Typha latifolia resilience to high metal stress: antioxidant response in plants from mine and flotation tailing ponds

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Abstract: *Typha latifolia* (cattail) forms natural stands in the transition zone of artificial flotation and mine tailings ponds and is contaminated with extremely high concentrations of metals. We assessed the absorption capacity of the plant, metal transfer to leaves, and the effects of elevated metal concentrations on antioxidant enzyme activities. Soil acidity, the pseudo-total and available metal content of the substrate, and metal concentrations in plants were examined. The effects of elevated metal concentrations in plants on antioxidant enzyme activities (superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase) were assessed. Cattails exhibited high metal accumulation levels in roots and a low transfer rate to the leaves. The effects of metal concentrations on antioxidant enzyme activities were found to depend on the type of enzyme, metal concentrations in the plant and their molar ratios, as well as on the pH of the substrate. High activities of antioxidant enzymes indicate increased generation of reactive oxygen species (ROS) and show that metal detoxification mechanisms are insufficient to restrain their toxicity. Pronounced resistance to elevated metal concentrations and high efficiency in metal phytostabilization show that cattail could be a valuable component of biological treatment systems for removing metals from multi-metal and heavily contaminated substrates in the pH range from ultra-acidic to neutral.

Keywords: cattail; metal antagonism; metal tolerance; oxidative stress, phytoremediation

Keywords: bioconcentration factor (BCF); translocation factor (TF); acid mine drainage (AMD); superoxide dismutase (SOD); catalase (CAT); ascorbate peroxidase (APX); guaiacol peroxidase (OD); glutathione reductase (GR); reactive oxygen species (ROS)

INTRODUCTION

The ever-increasing demand for met-als in various industries requires continuous exploitation of mineral resources, resulting in millions of tons of created waste being disposed of in the immediate vicinity of the mine. Fine, metal-rich particles are continuously introduced into surrounding natural terrestrial and aquatic ecosystems by wind and water erosion. Because of the dissolution of their minerals in the presence of oxygen, water, and microorganisms, they release their metal ions into the water solution. As metals cannot be transformed by any chemical and biological processes, they continuously accumulate in the environment leading to multi-metal environmental contamination that causes long-term adverse changes in soil chemical and biological properties [1].

Chronic exposure of organisms to metal stress results in various adverse effects on the plants' biochemical and physiological processes, disrupting their growth, development, and ultimately their survival. The toxic effects of metals arise from (i) their direct interaction with proteins because of their affinity for thiol, histidyl, and carboxyl groups, leading to structural changes and catalytic and transport disorders in the cell; (ii) the replacement of essential cations at specific enzyme-binding sites leading to the breakdown of many cellular functions; (iii) elevated direct and indirect generation of reactive oxygen species (ROS) [2].

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One of the most important consequences of elevated metal concentrations and their action in the plant cell is excessive production of ROS, leading to the oxidative deterioration of different biomolecules and loss of their functions, from proteins and polyunsaturated fatty acids to nucleic acids [3]. Therefore, the highly efficient antioxidant system is crucial for maintaining redox equilibrium in metal-stressed cells. However, the enzymatic component of the antioxidant defense system is composed of different enzymes with specific roles in the removal of ROS species. It can also be a target of direct or indirect metal effects in cells. The impact of metals on the antioxidant enzyme activities often produces contrasting outcomes that can be either suppressive or activating, mainly depending on the metal species and its concentrations, but also on the plant's ability and specific actions performed to manage elevated metal concentrations.

Although many plants do not usually inhabit metal-rich soils, some have been found to be able to survive even in highly metal-polluted environments, such as *Phragmites australis*. This species has even been found in mine and flotation tailings and has therefore been suggested as valuable for the phytoremediation of metal-polluted municipal and industrial wastewater in constructed wetlands [4-6]. The adaptive mechanisms of such plants are always complex, from the molecular to the structural level, and involve various processes, including the highly efficient antioxidant system.

Cattail, *Typha latifolia* L. (Poaceae), is an herbaceous perennial plant with a cosmopolitan distribution due to its ability to tolerate a wide range of abiotic environmental factors. It grows preferentially in wetlands and transition zones of freshwater bodies. In this study, natural stands of cattail plants were found in the transition zone of artificial ponds in various mining and flotation wastes rooted directly in the substrate, indicating that the plants cope well with the unfavorable mineral composition of such metal-rich substrates. Accordingly, the main objectives of this study were to determine the metal concentrations in the *T. latifolia* below- and aboveground parts and to evaluate the effects of elevated concentrations of specific metals on antioxidant enzyme activities in roots and leaves.

MATERIALS AND METHODS

Site description and sampling

The control sediment samples and cattail plants were collected in the shallow waters of the coastal zone of the unpolluted nature reserve, Carska Bara, in northern Serbia (the control). The metal-polluted specimens were collected from the transition zone of two artificial ponds formed at the foot of the mine and flotation tailings at the Copper Mine Bor (Bor and Robule) in eastern Serbia, an acid mine drainage (AMD) generated from the overburden, eastern Serbia, and shallow water of the flotation tailings at Rudnik mine, central Serbia. The substrate and plant roots were sampled, corresponding to the rhizosphere area. Five plants were collected within a 10 m × 2 m plot from each sampling point, transported in barrels filled with water from the sampling site to the laboratory, and prepared for further analysis immediately after arrival. Sampling was performed in September 2018.

Determination of substrate pH

The substrate samples were dried at 65°C to constant weight. They were then pulverized using a mortar and pestle and sifted through a sieve (pore size diameter, 0.2 mm). Determination of soil pH was performed at a solid-liquid ratio of 1:2.5 (w/v) (ISO 10390:1994 method – soil quality determination of pH). To determine the active acidity (pH_a), 10 g of dry soil was weighed, and 25 mL of distilled water was added and stirred. After 30 min, the pH was measured directly in a suspension with a pH meter (Iskra MA 5730, Slovenia). Exchangeable acidity (pH_e) was determined in a 1 M KCl solution.

Determination of mineral element composition in the substrate

The pseudo-total metal concentrations of Pb, Zn, Cu, Mn, Ni, and Fe were determined after digestion of sieved samples in *aqua regia* ($HCl:HNO_3$, 3:1, v/v) at 150°C, according to the described method [7]. Available metal concentrations were extracted in a mixture of 1 M ammonium acetate and 0.01 M EDTA (pH 7.0) during 2 h of continuous stirring [8]. The absorbance of metals was detected by an atomic absorption spectrophotometer (AAS, Shimadzu AA 7000, Japan). The metal concentrations in samples were determined by comparison of the absorbance values with those of standard solutions (Carlo Erba, Italy). The analytical procedure was checked using the standard reference plant material NIST 1515. The accuracy of the procedure was verified by analyzing certified soil material (BIPEA – Bureau Interprofessionnel d'Etudes Analytiques, Soil 90-0115-0106).

Determination of metal content in plant

Plant material was washed in tap water and cleaned with an ultrasonic cleaner to remove residual substrate particles from the plant surface, especially roots. Plant samples were washed in deionized water, air dried, ground with a ceramic mortar and pestle, and sieved (pore diameter<0.5 mm). The powdered plant material was then dried at 105°C to constant weight and digested in 65% HNO_3 and 30% H_2O_2 (3:1, v/v) [9]. Elemental stoichiometry in plant samples was expressed as the molar ratio after the conversion of metal concentrations expressed in mg kg^{-1} to mol kg^{-1}.

Assessment of plant bioaccumulation and metal translocation capacity

The bioconcentration (BCF) and translocation factor (TF) were calculated as follows:

BCF = metal concentration in roots / pseudo-total metal concentration in substrate

The efficiency of acropetal metal transfer in plants was evaluated using the TF:

TF = metal concentration in leaves/metal concentration in roots

Estimation of the soluble protein content and assays of antioxidant enzyme activities

Plant material (0.5 g) was ground in liquid nitrogen and homogenized in 1 mL of extraction buffer containing 0.1 mM EDTA, 100 mM potassium phosphate buffer (pH 7.0), and 5% polyvinylpyrrolidone. Homogenates were centrifuged for 30 min at 13,000×g, at 4°C. The concentrations of proteins in the extracts obtained were determined spectrophotometrically at 595 nm (Shimadzu UV-1800, Japan), using bovine serum albumin as a standard [10]. The activity of each enzyme was detected at 25°C.

Superoxide dismutase (SOD) activity was determined by measuring of photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm [11]. The SOD activity was expressed as U mg^{-1} of protein. One unit of SOD activity corresponds to the amount of enzyme that inhibits the rate of photochemical reduction of NBT by 50%. Catalase (CAT) activity was determined by monitoring the decrease in H_2O_2 concentration at 240 nm in the reaction mixture [12] and is expressed as U mg^{-1} of protein. Ascorbate peroxidase (APX) activity was determined by monitoring the decrease in absorbance of ascorbic acid at 290 nm [13]. The activity of APX was expressed as U mg^{-1} of protein. The activity of total soluble peroxidases (POD) was determined by the formation of tetraguaiacol and the increase in its absorbance at 470 nm [14]. Glutathione reductase (GR) activity was determined after oxidation of NADPH to $NADP^+$ and measured as a decrease in absorbance at 340 nm [15]. The activity of CAT, APX, POD, and GR are expressed as U mg^{-1} of protein.

Statistical analysis

All data are expressed as the mean±standard deviation of three to six replicates. All data were primarily tested for distribution normality. Since the analyzed data had a normal distribution, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to determine the significance of the difference between groups of plants from different locations. $P<0.05$ was considered statistically significant, and statistically significant differences between the values were marked with different lowercase letters. Pearson's correlation test was used to analyze the correlations between the measured variables (heavy metal concentration and enzyme activity). All statistical analyses were performed with the software package R (Version 3.5.1; R Core Team 2018).

RESULTS

pH value and the metal concentrations in the substrate

Both active and exchangeable acidity was considerably different among the studied substrates. The pH of the control and the Bor mine tailings samples was neutral, the Rudnik flotation tailings were moderately acidic,

Table 1. pH value and metal concentrations in the analyzed substrates. Control, two mine tailings (Bor, Robule) and flotation tailings (Rudnik).

	Control	Bor	Robule	Rudnik
pH_a	7.33-7.48	7.02-7.16	3.44-3.59	6.13-6.33
pH_e	6.64-6.77	6.71-6.87	2.89-3.10	5.53-5.65
Pseudo-total concentrations (mg kg^{-1})				
Pb	10.8 ± 0.1^a	59.8 ± 4.5^c	26.1 ± 0.1^b	164 ± 0^d
Zn	54.8 ± 0.66^a	218 ± 23^c	60.9 ± 2.7^b	274 ± 5^d
Cu	15.7 ± 0.2^a	4703 ± 459^d	288 ± 20^c	115 ± 0^b
Mn	362 ± 2^b	413 ± 47^c	186 ± 2^a	849 ± 18^d
Ni	25.5 ± 0.3^a	12.5 ± 1.3^b	4.98 ± 0.01^c	613 ± 7^d
Fe (%)	2.52 ± 0.02^a	3.55 ± 0.38^b	4.86 ± 0.01^d	4.11 ± 0.10^c
Available concentrations (mg kg^{-1})				
Pb_{EDTA}	3.38 ± 0.33^b	12.3 ± 0.4^c	0.83 ± 0.04^a	84.7 ± 0.8^d
Zn_{EDTA}	1.30 ± 0.21^a	20.9 ± 1.3^c	2.09 ± 0.05^b	36.0 ± 8.9^d
Cu_{EDTA}	5.74 ± 0.2^a	774 ± 22^d	29.2 ± 0.25^b	31.56 ± 0.20^c
Mn_{EDTA}	41.69 ± 0.35^b	74.12 ± 2.51^c	14.22 ± 0.03^a	390 ± 7^d
Ni_{EDTA}	1.04 ± 0.1^a	0.15 ± 0.06^b	0.17 ± 0.07^b	18.55 ± 0.38^c
Fe_{EDTA} (%)	0.007 ± 0^a	0.003 ± 0^b	0.04 ± 0^c	0.03 ± 0^d

Statistically significantly different values are marked with different letters for each row (P<0.05).

Table 2. Concentrations of metals in roots and leaves of *T. latifolia* from an unpolluted pond (control) and metal-polluted mines (Bor, Robule) and flotation (Rudnik) tailing ponds, and their bioconcentration and translocation factors.

Metal concentrations (mg/kg)	Control	Bor	Robule	Rudnik
				Roots
Pb	24.2 ± 4.1^a	37.4 ± 1.5^b	37.0 ± 1.4^b	374 ± 4^c
Zn	80.4 ± 30.0^a	70.4 ± 0.4^a	86.7 ± 1.9^a	421 ± 1^b
Cu	14.0 ± 0.8^a	368 ± 25^c	348 ± 10^c	278 ± 4^b
Mn	1225 ± 16^d	177 ± 9^a	499 ± 19^b	868 ± 13^c
Ni	12.1 ± 3.1^a	< nd	11.7 ± 2.5^a	91.8 ± 0.4^b
Fe	20878 ± 625^{ab}	35553 ± 3713^c	23622 ± 1316^b	16908 ± 269^a
				Leaves
Pb	8.9 ± 0.6^a	17.3 ± 5.3^b	8.7 ± 1.2^a	21.3 ± 1.0^b
Zn	27.9 ± 0.8^c	24.1 ± 1.3^b	21.8 ± 0.1^a	44.9 ± 0.1^d
Cu	6.2 ± 1.4^a	20.5 ± 0.9^c	23.9 ± 1.3^d	16.5 ± 0.2^b
Mn	525 ± 23^a	570 ± 18^a	1664 ± 24^c	647 ± 37^b
Ni	13.1 ± 0.8^b	7.4 ± 0.3^a	5.2 ± 1.2^a	13.3 ± 2.0^b
Fe	451 ± 370^a	360 ± 4^a	220 ± 1^a	155 ± 24^a
Bioaccumulation factor				
Pb	2.2	0.6	1.4	2.3
Zn	1.5	0.3	1.4	1.5
Cu	0.9	0.1	1.2	2.4
Mn	3.4	0.4	2.7	1.0
Ni	0.5	0.0	2.3	0.1
Fe	0.8	1.0	0.5	0.4
Translocation factor				
Pb	0.37	0.46	0.24	0.06
Zn	0.39	0.34	0.25	0.11
Cu	0.434	0.06	0.07	0.06
Mn	0.43	3.24	3.34	0.75
Ni	1.14	-	0.44	0.15
Fe	0.02	0.01	0.01	0.01

Statistically significantly different values are marked with different letters for each row (P<0.05); < nd – lower than the detection limit

and the Robule AMD-generated pond from waste overburden was very acidic (Table 1). Although present in varying amounts, the pseudo-total concentrations of Pb, Zn, and Cu were far higher in the mine waste than in the control substrate (Table 1). The Rudnik substrate contained the highest concentrations of both pseudo-total and available Pb, Zn, Mn, and Ni compared to all other samples. The Bor substrate contained the highest pseudo-total concentration of Cu concentrations, which was 16- and 41-fold higher than in the Robule and Rudnik substrates, respectively, and as much as 300-fold higher than in the uncontaminated control substrate. The difference between the samples was smaller for the Cu concentrations, being 27-, 25-, and 135-fold higher in Bor than in Robule, Rudnik, and the control, respectively.

Metal accumulation in plants

Statistically significantly higher concentrations of Cu and Pb were detected in the root samples from all studied tailings compared to the control plants (Table 2). Namely, the highest concentrations of Cu were detected in the roots of plants from the Bor and Robule mine tailings at the Bor copper mine, whereas the highest concentrations of Pb, Zn, and Ni were determined in the root samples from Rudnik. BCF>1 was detected for several elements in plants from Robule (Pb, Cu, Zn, Mn, Ni) and Rudnik (Pb, Cu, Zn) tailings.

In the leaves, the concentrations of Cu were significantly higher in all metalliferous plants compared to the control (Table 2). The content of Mn in the leaves was highest in Robule plants, the Pb content

Table 3. Element stoichiometry in roots and leaves of *T. latifolia* plants from unpolluted (control) and metal-polluted ponds (Bor, Robule, and Rudnik).

		Mn:Cu	Mn:Zn	Mn:Pb	Fe:Mn	Fe:Pb	Fe:Zn	Fe:Cu	Cu:Pb	Zn:Pb	Cu:Zn
Roots	Control	101±7	20.1±7.9	194±31	16.8±0.3	3259±462	335±127	1700±146	1.9±0.4	10.3±2.3	0.20±0.09
	Bor	0.6±0.0	3.0±0.1	17.9±1.7	199±31	3518±222	592±65	111±19	32.1±3.5	6.0±0.3	5.3±0.3
	Robule	1.7±0.0	6.8±0.1	51.0±3.9	46.5±0.8	2374±222	319±11	77.2±2.0	30.7±2.1	7.4±0.4	4.1±0.0
	Rudnik	3.6±0.0	2.4±0.0	8.7±0.0	19.2±0.0	167±0.7	47.0±0.6	69.2±0.1	2.4±0.0	3.6±0.0	0.68±0.01
Leaves	Control	101±19	22.4±0.3	222±24	0.83±0.66	194±168	18.6±15.0	75.5±51.6	2.3±0.7	9.9±0.9	0.23±0.04
	Bor	32.2±0.5	28.3±2.5	132±37	0.62±0.03	82.8±26.4	17.5±0.8	20.0±1.1	4.1±1.1	4.8±1.7	0.88±0.09
	Robule	80.6±5.6	90.9±1.7	730±111	0.13±0.00	95.1±13.6	11.9±0.1	10.5±0.6	9.0±0.7	8.0±1.1	1.1±0.1
	Rudnik	45.4±3.3	17.2±1.0	115±12	0.24±0.05	27.0±3.0	4.1±0.6	10.7±1.5	2.5±0.1	6.7±0.3	0.38±0.00

Table 4. Pearson's correlation coefficients for the metal concentrations and antioxidant enzyme (APX, POD, CAT, GR, SOD) activities in *Typha latifolia* roots and leaves (marked in bold, and bold-gray are correlations significant at P<0.05 and P<0.01, respectively).

Roots	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	**APX**	**POD**	**CAT**	**GR**	**SOD**
Cu	0.446	**-0.897**	0.028	0.142	0.099	**0.622**	**0.529**	0.361	**0.615**	0.432
Fe		**-0.782**	**-0.690**	**-0.572**	**-0.613**	0.330	-0.119	-0.382	-0.047	-0.044
Mn			0.354	0.225	0.275	**-0.596**	-0.248	-0.080	-0.390	-0.280
Ni				**0.987**	**0.993**	-0.147	**0.552**	0.235	0.076	-0.179
Pb					**0.996**	-0.062	**0.611**	0.186	0.076	-0.207
Zn						-0.092	**0.581**	0.203	0.073	-0.190
APX							0.298	-0.001	0.197	0.189
POD								0.310	0.556	0.211
CAT									**0.795**	**0.766**
GR										**0.876**
Leaves	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**	**APX**	**POD**	**CAT**	**GR**	**SOD**
Cu	-0.302	0.653	**-0.785**	0.191	-0.257	0.231	-0.041	0.198	-0.600	**-0.669**
Fe		-0.267	0.160	-0.299	-0.285	-0.111	-0.382	-0.422	0.317	**0.784**
Mn			-0.704	-0.456	-0.431	-0.425	-0.304	0.043	-0.396	-0.474
Ni				0.287	**0.734**	0.249	**0.611**	0.369	0.215	0.432
Pb					0.649	**0.706**	**0.675**	0.551	-0.159	-0.309
Zn						0.469	**0.921**	**0.670**	-0.299	0.004
APX							**0.665**	**0.600**	-0.408	-0.332
POD								**0.859**	-0.437	-0.246
CAT									-0.481	-0.518
GR										0.440

in Bor and Rudnik plants, and the content of Zn in the leaves of plants from Rudnik flotation tailings. The concentrations of all analyzed metals were much lower in the leaves than in the roots, except for Mn in Bor and Robule plants and Ni in Bor plants, the contents of which were several times higher in leaves than in the corresponding roots (Table 2). Translocation factors<1 for Pb, Zn, Cu, and Fe showed limited acropetal transfer of the listed metals in all analyzed cattail plants. Exceptions were the TFs for Mn in Bor and Robule plants (TFs>3) and Ni in the control, the value of which was only slightly greater than 1 (Table 2).

The analysis of elemental stoichiometry in the roots and leaves was focused on the ratios of metals that could affect the activities of antioxidant enzymes (Table 3). Significantly higher molar ratios of Pb:Mn, Cu:Pb, and Cu:Zn were found in the roots of plants from Bor and Robule compared to the control. Lower stoichiometries of Mn:Cu, Mn:Zn, and Mn:Pb were observed in all analyzed plants from metalliferous substrates, in addition to lower Fe:Pb and Fe:Zn in plants grown in Rudnik flotation tailings. In the leaves, the ratios of Mn:Zn, Mn:Pb, and Cu:Pb were 2- to 6-fold higher in Robule plants than in plants from the other

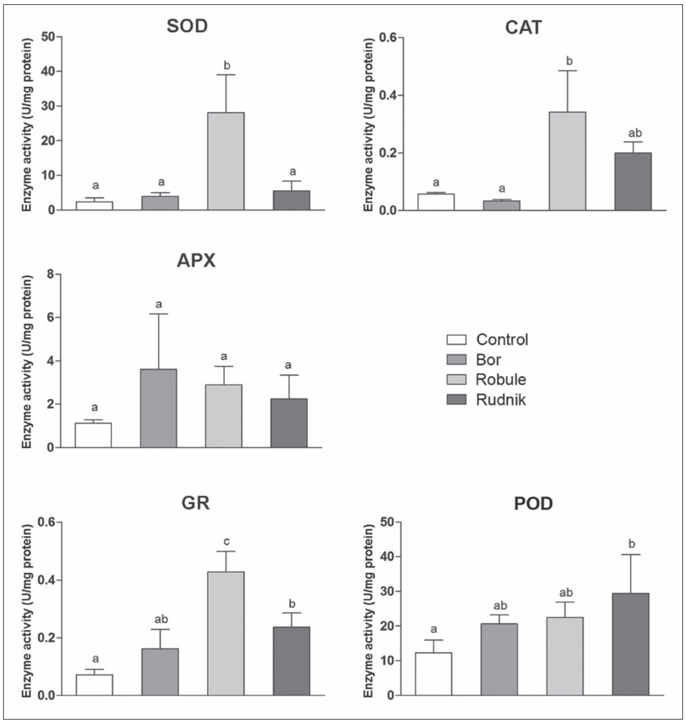

Fig. 1. Activities of antioxidant enzymes (SOD – superoxide dismutase, CAT – catalase, APX – ascorbate peroxidase, GR – glutathione reductase, POD – guaiacol peroxidase) in roots of *Typha latifolia* from unpolluted (control) and metal-polluted ponds (Bor, Robule and Rudnik). Statistically significantly different values are marked with different letters (P<0.05).

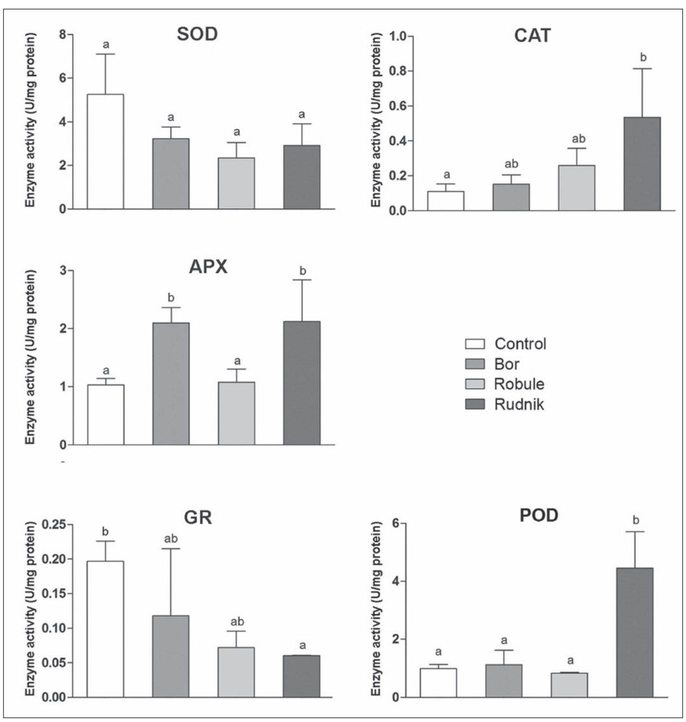

Fig. 2. Activities of antioxidant enzymes (SOD – superoxide dismutase, CAT – catalase, APX – ascorbate peroxidase, GR – glutathione reductase, POD – guaiacol peroxidase) in leaves of *Typha latifolia* from unpolluted (control) and metal-polluted ponds (Bor, Robule, and Rudnik). Statistically significantly different values are marked with different letters (P<0.05).

three sites. In contrast, the molar ratios of Fe:Cu were several-fold lower in plants grown in tailings compared to the control site, while lower ratios of Fe to Pb and Zn ratios were found in Rudnik plants.

Protein content and the activity of antioxidant enzymes

The highest activities of SOD, CAT, and GR in roots were detected in Robule plants, which were as much as 12-, 6-, and 4.5-fold higher than in the control site, respectively (Fig. 1). The APX, GR, and POD activities in roots of all metal-stressed plants were also higher compared to the control, although in some cases without statistical significance.

Correlation analysis of metal concentrations and enzyme activities in roots exhibited strong ($P<0.01$) positive correlations between POD activity and Pb content ($\rho=0.741$). Also, positive correlations ($P<0.05$) were found between APX and Cu ($\rho=0.622$), POD activity and Cu ($\rho=0.529$), Ni ($\rho=0.552$), Pb ($\rho=0.611$), and Zn concentrations ($\rho=0.581$), between GR and Cu ($\rho=0.615$), and negative correlations between APX and Mn ($\rho=-0.596$) (Table 4). Positive correlations were also observed between the activities of GR and CAT ($\rho=0.795$), and between GR and CAT ($\rho=0.766$) or SOD ($\rho=0.876$).

As for the enzyme activities in leaves, they displayed different patterns of change compared to those observed in roots. The highest activity of SOD was detected in the control plants, but without statistically significant differences as compared to plants grown in mine and flotation tailings (Fig. 2). GR also showed the highest activity in plants from the control site, which was statistically significantly higher only compared with Rudnik plants. CAT and POD activities in roots showed similar patterns and were significantly higher in Rudnik plants than in plants from other sites. Plants from the Rudnik flotation tailings showed the highest activities of CAT, APX, and POD in leaves, which were 5-, 2-, and 4.5-fold higher than the enzyme activities in the control. Whereas SOD activity was negatively correlated with Cu ($\rho=-0.669$), the activities of APX with Pb ($\rho=0.706$), POD with Ni, Pb, and Zn ($\rho=0.611$, $\rho=0.675$, $\rho=0.921$), and CAT with Zn ($\rho=0.670$) were positively correlated (Table 4). Correlation analysis showed negative correlations between CAT activity and the activities of POD and APX ($\rho=-0.665$ and $\rho=-0.600$, respectively).

DISCUSSION

The mine and flotation tailings studied typically contain high pseudo-total and available concentrations of metals that are characteristic constituents of polymetallic ores and, as expected, far exceed the concentration limits for metals in uncontaminated control soils. The Bor and Robule mine waste contains the highest concentrations of Zn and Cu, while the Rudnik flotation tailing is particularly burdened with Pb, Zn, Cu, and Ni. In contrast to the neutral (Bor) and slightly acidic (Rudnik) pH reactions of technosols, the Robule had an ultra-acidic reaction [16] due to the dissolution of sulfide minerals and consequent inflow of acidified water that drains from the surrounding overburden [17].

Metal status in cattail plants

Typha latifolia plants that spontaneously grow rooted directly in the technosol of the mine and flotation tailings ponds contain significantly higher concentrations of all metals studied in their roots than control plants, except for Mn and Fe. The highest concentrations of Cu detected in the roots of plants from Bor and Robule and the highest concentrations of Pb, Zn, and Ni detected in the roots of plants from Rudnik are directly governed by the extremely high concentrations of these metals in the corresponding technosols and suggest their passive absorption by roots [18]. The concentrations of several metals detected in the roots of plants from Bor (Cu=368 mg kg^{-1}; Pb=37.4 mg kg^{-1}), Robule (Cu=348 mg kg^{-1}; Pb=37.0 mg kg^{-1}), and Rudnik (Cu=278 mg kg^{-1}; Pb=374 mg kg^{-1}; Ni=91.8 mg kg^{-1}, Zn=421 mg kg^{-1}) are above the toxicity limits (Cu>20-30 mg kg^{-1}; Pb>30 mg kg^{-1}; Zn>300-400 mg kg^{-1}; Ni>10 mg kg^{-1} in sensitive or >50 mg kg^{-1} in moderately tolerant plants) [18-20], which is in agreement with previously reported data on the accumulation of metals (Cd, Zn, Cu, Pb) in cattail roots [21-23]. Although the concentrations of the analyzed metals were in ranges that are toxic to most plants, the concentration limits established mainly refer to aboveground plant parts and are therefore not necessarily applicable to roots [18].

Of all the metal-stressed plants analyzed, the highest level of bioaccumulation in the roots was observed in plants grown in the Robule acidic mine tailings. Bioconcentration factors higher than 1, which were detected for most metals in Robule, but also in Rudnik plants, point to the accumulation of metals in roots in concentrations higher than their pseudo-total concentrations in the corresponding substrate. In Robule plants, the concentrations of Cu, Pb, Mn, and Ni in the roots were 12-, 44-, 35-, and 69-fold higher, respectively, than their EDTA-available concentrations in the corresponding substrate. Such pronounced metal accumulation could result from the ultra-acidic (pH<3.5) reaction of the Robule substrate and its influence on metal mobility and availability. It is known that the redox behavior of elements in soils is very complex and depends on various environmental factors, of which pH is the most important; low pH increases the mobility of Ni, Mn, and Pb and promotes both their uptake by roots and acropetal translocation within the plant [24]. Additionally, in poorly drained and aerated substrates, such as the studied ones, the transition metals Mn and Pb occur in predominantly reduced forms (Mn^{2+}, Pb^{2+}), which are more mobile and bioavailable and thus potentially more toxic than their oxidized forms (Mn^{4+}, Pb^{4+}) [18]. Moreover, acidic conditions increase the rate of chemical weathering of minerals, whereas H^+ ions exchange well with monovalent and divalent cations on negatively charged sites of substrate particles, making them more available for plant uptake [25].

As a result of apparently efficient metal immobilization within the roots, their concentrations in the leaves of all tested cattail plants were several times lower. Plants that retain metals in roots are known to immobilize and sequester them predominantly in the cell walls and vacuoles of the parenchyma cells of the root cortex, in addition to limiting their acropetal transport by the hydrophobic Casparian strips of the endoderm [26]. This reduces both the toxic effects of high metal concentrations on root cell metabolic activities and their further transport to aboveground plant parts. Our results are consistent with previously reported data for *T. latifolia*, which maintained low concentrations of Zn, Pb, and Cd in its leaves, regardless of their high concentrations estimated in the roots and soil [27]. However, in plants grown in Bor and Robule, Cu concentrations in leaves (20.5 mg kg^{-1} and 23.9 mg kg^{-1}) were at the upper toxicity limit (20-30 mg kg^{-1}) [18]. The low transport rate of metals from roots to aboveground plant parts was confirmed by the low TFs, which were less than 1 in all samples, except for Mn in plants from both the Bor and Robule tailings (TF=3.2 and TF=3.3, respectively). The highest Mn concentration in leaves detected in plants from the ultra-acidic Robule AMD (1664 mg kg^{-1})

was at a concentration that causes physiological problems in most plants (Mn>400 mg kg-1), which, together with the highest BCFs, points to the specific environmental conditions that promote Mn uptake by plants [28-30]. The pronounced transfer of Mn to aboveground plant parts may also be related to its known high mobility within the plant and its role in the biosynthesis of ATP, fatty acids, and proteins [31].

Antioxidant enzymes activities in cattail roots

Significantly higher activities of antioxidant enzymes detected in all plants from metalliferous substrates for most of the enzymes analyzed point to metal-induced ROS and oxidative stress, one of the first consequences of metal toxicity [32]. Increased SOD, CAT, and GR activities and their positive correlations indicate increased production of H_2O_2 by SOD during $O_2^{\cdot-}$ removal and subsequent degradation by CAT and GR [33]. Despite the similar metal composition in the roots of Bor and Robule plants, the significantly higher SOD and CAT activities detected in the latter plants suggest that the ultra-acidic pH of the Robule tailings may stimulate enzyme activities. It is known that a high H^+ concentration in roots leads to excessive ROS generation, including superoxide radicals and hydrogen peroxide, triggering additional oxidative stress in plants [34,35]. Therefore, root cells in Robule plants must resolve two issues: reduce the negative consequences of high metal concentrations and maintain a favorable pH in their tissues and cells, which is challenging in such an environment. Increases in APX, POD, and GR activities were previously reported for rice and cucumber roots exposed to an ultra-acidic substrate [34,36]. In addition, the highest SOD activity in Robule plants may also be supported by favorable Mn:Zn and Mn:Pb ratios that are markedly higher than in plants from the other two tailings, indicating a lower probability of Mn replacement in the active site of Mn-SOD by elevated Zn and Pb, and a potentially lower inhibition rate of SOD [6,31]. An adequate Mn content has been shown to stimulate the activities of SOD and peroxidase, thus positively affecting the efficiency of the antioxidant system [34].

Antioxidant enzymes activities in cattail leaves

The statistically significantly higher CAT, APX, and POD activities detected in Rudnik plants indicate higher production of H_2O_2 compared to other plants. The upregulation of all three enzyme activities suggests a synergistic effect of elevated Pb and Zn concentrations in leaves. In addition, APX activity was statistically significantly higher in plants from Bor compared with the control. This increase in APX in Bor and Rudnik plants could be related to a slightly higher Pb content in their leaves (Bor: 17.3 mg kg^{-1}; Rudnik: 21.3 mg kg^{-1}). Although Pb concentrations in leaves do not exceed critical Pb levels, even low Pb concentrations could have negatively influenced cell functionality and increased APX and POD activities, as has been demonstrated in the roots and shoots of rice [37]. Our results are consistent with data reported for *Tetraena qataranse* and *Ceratophyllum demersum* showing the progressive increase in CAT, APX, and POD activities induced by increasing Pb concentrations [38,39].

GR was the only enzyme that exhibited a reduction in activity in all metal-stressed plants compared to the control. This decline in activity could result from its irreversible inhibition by metal-glutathione conjugates, which consist of two oxidized glutathione molecules linked by a metal. When a metal-glutathione conjugate is at the active site of GR, the heavy metal binds to the active site of the enzyme instead of sulfur from oxidized glutathione (GSSG), leading to irreversible inhibition of GR activity.

CONCLUSION

While *Typha latifolia* does not typically inhabit metalliferous substrates, it shows pronounced tolerance to extremely harsh chemical conditions characteristic of mine and flotation tailings. The efficient response of antioxidant enzymes as part of a broader adaptive response ensures the long-term survival of cattail plants in the mine and flotation tailing technosol. This study suggests that cattail plants and other plant species could be efficiently used for phytostabilization and purification of wastewater heavily contaminated with various metals prior to its release into the environment, such as outflow water from the mine and flotation ponds. However, the extent to which this tolerance is supported by high ecological plasticity and genetic differences among different cattail ecotypes remains to be clarified.

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Data availability: Data underlying the reported findings have been provided as a raw dataset which is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Grdovic%20et%20al_Data%20Set.pdf

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