb	0.83	0.01	0.00	-	4.24	0.05
Zn	2.28	0.08	4.67	0.21	3.50	0.49
Cu	6.59	0.04	8.74	0.07	12.2	0.15
Mn	1.34	0.52	3.08	0.59	0.94	1.33
Ni	6.19	0.00	7.69	0.40	-	-
Cd	1.50	0.00	10.9	-	6.48	0.01
Co	12.7	0.41	-	-	-	-

of roots from the "Rudnik" mine, which were positively correlated with the greatest amounts of almost all of the investigated metals, should be emphasised. The PC2 axis showed discrimination between roots from "RTB Bor" in the positive part and plants from the "Lece" mine in its negative part. The projection of variables showed that belowground organs of the common reed from "RTB Bor" were positively correlated with the greatest amounts of available Fe and Cu, whereas those from the "Lece" mine were positively correlated with Zn. Regarding PCA ordination of the content of elements in aboveground organs, PC1 showed a significant positive correlation with Co ($r = 0.85$) and a negative correlation with Cu ($r = -0.80$). Moreover, it revealed a gradient from "RTB Bor", where the plants had the highest concentrations of copper in stems and leaves, to the "Rudnik" mine, with the highest concentrations of cobalt in the aboveground plant parts (Fig. 2B). The second principal component was positively correlated for the most part with Ni ($r = 0.69$) and Zn ($r = 0.68$), and significantly negatively correlated with Fe ($r = -0.84$). It most prominently discriminated plants from the "Lece" mine, which had the greatest amounts of Ni and Zn and lowest content of Fe in the aboveground organs.

DISCUSSION

Phragmites australis is a cosmopolitan species that inhabits freshwater and brackish wetlands in different climatic regions of the world. Its success is ensured by high biomass production, tolerance in relation to most abiotic stressors, efficient adaptation to new ranges and rapid expansion through clonal and sexual reproduction. Owing to its high ecological plasticity, common reed naturally grows in shallow water of highly contaminated mine tailing ponds as well. Mine tailings from three mines in Serbia-- "Rudnik", "RTB Bor" and "Lece"-- differ with respect to their chemical parameters. The main differences between them arise both from chemical properties of the processed polymetallic ores and from their chemical treatments. To be specific, the main metals extracted from ore from the "Rudnik" mine are Pb, Zn and Cu; the main metal extracted from ore from "RTB Bor" is Cu; and the main metals extracted from ore from the "Lece" mine are Cu, Pb, Zn and Au.

Heavy metal concentration in bottom sediments. The pH of sediment from the "Rudnik" mine was around neutral due to the presence of residues of the organic reagent xanthate, an organosulphur compound commonly used in mineral processing that keeps the pH reaction of the flotation process neutral. In contrast, sulphuric acid significantly decreases pH of the substrate and has a strong negative impact on the environment. Sediments from "RTB Bor" and the "Lece" mine were ultra- and extremely acidic, with pH_{H2O} 3.0 and pH_{H2O} 4.67, respectively. It is known that the pH value strongly affects the precipitation of metals and their bioavailability. The highest available/total metal concentration ratios were detected for Fe, Mn, Ni, Cd and Cu in the ultra-acidic sediments from "RTB Bor", which is in accordance with the fact that most heavy metals are more available at lower pH. As expected, all three mine tailings were extremely poorly provided with organic matter and nutrients, which makes them very unfavourable for plant growth. Although organic matter is completely absent from the mine spoils, slightly higher concentrations of organic C and available P and K were detected in flotation tailings from the "Rudnik" mine. However, they do not originate from natural organic matter, but rather from residual xanthate used in the flotation process.

Because the bottom sediments in all three mine tailing ponds are composed exclusively of mine tailings generated by ore processing, concentrations of the analysed metals mainly exceeded the amounts detected in most unpolluted soils. In all of the investigated sediments, the total and available amounts of Fe, Zn Pb and Cu were notably high, with their upper concentrations close to phytotoxic levels (Kabata-Pendias 2011). Thus, iron, one of the major constituents of the lithosphere, was present in sediments from the "Rudnik" mine (6.79%) and "RTB Bor" (5.10%) in concentrations that are considered very high. To be specific, Fe content in uncontaminated soils ranges from 0.7 to 5.5% (Nagajyoti *et al.* 2010). Zinc concentrations detected in sediments from the "Rudnik" and "Lece" mines also strikingly exceeded amounts that are typical for uncontaminated soils (2–200 mg/kg) (Nagajyoti *et al.* 2010). Due to the very poor mobility of Pb in soil and its known very low rate of translocation to organs of the aboveground part, it is quite difficult to evaluate the levels of Pb in soils that are toxic to plants. However, several authors have given similar threshold values that range from 2 to 200 mg/kg (Balks 1961; Bowen 1979; Berrow & Mitchell 1980). Thus, the amounts of Pb detected in sediments from the "Rudnik" (1949 mg/kg) and "Lece" (1304 mg/kg) mines were at phytotoxic levels. Furthermore, large amounts of Cu were found in both the "Rudnik" mine (761 mg/kg) and in "RTB Bor" (232 mg/kg), which are copper mines. The content of copper in unpolluted soils ranges from 2 to 100 mg/kg, but its concentrations in polluted soils can reach extremely high values of about 3500 mg/kg in soils contaminated by industrial sources of pollution, and of about 1500 mg/kg in soils contaminated by agricultural sources of the metal (Kabata-Pendias 2011).

Heavy metal concentration in common reed. Metal accumulation in aquatic plants depends on various factors, including the concentration of a metal and its availability in the substrate, physical and chemical properties of the water and sediments, species-specific uptake, plant growth conditions, sampling time and the process of translocation within the plant (Du Laing *et al.* 2009; Bonanno & Giudice 2010; Teuchies *et al.* 2013). In the present study, we found a significant linear correlation between available metal concentrations in sediments and roots of *P. australis* from all three mine tailings, which is in agreement with data previously reported by Bonanno & Giudice (2010).

Rooted aquatic plants absorb and often accumulate heavy metals in their roots, which play a significant role in metal immobilisation (Baldantoni *et al.* 2004; Benavides *et al.* 2005). The common reed plants from all three mine tailings accumulated the greatest amounts of Fe, Pb, Zn, Cu and Cd in their roots, significantly exceeding values in other plant organs. Only Mn was found in similar concentrations in roots and other plant organs, especially leaves.

Iron content in *P. australis* roots was extremely high, even up to 11000 and 16000 mg/kg at the "Rudnik" and "RTB Bor" localities, respectively. These exceptionally high amounts of Fe found only in roots, together with low TF values, indicate its apoplastic transport and precipitation within the root cortex, and very low translocation to shoots. The detected amount of 2000 mg/kg in leaves exceeds the threshold value of 1000 mg/kg that in sensitive plants usually causes necrotic spots and other visible leaf injuries (Kabata-Pendias 2011), which were not observed in any of the investigated common reed plants. The detected amounts of Fe were far greater than those detected in common reed growing in shallow lakes (Klink 2017) and soils affected by mining activities (Pérez-Sirvent *et al.* 2017), but still smaller than in plants growing on acidic mine drainage (Guo *et al.* 2014).

The greatest amounts of Pb were detected in roots of plants growing on Pb-rich mine tailings at the in "Rudnik" and "Lece" localities, viz., 675.3 and 117.3 mg/kg, respectively. According to Ross (1994), these two concentrations are in or above the range that is phytotoxic to most plants (30–300 mg/kg). Whereas the amounts of Pb in stems of plants from the "Rudnik" and "RTB Bor" localities were under the detection level, they were above the average content reported as toxic to plants (5 mg/kg) in stems of plants from the "Lece" mine (Markert 1992). In view of the very low mobility of Pb from roots to shoots, some other possible sources of this metal in leaves should be considered. To be specific, mine tailing ponds are always surrounded by mine tailings composed of very fine particles that are subject to wind dispersal. Consequently, some portion of Pb in leaves might derive from particle deposition on the leaf surface and their entrance into the leaf through the stomata (Kabata-Pendias 2011; Rzymski *et al.* 2014).

Zinc concentrations in roots of plants from the "Rudnik" (442.7 mg/kg) and "Lece" (425.2 mg/kg) localities were close to, but still lower than the phytotoxic range (500–1500 mg/kg) proposed by Chaney (1989). The amounts detected in roots correspond to those reported elsewhere for common reed from mine tailings (Stoltz & Greger 2002) and are greater than in plants exposed to lower Zn contamination (Bragato *et al.* 2006, Bonanno & Giudice 2010).

The amounts of Cu detected in roots of common reed from the "Rudnik" and "RTB Bor" localities (298 and 329 mg/kg, respectively) were far above phytotoxic concentrations (25–40 mg/kg; Chaney 1989), which is a consequence of their growth on the Cu-rich mine tailings at those localities. Owing to its precipitation within the root cortex and low translocation from the roots, the content of Cu in the rhizome and aboveground organs remained low and below toxic levels. The same pattern of Cu uptake and its distribution within the plant were previously reported for common reed by Ali *et al.* (2002) and Stoltz & Greger (2002).

Manganese content in common reed from all three tailing ponds was lower than the threshold concentration (500 mg/kg) that was found to negatively affect plants (Kabata-Pendias 2011). The amounts of Mn and its distribution among plant organs were very similar to those reported by Klink (2017) for plants collected from lakes in Poland, soils polluted by mining activities (Pérez-Sirvent *et al.* 2017), heavy metal-contaminated estuarine sediments (Cicero-Fernández *et al.* 2017) and rivers influenced by wastewaters from nearby cities (Bonanno & Giudice 2010), but lower than in reed from a site of acid mine drainage (Guo *et al.* 2014). The distribution of Mn within the plant differed from those of the other investigated metals as a consequence of higher translocation to aboveground plant organs, as can be seen from distinctly higher TF values compared to those of the other heavy metals. Thus, the amount of Mn in leaves in plants from the "Rudnik" and "RTB Bor" localities was cca half of the amount detected in roots, whereas in plants from the "Lece" mine Mn was almost uniformly distributed within the plant, with the greatest amounts detected in leaves.

Nickel concentrations higher than 10 mg/kg, which are the amounts that are known to be toxic to most plants (Kabata- Pendias 2011), were found only in roots of common reed from the "Rudnik" mine. The amounts of Ni in their roots (39 mg/kg) were far greater than in plants from rivers influenced by municipal wastewaters (Bonanno & Giudice 2010), constructed wetland (Bragato *et al.* 2006) and heavy metal-contaminated estuarine sediments (Cicero-Fernández *et al.* 2017). Nickel concentrations in shoots of the same plants were below the detection limit, which points-as in case of Fe and Pb-to negligible translocation of Ni to aboveground plant organs.

The detected cadmium concentrations were close to the lower limit of phytotoxicity (5–700 mg/kg) reported by Chaney (1989), which is correlated with low levels of available Cd in the mine tailings. With the exception of its amounts in roots ("Rudnik"-6.06 mg/kg, "Lece"-5.64 mg/kg), the concentrations of Cd in other plant organs correspond to those detected in plants from unpolluted environments (0.01–0.3 mg/kg) (Allen 1989).

The extremely high concentrations of heavy metals in roots of the common reed, which were found to be positively correlated with their amounts in the investigated mine tailings, indicate their apoplastic movement and accumulation within the root cortex. The conspicuously high metal concentration in roots in comparison to rhizomes, stems and leaves clearly indicates that they were strongly sequestrated within the root cortical tissues and were not transferred across the endodermis. To judge from the high BAF values detected in all of the examined plants, *P. australis* is an excluder for all of the investigated heavy metals. Translocation factors were <1, except for Mn in plants from "Lece" (TF = 1.33), and indicate heavy metal immobilisation in the plant roots.

CONCLUSIONS

The successful life and vegetative propagation of common reed on such extreme substrates as mine tailings confirm its incredible potential ability to efficiently adapt to a large variety of ecological conditions. The present study shows that accumulation of heavy metals and their concentrations in roots of common reed strongly depend on the mine tailings′ chemical properties and indicates the potential usefulness of this species in heavy metal biomonitoring in different environments. The study also showed that behaviour of the metals within the plant and their toxicity are not simply a function of their total concentrations, but are also influenced by the plant species and by mechanisms involved in sequestration and translocation of the given metals within the plant. The greatest amounts of heavy metals accumulated in plant roots, together with high BAF values and $TF<1$, clearly indicate that common reed can be efficiently used in rhizofiltration of wastewaters, and in phytostabilisation and restoration of sites contaminated by the presence of heavy metals.

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Botanica SERBICA

REZIME

Potencijalna uloga *Phragmites australis* u bioakumulaciji teških metala i fitoremedijaciji visoko zagađenih jezera flotacione i rudničke jalovine u Srbiji

Milijana Prica, Gordana Andrejić, Jasmina Šinžar- Sekulić, Tamara Rakić i Željko Dželetović

Zagađivanje akvatičnih ekosistema teškim metalima predstavlja jedan od globalnih problema. Obrada mineralnih ruda i ekstrakcija metala proizvode ogromne količine veoma finih čestica stene koje se odlažu na posebnim odlagalištima rudničke i flotacione jalovine. Ove sitne čestice jalovine predstavljaju dugotrajan izvor zagađenja okolnih prirodnih ekosistema teškim metalima zbog lakog prenošenja vetrom i vodom, kao i zbog postepene hemijske dezintegracije čestica i spiranja metala u podzemne vodotokove. Makrofite imaju izuzetno važnu ulogu u prečišćavanju prirodnih voda kroz usvajanje i akumulaciju teških metala u pojedinim biljnim organima. U ovom istraživanju su ispitivane koncentracije teških metala (Fe, Pb, Zn, Cu, Mn, Ni, Cd, Co) u sedimentu, kao i u korenu, rizomu, stablu i listovima *Phragmites australis* koja spontano raste u plitkim vodama visoko zagađenih jezera flotacione i rudničke jalovine rudnika „Rudnik", „RTB Bor" i „Lece". Ciljevi ovog istraživanja su bili da se (i) odrede hemijske odlike sedimenta/jalovine, (ii) odredi količina i distribucija metala u biljci i (iii) proceni fitoremedijacioni potencijal trske na osnovu bioakumulacionog i translokacionog faktora. U ispitivanim sedimentima su detektovane izrazito visoke koncentracije ukupnih i dostupnih Fe, Pb, Zn i Cu, čije su gornje koncentracije bile u nivou fitotoksičnih. Najveće količine gotovo svih metala u trsci su nađene u korenu biljaka, u koncentracijama koje su pozitivno korelisane sa dostupnom količinom metala u pripadajućem sedimentu. Daleko više koncentracije metala u korenu u odnosu na ostale biljne organe ukazuju na efikasnu sekvestraciju metala u korteksu korena i njihov slab transport kroz endodermis. Visoke koncentracije metala u korenu, visok bioakumulacioni i nizak translokacioni faktor (<1) jasno ukazuju da je trska ekskluder teških metala, da ima veliki fitostabilizacioni potencijal i da se može koristiti za efikasnu rizofitraciju metalima zagađenih voda i sedimenta.

Ključne reči: toksični metali, trska, fitostabilizacija, rizofiltracija