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Original scientific paper

Seasonal changes in metal accumulation and distribution in the organs of *Phragmites australis* (common reed) from Lake Skadar, Montenegro

VLATKO KASTRATOVIĆ[1]*, SLAĐANA KRIVOKAPIĆ[1], DIJANA ĐUROVIĆ[2#] and NADA BLAGOJEVIĆ[3]

[1]*Faculty of Natural Sciences and Mathematics, University of Montenegro, G. Washington Street, P. O. Box 5455, 81000 Podgorica, Montenegro,* [2]*Institute of Public Health of Montenegro, Ljubljanska bb, 81000 Podgorica, Montenegro and* [3]*Faculty of Metallurgy and Technology, University of Montenegro, G. Washington Street, P. O. Box 5455, 81000 Podgorica, Montenegro*

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Abstract: Due to its ability to accumulate metals, availability throughout the year and large biomass, *Phragmites australis* (common reed) is suitable for biomonitoring studies for the evaluation of load levels of trace metals in aqueous ecosystems. The heavy metals concentration in *P. australis* tissue can be several ten to several thousand times higher than those in the surrounding water. In this study, the content of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn, Sr and V) in sediment, water and different organs of *Phragmites australis* collected from Lake Skadar, Montenegro, during different seasons of the year 2011, were examined. The highest concentrations of Sr were found in the leaves, while the other studied metals showed their highest concentrations in the roots. Thus, *P. australis* is considered a root bioaccumulation species. For most metals, the concentrations in the roots and stems increased over time until the end of the growing season and then decreased, while the concentrations in the leaves increased even after the growing season of the plant. If *P. australis* is used for phytoremediation purposes, then it should be harvested after the growing season because then the concentrations of metals in the above-ground parts are maximal.

Keywords: phragmites australis; heavy metals; Lake Skadar; bioaccumulation; phytoremediation.

* Corresponding author. E-mail: vlatkok@ac.me
# Serbian Chemical Society member.
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INTRODUCTION

Water systems are the main destination of pollutants, directly or indirectly, and heavy metals are certainly the most important contaminants.[1] The ability of aquatic plants to accumulate heavy metals is being increasingly used for the evaluation of changes in aqueous systems resulting from environmental pollution.[2] The important role of aquatic flora results from the fact that the concentrations of metals in the macrophyte tissues can be 10^5 times higher than their concentration in the surrounding water.[3]

Chemical absorption and distribution of metals in plants depend on many factors: the plant species and its characteristics, the type of metal and its physical and chemical properties and ecological environmental factors.[4–8]

Unlike water and sediments, plants show significant changes in metal concentration over time. For phytomanagement, it is necessary to take into consideration seasonal and annual concentration changes in plants.[9–11] The use of macrophytes provides a relatively quick way to determine the space and time variations of the bioavailability of heavy metals, which makes plants superior compared to water or sediment samples.[12]

Immersed plants take up metals mainly by roots from the sediment, and considerably less by stem and the leaves from the water and air.[13] Based on a variety of trace metals translocation from roots to shoots, macrophytes form three groups:[14] 1) somewhat uniformly distributed between roots and shoots, *e.g.*, Zn, Mn, Ni and B; 2) usually more in the roots than in the shoots with moderate to sometimes large quantities in the shoots, *e.g.*, Cu, Cd, Co and Mo; 3) mostly in roots with very little in the shoots, *e.g.*, Pb, Sn, Ti, Ag and V. This groupation however may change with plant species, high levels of element in the sediment, location and testing season. The identification of the time that corresponds to maximum accumulation of heavy metals in the above-ground parts of plants is of importance for the optimization of the potential of plants for metal removal.

Phragmites sp. is one of the most widely distributed species in the world. It grows fast, is easy to collect and find on the ground, and it can withstand extreme environmental conditions, including the presence of heavy metals.[15] It accumulates heavy metals both from sediment and water, concentrating them in its tissues and thus reflecting the degree of environmental pollution. It is a continually good bio-indicator over a longer investigation period. The biomass of the above-ground part is 700–4000 g m^{-2},[16] which makes it suitable for potential phytoremediation. Over the past two decades *P. australis* has been widely used in constructed wetlands for the treatment of industrial wastewaters containing metals.[17]

There are only a small number of studies concerning the concentration of trace metals in plant species of Lake Skadar,[18–20] and especially little data on their concentration in different parts of aquatic macrophytes and their seasonal variations.

In this study, the content of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn, Sr and V) in sediment, water and different organs of *P. australis* collected from Lake Skadar, Montenegro, during the different seasons of the year 2011 were investigated.

The aim of this study was to determine the dynamics of the distribution of metals in different organs of the plant *P. australis*, and particularly to determine the time of maximum accumulation in the above-ground tissue. Another aim was to use the chemical composition (heavy metals content) of the dominant plant species as an indicator of the degree of the metal load of water and lake sediment over a longer period.

EXPERIMENTAL

Study area

Lake Skadar (19°03′–19°30′E, 42°03′–42°21′N) is the largest lake on the Balkan Peninsula. It is located on the border between Montenegro and Albania. Two-thirds of the lake lies in Montenegro. During the summer, Lake Skadar has a surface of 370 km^2, which expands to 540 km^2 in the winter. The lake is 44 km long and 13 km wide.

Sediment samples from Lake Skadar were collected at 6 locations (Fig. 1): 1 – Raduš, 2 – Right estuary of Morača, 3 – Left estuary Morača, 4 – Plavnica, 5 – Crni Zar and 6 – Crnojevića River. The right and left estuaries of the Morača and the Plavnica and Crnojevića Rivers feed the Lake. The Plavnica and Crnojevića Rivers are popular tourist destinations, especially in the last few years. Raduš is the deepest of many underwater springs of the Lake, while Crni Zar is a special nature reservation.

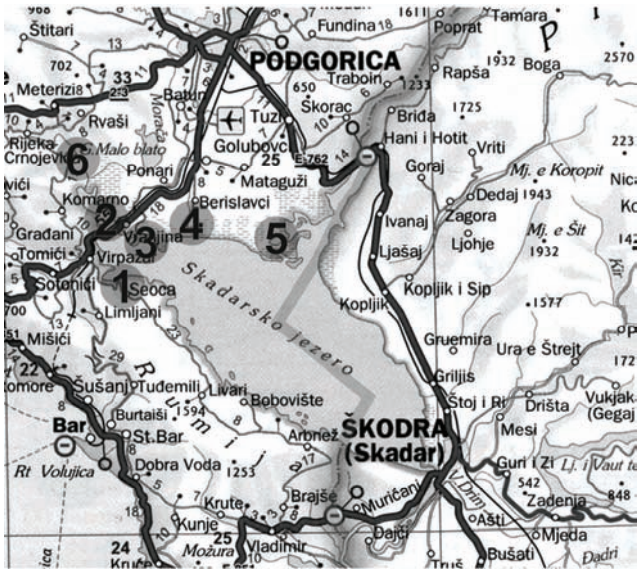

Fig. 1. Location of the sampling station in Lake Skadar.

Sampling collection

The samples of sediments, water and *P. australis* were taken four times during 2011, from early April to late October, every 60 to 70 days, from 6 locations on Lake Skadar.

P. australis was sampled at locations with a high density and coverage of the plant under a clear sky with no wind. At each location of about 25 m^2, 3–4 whole healthy plants of similar size, shape and weight were sampled manually in order to repeat the results for each site.

Sediment and water samples were also taken from the same locations as *P. australis.* Sediment sampling was realize with an Ekman dredge from the depth of 0–20 cm. Water samples were collected from the depth of 0.5–1 m using 1.5 L PET bottles. The samples were stored in a refrigerator (5±2 °C).

Data analyzes

The sampled plant material was washed in the laboratory first with tap water, and then twice with deionized water. The plant parts were cut with stainless scissors into roots, stems and leaves of *P. australis*, for determination of the bioaccumulation diversity of the plant organs. The plant material was subsequently dried at 75 °C for 48 h and then ground into a fine powder and homogenized. In order to avoid the influence of the matrix, the samples were mineralized. Thus, the prepared samples (0.5±0.0001 g) were mineralized with a mixture of 5 mL HNO_3 and 2 mL H_2O_2 in a Milestone Ethos 1 Microwave digestion system. After digestion, the solutions were diluted with deionized water to a final volume of 25.0 mL.

Sediment samples were dried at 75 °C for 48 h under air in a dryer. The dried sediment samples were ground in an agate mortar and sieved to <1.5 mm. The sediment samples (0.5±0.0001 g) were mineralized under pressure and high temperature using a mixture of HCl:HNO_3:HF (6 mL:2 mL:1 mL). After mineralization, the solutions were diluted with deionized water to a final volume of 25.0 mL.

Water samples were filtered through a 0.45 μm Millipore filter and stored in 1 L plastic bottles after addition of 2 mL HNO_3.

All samples of plants parts, sediments and water were prepared three times. In each batch of ten samples, a blank solution was included. The concentrations of heavy metals (Cd, Cu, Co, Cr, Mn, Ni, Pb, Zn, Sr and V) were determined using the ICP-OES technique on a Spectro Arcos instrument. The analytical accuracy was determined using certified reference materials from the National Institute of Standards and Technology (USA), a standard for trace elements in lake sediments (SRM 2709) and for plants, Tea Leaves (INCT-TL-1). The recoveries were within 10 % of the certified values.

Statistical analysis

The Microsoft Excel 2000 package was used for the calculation of the mean, standard deviation and variation coefficient. One-way ANOVA with the value of $p < 0.05$ was performed between the content of each metal in roots, stems and leaves and between the content of each metal in some parts of *P. australis* with regard to the sampling season. If the differences between the mean values were significant at the 5 % level, the post hoc Duncan test was used to determine the minimum allowable differences between particular result groups. All calculations were performed using the SPSS (version 11.5) software package (SPSS Inc., Chicago, USA).

The ability of plants to absorb and accumulate metals from the growth media was evaluated using the bioconcentration factor (*BCF*). The *BCF* value was calculated as the ratio of the concentrations of metals in plants and sediments:

$$BCF = [\text{Metal}]_{\text{plant}}/[\text{Metal}]_{\text{sediment}}.$$

A higher *BCF* implies a greater phyto-accumulation ability of the plant.

The possibility of plants to transport metals from the roots to the above-ground organs was estimated using the translocation ability (*TA*). The value of the translocation ability was calculated as the ratio of the concentrations of metals in roots and a part of the plant:

$$TA = [\text{Metal}]_{\text{root}}/[\text{Metal}]_{\text{part of the plant}}.$$

A higher *TA* means a smaller translocation ability.

RESULTS

The determined values of the metal concentrations in water and sediment are given in Table I, respectively.

TABLE I. Seasonal minimal, maximal and average concentrations ± standard deviation (*SD*) of metals in water (mg L^{-1}) and sediment (mg kg^{-1})

Metal	Concentration type	Month			
		April	June	August	October
		Water			
Cd	–	<0.001	<0.001	<0.001	<0.001
Cu	Min.–max.	0.003–0.012	0.002–0.012	0.002–0.013	0.002–0.014
	Average ± *SD*	0.007±0.004	0.007±0.004	0.007±0.004	0.007±0.004
Co	–	<0.001	<0.001	<0.001	<0.001
Cr	–	<0.002	<0.002	<0.002	<0.002
Mn	Min.–max.	0.006–0.013	0.005–0.013	0.007–0.014	0.006–0.014
	Average ± *SD*	0.009±0.003	0.010±0.003	0.011±0.003	0.010±0.003
Ni	–	<0.001	<0.001	<0.001	<0.001
Pb	–	<0.005	<0.005	<0.005	<0.005
Zn	Min.–max.	0.002–0.008	0.002–0.007	0.003–0.008	0.003–0.008
	Average ± *SD*	0.005±0.002	0.005±0.002	0.005±0.002	0.005±0.002
V	Min.–max.	0.002–0.007	0.002–0.007	0.002–0.006	0.002–0.005
	Average ± *SD*	0.004±0.002	0.004±0.002	0.004±0.002	0.004±0.001
Sr	Min.–max.	0.023–0.047	0.019–0.046	0.020–0.052	0.020–0.051
	Average ± *SD*	0.035±0.009	0.034±0.010	0.035±0.011	0.037±0.012
		Sediment			
Cd	Min.–max.	0.27–0.66	0.29–0.63	0.28–0.65	0.28–0.65
	Average ± *SD*	0.40±0.15	0.41±0.14	0.41±0.14	0.40±0.15
Cu	Min.–max.	27.2–50.4	25.5–46.9	25.5–52.1	23.9–54.4
	Average ± *SD*	34.5±8.84	33.1±7.81	33.3±10.2	34.0±11.1
Co	Min.–max.	6.31–10.1	5.73–12.9	5.28–13.2	5.12–12.6
	Average ± *SD*	8.96±2.52	9.02±2.94	8.76±3.13	9.18±3.08
Cr	Min.–max.	42.4–127	42.3–117	39.8–122	35.6–126
	Average ± *SD*	69.7±31.4	67.0±27.6	68.7±30.2	68.2±32.0
Mn	Min.–max.	99.0–424	120–357	118–379	95.4–419
	Average ± *SD*	232±122	221±91.4	223±101	239±128
Ni	Min.–max.	29.3–131	34.8–110	30.1–113	34.6–125
	Average ± *SD*	79.0±44.3	73.1±36.3	74.5±40.6	83.4±47.6
Pb	Min.–max.	19.1–43.2	16.6–37.6	19.4–43.5	17.6–46.2
	Average ± *SD*	29.0±10.2	25.7±9.39	27.6±9.85	30.2±12.1

TABLE I. Continued

Metal	Concentration type	Month			
		April	June	August	October
		Sediment			
Zn	Min.–max.	47.6–117	56.1–135	59.1–128	53.2–108
	Average ± *SD*	75.4±24.0	79.1±29.7	76.2±26.1	73.8±19.7
V	Min.–max.	20.4–46.7	20.1–45.3	18.8–49.5	18.1–45.5
	Average ± *SD*	29.4±9.93	28.9±10.6	27.9±11.7	26.9±9.92
Sr	Min.–max.	17.7–101	20.4–101	18.2–113	16.8–105
	Average ± *SD*	55.1±30.6	55.8±30.7	56.2±34.2	57.6±32.3

Based on the results given in Table I, it could be concluded that there were no time variations of the results in the water and sediments, at the 95 % level of confidence. There were considerable space variations of the results, reflected in high standard deviation and variation coefficient, sometimes over 50 %. Low concentrations of metals were registered in the water samples. Cd, Co and Pb were below the *LOQ* value at all sampling locations, Cr at one location and Ni at three of the six sampling locations. The results for the individual parts of *P. australis* per season are given in Table II. It could be concluded that the concentrations of metals in water, sediment and the organs of *P. australis* differed significantly. Depending on the type of the sample, different trends of metal concentration reductions were registered:

- in water: Sr > Mn > Cu > Zn > V > Pb, Cr, Ni, Co, Cd
- in sediment: Mn > Ni ≈ Zn > Cr > Sr > Cu > Pb ≈ V > Co > Cd
- in root: Mn > Zn > Cu > Pb ≈ Ni > Cr > Sr > V > Co > Cd
- in stem: Mn > Zn > Cu > Pb > Sr > Cr ≈ Ni > V > Co > Cd
- in leaves: Mn > Zn> Sr > Cu > Pb > Ni > Cr > Co > V > Cd

In sediments and some parts of *P. australis*, the highest concentrations of Mn and Zn were found, the concentrations of which in the sediments did not differ statistically from those of Ni. Vanadium, Co and Cd showed the lowest concentrations in sediment and some parts of *P. australis* (Tables I and II).

Regardless of the sampling period, Zn, Cd and Co followed the concentration trend: root > leaf > stem (Table II). Ni, Pb, Mn and Cr in the first three sampling periods followed the trend: root > stem > leaf. After the vegetation period, their concentrations in leaves were higher than those in the stems. By the end of vegetation period, the highest concentrations of Cu were found in the roots, but at the end of October, the highest concentrations were registered in the leaves. Regardless of the sampling period, the highest concentrations of Sr were registered in leaves, then in the roots and finally in stems of *P. australis*. Almost all the V during the entire investigation period was stored in roots of the plant (Table II).

The seasonal changes in the bioconcentration factors (*BCF*) for the roots, stems and leaves are shown in Fig. 2. The BCF_{root} for all the studied metals increased until the end of the growing season and then decreased, except for Cd and Pb, which slightly increased even after completion of vegetation. The accumulation of metals by roots from the sediment follows the order: Mn > Zn > Cd > Cu > Co > Pb > V > Sr > Cr > Ni. The order is somewhat similar even considering the overall bioaccumulation for all plant parts, *i.e.*, average during the investigation period: Mn ≈ Zn ≈ Cu > Cd > Sr > Pb > Ni ≈ V ≈ Cr. Similarly, to root bioaccumulation, the BCF_{stem} increased from April to August, while it decreased in October. The BCF_{leaf} constantly increased during and after the vegetation period.

TABLE II. Seasonal changes in metal concentrations (min.–max., average ± *SD*, mg per kg of dry weight) in the parts of *P. australis*; minimal and maximal concentrations and average concentrations ± standard deviation (*SD*)

Metal	Part	Month			
		April	June	August	October
Cd	Root	0.05–0.11	0.07–0.24	0.11–0.35	0.12–0.39
		0.08±0.02 a[a] (b)[b]	0.13±0.06 a (ab)	0.22±0.08 a (ab)	0.24±0.10 a (ab)
	Stem	0.02–0.08	0.03–0.11	0.04–0.12	0.04–0.14
		0.06±0.02 a (a)	0.07±0.03 b (a)	0.09±0.03 b (a)	0.10±0.03 b (a)
	Leaf	0.02–0.09	0.04–0.10	0.07–0.13	0.09–0.17
		0.06±0.03 a (b)	0.08±0.02 b (b)	0.10±0.03 b (a)	0.12±0.03 b (a)
Cu	Root	6.68–14.5	9.00–17.7	16.9–23.0	12.4–20.5
		8.92±2.89 a (c)	12.3±3.91 a (bc)	19.3±2.04 a (a)	16.9±3.53 a (ab)
	Stem	3.46–13.4	8.45–15.5	8.60–17.7	7.13–16.1
		6.95±3.61 a (b)	11.2±3.03 a (a)	11.5±3.41 b (a)	10.6±2.95 b (a)
	Leaf	4.75–13.6	4.21–16.4	6.79–20.7	14.9–28.7
		8.72±3.44 a (b)	10.1±4.98 a (b)	12.4±5.99 b (ab)	20.6±5.38 a (a)
Co	Root	0.40–0.90	0.20–2.96	3.86–8.20	2.58–5.40
		0.60±0.19 a (c)	1.34±1.03 a (bc)	5.57±1.98 a (a)	3.71±1.03 a (ab)
	Stem	0.04–0.09	0.05–0.13	0.07–0.29	0.04–0.24
		0.06±0.02 b (c)	0.09±0.04 b (bc)	0.16±0.09 b (a)	0.14±0.08 b (ab)
	Leaf	0.08–0.24	0.12–0.40	0.19–0.92	0.18–0.98
		0.14±0.06 b (a)	0.20±0.10 b (a)	0.41±0.26 b (a)	0.46±0.28 b (a)
Cr	Root	1.25–4.73	3.27–19.1	4.00–12.4	2.37–10.7
		3.32±1.21 a (c)	10.3±6.31 a (a)	7.98±2.80 a (ab)	5.90±2.32 a (bc)
	Stem	0.49–1.77	1.28–6.16	0.80–3.75	0.40–3.20
		1.05±0.52 b (b)	2.68±1.76 b (a)	2.09±1.22 b (ab)	1.26±1.03 b (b)
	Leaf	0.21–0.38	0.28–0.88	0.41–1.09	0.70–1.99
		0.28±0.06 b (b)	0.48±0.22 b (ab)	0.66±0.24 b (ab)	1.24±0.43 b (a)
Mn	Root	66.1–143	52.4–279	117–239	61.4–176
		95.8±37.1 a (b)	148±84.9 a (ab)	181±52.4 a (a)	95.8±42.7 a (b)
	Stem	11.6–47.1	27.4–76.0	33.9–90.9	23.8–94.8
		30.2±12.3 b (b)	40.6±18.5 b (ab)	54.4±20.0 b (a)	47.3±27.4 a (ab)

TABLE II. Continued

Metal	Part	Month			
		April	June	August	October
Mn	Leaf	11.0–22.1	16.9–33.5	23.4–83.5	45.1–169
		16.5±4.49 b (c)	23.8±7.47 b (bc)	43.4±21.6 b (b)	86.1±46.0 a (a)
Ni	Root	0.89–7.79	3.54–9.19	5.02–13.5	3.78–10.7
		4.38±2.91 b (d)	6.50±2.59 a (c)	9.81±3.32 a (a)	8.00±3.00 a (b)
	Stem	0.49–2.28	1.09–2.29	1.49–3.58	1.28–2.20
		1.02±0.68 b (b)	1.81±0.48 b (ab)	2.42±0.74 b (a)	1.72±0.35 b (ab)
	Leaf	0.30–2.16	0.50–2.39	1.09–2.96	1.96–4.46
		1.31±0.65 b (b)	1.52±0.72 b (b)	2.11±0.61 b (ab)	3.00±1.08 b (a)
Pb	Root	1.47–6.46	3.90–9.01	4.64–13.1	4.36–14.8
		3.68±1.84 a (c)	6.30±1.79 a (bc)	8.94±2.98 a (ab)	10.1±4.46 a (a)
	Stem	0.58–4.26	3.09–6.26	4.48–9.18	3.54–9.93
		2.04±1.71 ab (c)	4.22±1.15 ab (b)	6.75±1.69 a (a)	6.64±2.50 a (a)
	Leaf	0.32–1.67	0.20–6.05	1.38–9.76	5.43–11.4
		0.88±0.45 b (b)	2.63±2.18 b (b)	6.57±3.07 a (a)	9.67±2.15 a (a)
Zn	Root	18.4–40.2	25.3–49.1	36.3–72.9	21.1–79.4
		28.4±8.33 a (c)	37.5±9.15 a (b)	52.1±15.8 a (a)	45.8±21.6 a (ab)
	Stem	5.34–22.5	17.1–29.1	13.0–31.7	8.28–33.4
		14.8±5.68 b (b)	24.4±4.21 b (a)	22.0±7.67 b (a)	16.3±9.68 b (b)
	Leaf	12.8–31.7	13.1–44.8	17.4–48.4	9.42–48.3
		22.4±6.86 ab (b)	29.0±11.1 ab (a)	30.7±11.1 b (a)	25.2±12.9 ab (ab)
V	Root	0.29–2.19	1.58–4.05	6.91–9.24	2.66–8.12
		1.28±0.66 a (c)	2.62±0.84 a (c)	8.15±0.95 a (a)	4.50±2.04 a (b)
	Stem	0–0.18	0.13–0.32	0.18–0.69	0.10–0.23
		0.12±0.07 b (b)	0.22±0.07 b (ab)	0.30±0.19 b (a)	0.16±0.04 b (b)
	Leaf	0–0.20	0.15–0.37	0.04–0.31	0.02–0.12
		0.09±0.07 b (c)	0.23±0.08 b (a)	0.15±0.10 b (b)	0.05±0.04 b (d)
Sr	Root	1.87–5.62	3.69–6.53	4.76–13.7	3.14–10.8
		3.80±1.30 a (b)	5.28±1.13 b (ab)	8.86±3.51 b (a)	5.93±2.71 b (ab)
	Stem	0.77–4.13	1.87–5.81	1.97–5.14	1.99–9.28
		2.78±1.19 a (b)	3.41±1.54 b (ab)	3.62±1.34 b (ab)	5.54±2.50 b (a)
	Leaf	0.58–11.7	5.56–26.9	21.5–34.9	25.5–50.8
		6.45±4.13 a (c)	14.4±8.12 a (bc)	27.9±5.79 a (ab)	35.8±9.14 a (a)

[a]The values of individual metals with the same first letter(s) are not significantly different at $p = 0.05$ in the column (*i.e.*, between the different parts of the plant); [b]the values in individual parts of the plant with the same letter(s) in parentheses are not significantly different at $p = 0.05$ in the row (*i.e.*, between seasons)

The values of metal translocation ability are given in Table III. Translocation between different parts of *P. australis* depended on the type of metal and the sampling period. All the investigated metals showed the highest translocation from the stem to the leaves, particularly in the post-growing season. From the root to the above-ground organs, the most mobile metals were Sr and Cu, while Cr, V and Co showed the lowest mobilities.

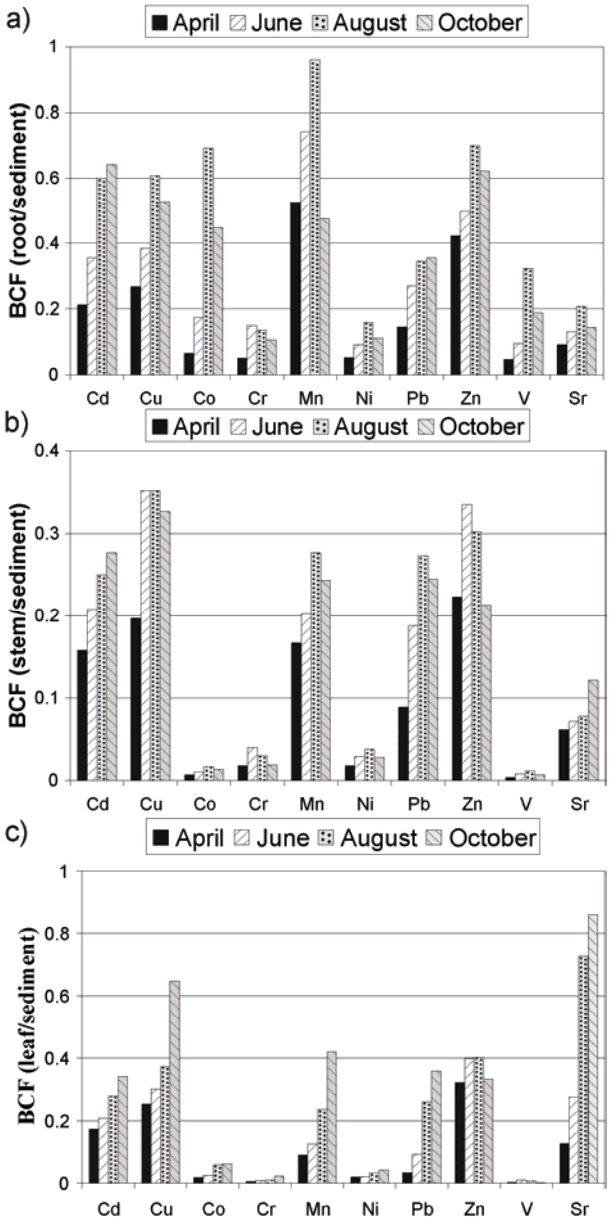

Fig. 2. Seasonal changes in the bioconcentration factor (*BCF*): a) root, b) stem and c) leaf.

DISCUSSION

Cd is a toxic element. In sediment, water and plants it comes mainly from natural sources. Possible anthropogenic input of Cd to the ecosystem of Lake Skadar comes from a metal industry located in the surroundings of the lake, as well

as from the use of agricultural fertilizers, pesticides and combustion of fossil fertilizers. The Cd contents in the sediment and parts of the plant were the lowest of all the investigated elements. Except in April, statistically the content of Cd in roots was significantly higher than in the above-ground parts of *P. australis*. Scierup and Larsen[21] reached a similar conclusion, as did Bonanno and Guidice.[22] Fediuc and Erdei[23] found higher Cd concentration in the shoots of *P. australis*.

TABLE III. Seasonal changes in the translocation ability (TA)

Metal	Part	Month			
		April	June	August	October
Cd	Root/stem	1.53	2.01	2.51	2.60
	Root/leaf	1.74	2.09	2.21	1.89
	Stem/leaf	1.14	1.04	0.88	0.73
Cu	Root/stem	1.58	1.18	1.76	1.68
	Root/leaf	1.15	1.55	1.82	0.86
	Stem/leaf	0.73	1.31	1.03	0.51
Co	Root/stem	10.1	18.0	41.4	37.1
	Root/leaf	4.94	7.17	18.1	11.2
	Stem/leaf	0.49	0.40	0.44	0.30
Cr	Root/stem	4.10	4.64	5.28	6.39
	Root/leaf	12.1	25.8	12.8	5.34
	Stem/leaf	2.95	5.56	2.42	0.84
Mn	Root/stem	3.49	4.08	3.81	2.28
	Root/leaf	6.00	6.07	5.03	1.16
	Stem/leaf	1.72	1.48	1.32	0.51
Ni	Root/stem	5.32	3.69	4.11	4.65
	Root/leaf	3.42	4.74	4.88	2.80
	Stem/leaf	0.64	1.28	1.19	0.60
Pb	Root/stem	2.74	1.52	1.38	1.63
	Root/leaf	6.22	7.52	1.93	1.18
	Stem/leaf	2.27	4.95	1.40	0.72
Zn	Root/stem	2.13	1.60	2.50	3.36
	Root/leaf	1.36	1.42	1.75	1.93
	Stem/leaf	0.64	0.88	0.7	0.57
V	Root/stem	8.98	12.3	33.0	31.8
	Root/leaf	16.7	12.6	95	165
	Stem/leaf	1.86	1.02	2.88	5.19
Sr	Root/stem	1.51	1.73	2.51	1.24
	Root/leaf	1.13	0.54	0.32	0.16
	Stem/leaf	0.75	0.31	0.13	0.13

There were no significant seasonal changes in the Cd concentrations in the stems and leaves of *P. australis*, while the concentration of Cd in the roots increased until the end of the vegetation period (Table II). The accumulation factor showed a similar trend (Fig. 2). The accumulation at the end of the exa-

mination period was higher probably due to decreased metabolic processes and smaller changes in plant biomass. Thus, with a similar absorption or translocation and bioavailability of metals, the concentrations changed significantly.

Cu is an important essential microelement for plants, but it can be toxic at higher concentrations. Cu contributes to several physiological processes in plants.[24]

In the investigated samples of *P. australis*, the lowest average concentration of Cu was recorded in the stems in April and the highest in the leaves in October (Table II). Numerous studies agreed on Cu accumulation in the root of *P. australis.* However, the conclusions about the further fate of Cu, *i.e.*, about its translocation through the plant, were different. The values of Cu accumulation in roots and above-ground parts of *P. australis* taken from Lake Skadar were similar in April and June. Sampling in August showed greater accumulation in roots, while the concentrations in stems and leaves were similar (Table II). In October, the concentration of Cu in the leaves was higher than in the roots and stems, which is in agreement with the statement of Windham *et al.*[25] Yongxia *et al.*[26] found the following Cu trend: sediment > above-ground part > underground part in *P. australis*. Fitzgerald *et al.*[27] found that *P. australis* translocated Cu more to the shoots. Bragato *et al.*[6] found that the accumulation of Cu in the leaves of *P. australis* was comparable to those measured in the stems and rhizome from July to October.

It can be assumed that the plant has an effective translocation root–shoots system, which is activated at the end of the growing season, allowing a higher concentration of elements in the aging tissues. In this way, plants can eliminate heavy metals through phonem during the winter.[28]

Co, after Cd, is the least present of the examined elements in the sediment of Lake Skadar, in roots and stems of *P. australis*, and after V in the leaves. Co showed relatively high root/sediment mobility in August and October (Fig. 2a). A similar mobility Co from sediment to roots was found by Bonanno[29] in Italy and Kumar *et al.*[30] in India.

However, the translocation of Co within *P. australis* was low (Table III). Co has the lowest root/stem translocation of the analyzed elements throughout the whole investigation period. Bonanno[29] also found relatively low translocation of Co through the organs of *P. australis*.

It is not completely clear if Co is essential for higher plants, although there is some evidence that it favorably effects plant growth.[31] However, Co is one of the most important toxic metals, and the fact that Co was mostly absorbed by the roots might mean that the root acted as a filter for undesirable effects on other organs of the plant. The high accumulation in the roots could be a reaction of the plant to the toxicity of metals, which are immobilized in the vacuoles of the roots.[32]

Cr is not essential for plants and it is toxic even at low concentrations.[33] In these investigations, Cr had the lowest bioaccumulation capacity of the examined elements (Fig. 2) in April, August and October. The average values of the *BCF* for Cr during the entire investigation period, together with Ni and V (Fig. 2), also were the lowest. The results of this study show that most of the adopted Cr was found in the roots (Table II). The highest root and stem accumulation was recorded in June, and then decreased. The Cr concentration in the leaves of *P. australis* increased slightly during the entire investigation period.

Bragato *et al.*[6] found Cr was accumulated in the stems and rhizome of *P. australis* mostly in July, and then decreased and became constant from August to December.

In addition to its lower bioaccumulation compared to the other elements, Cr also showed low mobility, as did Co and V (Table III). During the growing season, the translocation ratio decreased (Table III), indicating limited transport of toxic metals from the roots to the shoots. Some authors[34–36] indicated low mobility of Cr from roots to shoots and leaves. It is obvious that there is a physiological barrier, *i.e.*, the absence of a transport mechanism of this element from roots to the green parts of the plant. Relatively low concentration of Cr in foliar tissues during all the sampling period is probably the result of the need of the plant to prevent pollution of its photosynthetic apparatus, as suggested by other authors.[37,38]

Mn is an essential nutrient for plants. It is a functional component of nitrate assimilation and indispensable element of many enzyme systems in plants.

Mn is the most abundant of all the investigated elements in the sediment of Lake Skadar and in all parts of *P. australis* (Tables II and III). After Zn, together with Cu, it has the highest bioaccumulation capacity, as well as the average value for the entire investigation period (Table II). The largest amount of accumulated Mn is in the roots, but a significant part is translocated to the stems and leaves. Both, space and time variations of Mn content are present, except between some organs of *P. australis.* Duman *et al.*,[39] of the seven investigated metals, also found the highest concentration of Mn in the sediment and some parts of *P. australis.* During all the sampling period, the Mn concentration in the roots of *P. australis* was higher than in the sediment. Bonanno and LoGiudice,[22] of the eight examined elements, also found the highest concentration of Mn in each organ of *P. australis.*

There is a tendency of many elements to show their highest concentration at the end of the growing season due to continuous accumulation during vegetation. Nikolaidis *et al.*[9] reported that heavy metals showed increased accumulation in *P. australis* during the growing season, with the maximum values in August and September, and then continuously decrease. Marchand *et al.*[17] evidenced more active metabolism of the plants in the summer than in the winter.

Although Khan and Moheman[40] were of the opinion that Ni was not necessary for healthy plant growth, recent research suggests that Ni is an essential element in many plant species.[41] It helps in the transport of oxygen, stimulates metabolism and it is a key metal for some enzymatic systems of some plants.[42]

According to these studies, Ni is a poorly accumulated element of sediment and, together with Cr and V, has the lowest bioconcentration ratio. The *BCF* for Ni had a maximum value in August, and as the sum of the three *BFC*s (Fig. 2) its value was 0.23. Kumar *et al.*[30] gave a value of *BCF* = 1.17 for *P. australis.* Duman *et al.*[39] found in spring and summer the *BCF* was less than 1, while it was greater than 1 in autumn and winter.

These results show an increase in the concentration of Ni in the plant during the growing season, while the concentration decreased in October (Table II). Most of accumulated Ni was found in the root. The concentrations in the stems and leaves were similar during season, with slightly higher content found in the leaves in October. Bragato *et al.*[6] examined *P. australis* from July to December and reported low Ni contents in leaves from July to October, while in December, the content was 10 times higher.

The mobility of Ni in plants varies among the species, from mobile[43,44] in some plants to immobile[45] in others. In the present investigation, the ratio root/ /leaf and root/stem varied depending on the season (Table III).

Pb is a potentially dangerous and toxic metal for most forms of life, and it is relatively accessible to aquatic organisms. Pb is particularly present in aquatic environments in areas with heavy traffic and neighboring cities.

In these tests, bioaccumulation ability of three investigated organs of *P. australis* for Pb increased during the whole investigation period (Fig. 2) and the sum of all three *BCF* values (Fig. 2) reached a value close to 1 in August and October. The average *BCF* value was 0.66 during the whole investigation period. Kumar *et al.*[30] found *BCF* = 0.59.

Most of the absorbed Pb was in the roots and the amount significantly increased during the season (Table II). The concentration in the stems increased significantly until August but thereafter remained almost constant until October. The concentration of Pb in the leaves increased significantly from April to October.

The absorbed Pb, mainly from sediment, was mostly translocated to the stems during the investigation period with the highest *TA* in August (Table III). The translocation from roots to leaves was low during April and June, while in August and October, it is significantly higher. Several papers[27,46,47] indicate great mobility of Pb through the organs of *P. australis* and its translocation to the shoots. On the contrary, other papers[34,35,48] evidenced the highest concentration of Pb in the roots, while only small amounts were transported to other parts of *P. australis*.

The significantly increased concentration ratios leaf/root in August and October were mainly caused by the increased mobility in the plant, but it could also be the result of foliar adsorption of the metal during the peak tourist season on Lake Skadar and usage of leaded gasoline by small boats and boats.

Zn is an essential and useful element for plants, mainly as a part of various metallo-enzymes. In most aquatic ecosystems, Zn^{2+} can be toxic for the organisms.

Among the examined elements, Zn was the most abundant element after Mn, in sediment and some parts of *P. australis* (Tables II and III). According to the present study, the roots of *P. australis* actively adsorbed Zn, which contained the most Zn during all the sampling period. The sum of the bioconcentration factors for Zn was the highest of all the investigated elements during April and June (Fig. 2) and the average values were the highest during the whole investigation period. Apart from April, when the value was ≈1, in the other seasons the *BCF* value was > 1. The greatest bioaccumulation was noticed at the end of the growing season. Kumar *et al.*[30] reported a *BCF* value of 1.79.

A significant translocation of Zn from the roots to the above-ground organs was registered. The average seasonal ratio root/stem was 2.39, while root/leaf ratio was 1.61 (Table III). Baldantoni *et al.*[35] found a root/leaf ratio of 4.2, while the root/shoot ratio was 1.6. Świerk Szpakowska[46] reported low Zn mobility, with a rhizome/leaf ratio of 3.7.

The distribution of metals in some parts of the plant is the result of differences in the amount and rate of metals input, primarily by root pressure and their release into the environment, mainly through the transpiration of the leaves.[49,50] Some metals are accumulated in roots, probably because of some physiological barriers for the transport of toxic elements in traces. The metals essential for metabolic needs are easily transported to the above-ground parts of the plant.

In recent years, V, together with some other metals, has become a point of increased interest due to its negative impact on the environment.[31]

The bioaccumulation of V, together with Ni and Cr, was the lowest during the investigated period (Fig. 2). The average *BCF* value of during the season was 0.18, almost identical to the value reported by Bonanno.[29] Almost whole amount of the accumulated V was found in roots of *P. australis* and significantly highest concentration was recorded in August (Table II). The phytotoxic level of V in roots in August and low mobility indicate that the root is tolerant to this metal and acts as a filter for the prevention of its toxic distribution in the plant. The translocation of V from roots to stems was, after Co, the lowest among the investigated elements, (Table III). The translocation of V from the underground part to leaves was the lowest among the studied metals. Variations in concentrations during the season in the stems and leaves, as well as of the maximum values during and at the end of the growing season, were noticed.

Bonanno[29] found V only in the underground organs of *P. australis* so that roots disabled its transport to other organs. Soluble forms of V in the sediment are quickly taken up by the roots, and, as well as some other plants, P. *australis* showed great ability to accumulate this metal in the roots.[24]

The absorption of Sr from sediment by *P. australis* in April and June was insignificant, while the sum of the three *BCF* values (Fig. 2) was ≥ 1 in August and October. The highest concentration of Sr was registered in October in the leaves (Table II). The concentrations of Sr in the parts of *P. australis* in April were similar, while from June to October, they were much higher in the leaves compared to in the stems and roots (Table II). The translocation of Sr from the roots to the stems was the highest among the analyzed elements in April and October, while it was highest from the roots to leaves and from stems to leaves from June to October (Table III). Bonanno[29] found the lowest bioaccumulation of Sr among the investigated metals. At the same time, Sr showed great mobility within the plant, which is consistent with the results of this study, taking in consideration the mean values during the season.

Underground organs, especially the roots, are mostly places for the storage of excess trace metals, but it is known that various trace metals are deposited even in the leaves.[51] The highest concentrations of Sr are common at the tops of the plants.[24]

CONCLUSIONS

The concentration of metals in various organs of *P. australis* varies depending on the location and time of sampling. For most metals, the concentrations in the roots and stems increased over time until the end of the growing season and then decreased, while the concentrations in leaves increased even after the period of plant growth. The results of this study showed that the concentrations of five (Cd, Cu, Mn, Zn and Sr) of the ten studied metals were higher in the plant than in sediment during and after the growing season. At the same time, the concentrations of metals in the plants were much higher than those in the water, which indicates sediment as the major source of the metals absorbed by the plant roots. The highest concentrations of Sr were found in leaves, while all other studied metals, the highest concentrations were found in the roots. Thus, *P. australis* could be considered a root bioaccumulation species. However, significant concentrations of the metals were found in both the stems and leaves. Thus, in addition to the absorption mechanism by root, transfer to the shoots must be taken into consideration. Generally, metal mobility through the plant, from roots to leaves, is generally higher than from sediment to the plant.

Due to its ability to accumulate metals, availability throughout the year and its large biomass, *P. australis* is suitable for biomonitoring studies for the evaluation of contamination of the lake with trace metals. If *P. australis* is used for

phytoremediation purposes, then it should be harvested after the growing season because the concentration of metals in the above-ground parts is then maximal.

Although the results show that the toxic values of the investigated heavy metals in Lake Skadar are not alarming at present, the control of possible anthropogenic inputs is recommended. For several years, the surrounding factories have not been working or have been working at reduced capacity; hence, the greatest attention should be paid to the prevention, control and drainage of the metal load from surrounding farms and municipal water utilities.

И З В О Д

СЕЗОНСКЕ ПРОМЕНЕ АКУМУЛАЦИЈЕ И ТРАНСЛОКАЦИЈЕ МЕТАЛА У ОРГАНИМА *Phragmites australis* (ТРСКА) ИЗ СКАДАРСКОГ ЈЕЗЕРА, ЦРНА ГОРА

ВЛАТКО КАСТРАТОВИЋ[1], СЛАЂАНА КРИВОКАПИЋ[1], ДИЈАНА ЂУРОВИЋ[2] и НАДА БЛАГОЈЕВИЋ[3]

[1]Природно–математички-факултет, Универзитет Црне Горе, Ц. Вашингтона, п. пр. 5455, 81000 Подгорица, Црна Гора, [2]Институт за јавно здравље Црне Горе, Љубљанска бб, 81000 Подгорица, Црна Гора и [3]Металуршко–технолошки факултет, Универзитет Црне Горе, Ц. Вашингтона, п. пр. 5455, 81000 Подгорица, Црна Гора

Phragmites australis (трска) је због способности акумулације метала, доступности током целе године и велике биомасе подесан у студијама биомониторинга за процену оптерећења воденог еко-система траговима метала. Концентрација тешких метала у ткиву *P. australis* може бити неколико десетина до неколико хиљада пута већа него у околној води. У овој студији испитиван је садржај тешких метала (Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn, Sr и V) у седименту, води и различитим органима *Phragmites australis* сакупљеним из Скадарског језера, Црна Гора, током различитих сезона 2011. године. Највеће концентрације Sr нађене су у листу а сви остали испитивани метали имају највећу концентрацију у корену што истиче *P. australis* као корен биоакумулаторску врсту. Код већине метала концентрација у корену и стаблу се повећава током времена до краја вегетационог периода а након тога опада, док се концентрација у лишћу повећава и након периода раста биљке. Уколико се *P. australis* користи и у фиторемедијационе сврхе, онда жетву треба извршити након сезоне раста јер је тада максимална концентрација метала у надземним деловима.

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