Lemna minor L. AS BIOINDICATOR OF HEAVY METAL POLLUTION IN SKADAR LAKE (MONTENEGRO)

Vlatko Kastratović¹, Željko Jaćimović², Dijana Đurović³, Miljan Bigović¹ and Slađana Krivokapić¹

¹University of Montenegro, Faculty of Natural Sciences and Mathematics, Džordža Vašingtona bb, 81000 Podgorica, Montenegro ²University of Montenegro, Faculty of Metallurgy and Technology, Podgorica, Montenegro ³Institute of Public Health of Montenegro, Podgorica, Montenegro E-mail: vlatkok@541.27:541.61ac.me

(Received February 20, 2015)

ABSTRACT. The use of aquatic plants to analyze the heavy metal pollution of a lake environment has a number of advantages over the standard chemical methods of the analysis of metal presence in water and sediment. The macrophyta Lemna minor L., sampled from Lake Skadar in Montenegro has been used as a bio-indicator. Floating macrophyte L. minor accumulates metals from water through its submerged roots and floated leaves. The contents of Cd, Cu, Co, Cr, Mn, Ni, Pb, Zn, Sr and V were determined in sediments, water and the plant. The results obtained indicated a very high capacity of L. minor for the accumulation of Mn. In addition, higher Zn concentration was found in root tissue comparing to the sediment. The concentration of the other metals under investigation decreases in the following order: sediment > L. $minor_{(root)}$ > L. $minor_{(leaf)}$ > water. Higher concentrations of all metals in the tissue of L. minor were observed at the end of the growing season. A higher metal content was recorded in the root compared to the leaf. In descending order, the concentrations in plant tissues were found for the following metals: Mn > Zn > Sr > Cu > Ni > Pb > Co > V > Cr > Cd, while the series of bioaccumulation is, in descending order: Mn > Zn > Ni > Co > Pb > Cu > Cr > V > Sr >Cd.

Key words: Lemna minor, Skadar Lake, heavy metals, bioaccumulation.

INTRODUCTION

The ability of aquatic plants to accumulate heavy metals is increasingly being used to monitor changes arising from environmental pollution (ELLES *et al.*, 2000). The mechanism of metals uptake is not entirely selective, meaning that plants, through roots and other organs, take in all the accessible ions from the environment, although all of them are not necessary for plant metabolism (JASTRZĘBSKA *et al.*, 2010). Depending on their type, the tissues of macrophytes may have a metal content equal to or higher than that present in the surrounding sediment (AYENI, 2010), and that content can be 10^5 times higher than the concentration in the surrounding water (ALBERS and CAMARDESE, 1993).

Aquatic macrophytes are often in contact with potential pollutants across their whole body. Being similar to all primary producers, macrophytes react to changes in the quality of the environment in which they live (water/sediment), and are good bioindicators of surface water condition. The chemical analysis of aquatic plants leads not only to an understanding of the current situation but also to the evaluation of the tendencies of environmental changes in time and space. In this way, conditions are created for monitoring the quality of the lake ecosystem over a longer period and timely action to prevent the consequences of unfavorable trends can be carried out.

The aquatic macrophyte which was chosen as an indicator plant (ZHOU *et al.*, 2008) in this paper is *L. minor* because it can consistently accumulate metals during the growing season (PRASAD *et al.*, 2006); it tolerates large amounts of metal without adverse impact on its growth and development; it is linked to one specific location, so it is the real representative of the area; it is easily available for collection, identification and handling; and the plant specimens are similar in size and age, making it easy to select a representative sample. Its disadvantage is that it does not have a long enough lifetime to fully exhibit the phenomenon of bioaccumulation.

In the present study, the content of various heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn, Sr and V) in sediment, water and different organs of *L. minor* from four localities around Lake Skadar.

The aim of this study was to determine the distribution of metals in macrophyte tissues and also to discover the degree of bioaccumulation of the investigated heavy metals, depending both on location and season. In particular, it aims to contribute to defining the mechanisms, paths and intensity of the bioaccumulation of metals. In addition, we expect to demonstrate the improving role of macrophyte vegetation in monitoringaquatic systems as well as the validation of the selected macrophyte plants as useful biological indicators of pollution studied in a lake environment.

MATERIALS AND METHODS

Study area

Skadar Lake is the largest freshwater lake in the Balkans. It is located at a distance of about 7 km from the Adriatic Sea. Depending on the amount of water within it, the surface of the lake varies from 379 to 530 km². Skadar Lake is 44 km long and 14 km wide. The average depth of the Skadar Lake is 5 meters. Two-thirds of the total area of Skadar Lake is situated in Montenegro (219 km²), and the rest is situated in Albania. Skadar Lake has been a National Park since 1983 and has had IBA status (as an area of international importance for birds) since 1989. In 1995 it was included on the List of Wetlands of International Importance – The Ramsar List. Skadar Lake is an ecological kingdom, an oasis of tranquility and of close contact between people and the natural environment. The clear water and protected environment has enabled the development of a very rich eco-system (KASTRATOVIĆ, 2013).

The geographic and economic position of Skadar Lake and its basin, as the largest karst lake in the Balkans, make it very interesting for the public. Monitoring sources of pollution and their effects are of particular importance when it comes to protecting the lake.

The largest water inflow of Skadar Lake is the Morača River, which supplies the lake with more than 60% of its water. The Morača River carries contaminants drawn from urban, agricultural and industrial sources of pollution. The most significant impact on the nature of the ecosystem of Lake Skadar is probably caused by the landfills of production process of the Aluminum Plant in Podgorica (KASTRATOVIĆ, 2013).

Plavnica, situated on a tributary of the lake, is a picnic area and tourist resort, which is located on the shores of Lake Skadar, surrounded by the living waters. The complex, which

includes a motel and a small marina, features a swimming pool, a boat-restaurant in the immediate vicinity and a small zoo.

Rijeka Crnojevića is located at the source of the eponymous river, which, among other things, is special because it is navigable after 1 km. A number of factors negatively affect the quality of the water and sediment of the river: extensive fishing and the presence of a fish processing plant, the development of a local fishing fleet, and the impact of numerous tourists and other visitors, who can number up to 1000 on a daily basis.

Data analyzes

Samples of *L. minor* were collected during two periods, throughout August and October, from four locations: 1 – The right estuary of the Morača $(42^{\circ} 16' 50,18" \text{ N}; 19^{\circ} 07' 38,92" \text{ E})$, 2 – The left estuary of the Morača $(42^{\circ} 15' 55,80" \text{ N}; 19^{\circ} 08' 31,49" \text{ E})$, 3 – Plavnica $(42^{\circ} 16' 17,48" \text{ N}; 19^{\circ} 12' 1,01" \text{ E})$ and 4 - Rijeka Crnojevića $(42^{\circ} 21' 6,03" \text{ N}; 19^{\circ} 02' 23,05" \text{ E})$. At each location, in an area of about 5 m², 3-4 healthy colonies of approximately 100 *L. minor* fronds were sampled manually, in order to be able to repeat the results for each site. The plant material was packed in polyethylene bags, labeled and transferred to the laboratory in the shortest possible period of time. At the same time, three subsamples of sediments and waters were taken from the same place as the plant material. Sediment sampling, of a weight of about 500 g, was conducted using an Eckman dredge to a depth of 0–20 cm. Water samples were collected from a depth of 0.5–1 m using 1.5 L PET bottles.



Fig. 1. Location of the sampling sites around Lake Skadar.

The plant material was separated in the laboratory into the root and leaf of *L. minor* with the intention of determining the plant part bioaccumulation and dried at 75°C over a 48 hour period. The samples were ground to a fine powder and homogenized. The samples were then mineralized to avoid the influence of the matrix. The prepared plant samples were mineralized in a Milestone Microwave Ethos 1, with mixture of HNO₃ and H₂O₂ (3:1).

The sediment samples were dried in air and then dried at 75°C for 48 hours. The dried sediment samples were chopped in an agate mortar and sieved through a sieve <1.5 mm. The sediment samples were mineralized using a mixture of HCl: HNO₃ (3:1) in a microwave furnace, a Milestone Microwave Ethos 1 (USEPA, 2007). The water samples were filtered through a 0.45 μ m Millipore filter stored in 1 L plastic bottles by adding 2 mL of HNO₃.

The determining of the concentration of the investigated metals in the samples of water, sediments and plant was conducted using the ICP-OES technique on a "Spectro Arcos" device. The detection limits of Cd, Cu, Co, Cr, Mn, Ni, Pb, Zn, V and Sr were 0.001, 0.001, 0.001, 0.001, 0.001, 0.001, 0.001, 0.001 and 0.001 mg/l respectively. All the samples of plants parts, sediments and water were prepared in triplicate and their average value was assessed.

The capacity of plants to absorb and accumulate metals from the growth media was evaluated using their bio-concentration factor (BCF). BCF was calculated as the ratio of the concentrations of metals in part of plant and water: BCF = [Metal] _{part of plant} /[Metal] _{water} (HAWKER and CONNELL, 1991). The possibility of plants transporting metals from the root to the leaf was estimated using translocation ability (TA). The Translocation ability was calculated as the ratio of the concentrations of metals in the leaves and in the roots: TA = [Metal]_{leaf} / [Metal] _{root} (DENG *et al.*, 2004).

RESULTS AND DISCUSSION

Table 1 shows the minimum and maximum values of the metal content in the lake water and sediments during the investigated period.

Table 1. – Minimum/maximum and average concentrations (mg/l; mg/kg d.w.dm³) ± standard deviation (S.D.) (n=3) of metals in water and sediments of Skadar Lake.

Matal		Wa	iter	Sediment			
Metal		August	October	August	October		
Cd	minmax.	< 0.001-0.001	$<$ LOD *	0.28-0.45	0.28-0.46		
Cu	Average±S.D.			0.34 ± 0.08	0.33±0.09		
Cu	minmax.	0.003-0.013	0.003-0.014	26.1-52.1	26.4-54.4		
Cu	Average±S.D.	0.008 ± 0.004	0.009 ± 0.006	37.0±10.9	37.7±12.1		
Ca	minmax.	< LOD	< LOD	6.90-13.2	8.13-12.6		
Co	Average±S.D.			10.3 ± 2.65	10.8 ± 2.06		
Cm	minmax.	<0.002.0.002	<0.002.0.002	40.4-122	45.5-126		
Cr	Average±S.D.	<0.002-0.002	<0.002-0.002	77.4±33.6	79.2 ± 33.8		
м	minmax.	0.008-0.013	0.006-0.012	118-379	125-419		
IVIII	Average±S.D.	0.011 ± 0.002	0.010 ± 0.003	255±112	283±132		
NI	minmax.		<0.001.0.004	30.1-113	48.0-137		
111	Average±S.D.	<0.001-0.004	<0.001-0.004	91.0±40.6	107 ± 40.0		
Dh	minmax.	< LOD	< LOD	19.4-43.5	17.6-46.2		
F D	Average±S.D.			26.2±11.6	28.0±12.5		
7	minmax.	0.003-0.008	0.003-0.008	59.1-128	61.0-108		
ZII	Average±S.D.	0.006 ± 0.002	0.005 ± 0.003	81.1±31.8	$78.4{\pm}21.8$		
N7	minmax.	0.002-0.006	0.002-0.005	18.8-32.6	19.4-28.4		
v	Average±S.D.	0.004 ± 0.002	0.004 ± 0.001	23.8 ± 6.36	24.5 ± 3.91		
C.	minmax.	0.020-0.052	0.020-0.051	28.6-113	30.0-105		
sr	Average±S.D.	0.034 ± 0.014	0.036 ± 0.014	66.5±36.3	66.1±32.8		

The temporal changes in metal concentrations (mg/kg dw) in the root and leaf of *L*. *minor* are given in Table 2.

The concentrations of metals in the root and leaf of *L. minor* were different from their concentrations in the water and sediment and follow the trend: sediment > *L. minor*_(root) > *L. minor*_(leaf) > water, except for Mn (root and leaf) and Zn (root), which showed a higher concentration than in the sediment.

The concentrations of metals in the root and leaf of *L. minor* followed a downward trend:

Mn>Zn>Sr>Cu>Ni>Pb>Co>V>Cr>Cd.

Higher metal contents were recorded at the locations to the left and right of the estuary of the Morača River when compared to those contents recorded at the other locations on Lake Skadar (Plavnice and Rijeka Crnojevića). The Morača River carries various impurities along its flow: sanitary landfill, wastewater and landfill waste, in particular from the production process of the KAP Aluminum factory. During the summer, Plavnica and Rijeka Crnojevića are visited throughout the whole day by a large number of tourists.

The presence of a large number of households, developed agricultural zones where fertilizers and chemicals are present, numerous roads towards urban areas, as well as restaurants and many other factors suggest that caution is in order when the protection of Skadar Lake is considered.

Matal		Aug	just	Octo	ober	
Metal		Root	leaf	root	leaf	
Cd	minmax.	0-0.83	0-0.05	0.05-1.63	0.10-0.89	
Cu	Average±S.D.	0.22 ± 0.41	0.02 ± 0.03	0.51±0.75	0.42 ± 0.36	
Cu	minmax.	12.4-33.1	7.94-22.3	9.12-47.8	9.02-26.5	
Cu	Average±S.D.	19.9±9.13	13.6±6.43	31.0±16.1	17.1±7.21	
Ca	minmax.	2.63-8.78	1.89-4.84	3.28-8.53	2.57-7.39	
Co	Average±S.D.	5.13±2.67	3.62 ± 1.26	5.60 ± 2.54	5.11±2.28	
Cm	minmax.	0.79-3.08	1.18-1.78	2.18-4.58	1.57-5.32	
Cr	Average±S.D.	1.60 ± 1.01	1.36±0.28	3.31±1.04	2.81±1.73	
Mn	minmax.	1 543-4 747	713-2 590	1 886-6 633	1 299-5 732	
IVIII	Average±S.D.	2718 ± 1415	1 490±789	4 136±1 965	2 960±1 936	
NI	minmax.	7.69-18.9	5.00-9.76	7.33-17.6	7.38-20.0	
INI	Average±S.D.	11.3±5.18	7.55 ± 2.25	11.6±4.64	11.4 ± 5.93	
Dh	minmax.	3.80-10.2	4.44-7.98	4.08-18.3	3.57-13.6	
PD	Average±S.D.	5.80 ± 2.96	6.51±1.59	9.70±6.59	7.67 ± 4.26	
Zn	minmax.	59.9-115	41.3-82.5	72.1-115	48.9-109	
ZII	Average±S.D.	78.6±25.2	58.6±18.2	97.8±18.6	83.2±27.7	
N7	minmax.	1.09-5.57	1.58-1.90	1.58-6.18	1.48-5.21	
v	Average±S.D.	3.18±2.18	1.71±0.13	4.22 ± 2.08	2.78 ± 1.68	
C.	minmax.	29.3-34.7	32.0-37.1	24.6-36.0	29.8-35.6	
sr	Average±S.D.	31.8±2.26	34.0±2.22	29.8 ± 4.86	33.4±2.63	

Table 2. - Seasonal changes in metal concentrations (mg/kg dw) in the root and leaf of *L. minor*; minimum and maximum concentrations and average concentrations \pm standard deviation (n=3).

Slight variations in the content in the tissues of *L. minor*, depending on the sampling, were shown on the part of Sr. In second place is Zn. The greatest differences in the content in relation to the timing of the sampling were shown by Cd.

Higher values of metals in the roots and in the leaves were recorded in October than in August for all the metals under investigation. These values are almost twice as high for Cd, Cu (root only), Cr and Mn. The bioaccumulation capacity of *L. minor* is shown through its bioaccumulation factors, indicating a decreasing order as follows:

for the root (BCF_{root/water}) : Mn > Zn > Ni > Co > Cu > Pb > Cr, V > Sr > Cd for the leaf (BCF_{leaf/water}) : Mn > Zn > Ni > Co > Pb > Cu > Cr > Sr > V > Cd

The difference in the order of the metal content in a plant compared to the sequence of their bioaccumulation ability can be seen, as well as their relative numbers. This difference suggests the different bioaccumulation capacity of macrophytes for certain metals. Plants accumulate certain metals irrespective of their concentrations in the water and sediment, which is obviously a characteristic provided by its capacity for the accumulation of each individual element.

There is a difference between the order of the metals' bioaccumulation capacity in the root and that in the leaf. This difference cannot be interpreted independently by a different metal translocation through the plant, but a different bioaccumulation capacity is most probably present in the leaves of individual metals when compared to the water.

The bioaccumulation capacity of *L. minor* for Mn is several times higher than it is for the other metals.

	Location	Right estuary of		Left es	stuary of	Pla	vnica	Rijeka		
	20000000	Mo	rača	Mo	orača			Crno	jevića	
Metal		August	October	August	October	August	October	August	October	
Cd	BCF _{root}	1.66	0.42	0	0.10	0.05	0.01	0	0.33	
	BCF _{leaf}	0	0.38	0	0.20	0.05	1.78	0.10	0.98	
Cu	BCF _{root}	1.41	2.31	3.31	3.68	4.13	3.04	2.63	6.94	
	BCF _{leaf}	1.72	1.89	1.45	1.33	2.65	3.01	1.61	3.12	
Со	BCF _{root}	17.6	17.1	10.6	13.8	7.60	6.56	5.26	7.38	
	BCF _{leaf}	7.20	7.68	9.68	14.8	8.32	13.3	3.78	5.14	
Cr	BCF _{root}	3.08	0.46	0.79	2.83	0.60	1.82	0.65	1.09	
	BCF _{leaf}	1.30	1.57	1.78	2.57	0.59	2.66	0.59	0.89	
Mn	BCF _{root}	253	609	197	364	365	663	154	171	
	BCF _{leaf}	324	955	101	225	103	210	713	118	
Ni	BCF _{root}	20.4	17.4	37.8	35.2	4.18	13.0	1.92	1.83	
	BCF _{leaf}	18.2	40.0	10.0	14.8	4.88	10.6	1.58	1.89	
Pb	BCF _{root}	4.08	7.32	1.52	4.56	1.85	1.63	1.83	2.01	
	BCF _{leaf}	3.19	2.97	2.45	1.43	3.00	2.44	1.78	5.44	
Zn	BCF _{root}	9.46	13.4	28.7	38.3	20.0	24.0	9.10	13.8	
	BCF _{leaf}	7.81	12.8	12.0	16.3	13.8	36.3	11.8	10.4	
V	BCF _{root}	2.24	2.76	2.78	2.06	0.18	0.71	0.27	0.39	
	BCF _{leaf}	0.79	2.60	0.95	0.62	0.28	0.51	0.28	0.37	
Sr	BCF _{root}	0.56	0.73	0.94	0.73	1.07	1.21	1.60	1.60	
	BCF _{leaf}	0.69	0.79	0.83	0.69	0.85	1.19	1.38	1.49	

Table 3. - Temporal and spatial changes in the bioconcentration factor for the root ($BCF_{root/water} \times 10^3$) and the leaf ($BCF_{leaf/water} \times 10^3$).

The average seasonal concentrations of metals were higher in the roots than in leaves, except for the concentration of Pb in August. Sr and Pb have the highest ratio of leaf/root translocation, while the smallest translocations were shown by Mn, V and Cu. Table 4 contains data on the seasonal changes in translocation ability (TA) for *L. minor*. It is important to note that with floating macrophyte, translocation is not uniquely determined, since in addition to translocation from the root, the leaf certainly contains metals absorbed from the water.

	Cd	Cu	Со	Cr	Mn	Ni	Pb	Zn	V	Sr
August	-	0.64	0.68	0.81	0.47	0.57	1.12	0.68	0.54	0.92
October	0.78	0.56	0.78	0.68	0.58	0.80	0.60	0.74	0.59	0.98

Table 4. - The average seasonal values of translocation ability (TA).

Metals in plant

Cadmium, Cd

Cadmium is a toxic element in aquatic environments mainly derived from natural sources. The possible anthropogenic input of Cd in the ecosystem of Lake Skadar is from the metal industry, which is located in the wider environment of the lake and from the use of agricultural fertilizers, pesticides and burning fossil fuel. Of all the studied metals, Cd has the lowest value of concentration in both the root and in the leaf of *L. minor* (Table 2). On the other hand, its bioaccumulation capacity is the lowest of all the metals we examined. The mean concentration in the root was 0.36 mg/kg and in leaf it was 0.22 mg/kg. At two sampling sites in August, the content in the root and the leaf was below the detection limit of the instrument. The bioconcentration degree was higher in October than in August, in both the root and the leaf.

Copper, Cu

For all the samples of *L. minor*, we found a higher content of Cu in October. The content of this metal was higher in the root than the leaf. An exception to this was at Plavnica. The mean value of the content in the roots was 25.4 mg/kg, while in the leaves it was 15.4 mg/kg. PAVLOVIĆ *et al.* (17) found that the content of Cu in whole plant was 29.9 mg/kg (sampled at the Memorial Park artificial lake in Kragujevac) and the 10.9 mg/kg (at Bubanj artificial lake). Copper showed variable potential for bioaccumulation in the tissues of *L. minor*. It also had a lower mobility from root to leaf in comparison with most of the studied metals. The distribution of metals in individual parts of the plant is the result of differences in the amount and rate of metal uptake, primarily due to root pressure, and its release into the environment, mainly through the transpiration of the leaves (KASTRATOVIĆ *et al.*, 2013).

Cobalt, Co

Cobalt is one of the main toxic metals. A lot of aquatic plants have the ability to develop tolerance and resistance mechanisms to toxic metals (MATTHEWS *et al.*, 2005; DENG *et al.*, 2009; MARCHAND *et al.*, 2010).

The mean seasonal and spatial concentration of Co in the root of *L. minor* is 5.37 mg/kg, while in the leaf it is 4.36 mg/kg. In the leaf, the concentration of Co slowly increased from August to October, depending on the sampling place. However, the impact of seasonal changes on the concentration of this metal in the root is not completely clear. Cobalt has shown a relatively high leaf/root concentration ratio. It is not entirely clear which part of the content of Co, and other metals, is present in the leaf due to translocation from roots and which is present through absorption from water. Due to the higher concentration of metals in the root, translocation processes probably dominate.

Chromium, Cr

Chromium, after Cd, showed the lowest content in the parts of *L. minor*. The mean value of Cr during the research period in the root is 2.46 mg/kg and 2.09 mg/kg in the leaf. The content of Cr in the leaf in October (5.32 mg/kg) significantly stands out from the other

results for Cr. The bioaccumulation ability of *L. minor* for Cr is moderate, as evidenced by other authors (ZAYED, 1998).

ATER *et al.* (2) observed the effectiveness of *L. minor* in the phytoremediation of Cr, especially for lower levels of contamination of the aquatic environment. At the same time, they find a higher degree of bioaccumulation for Cr compared to Cu. In studies in the natural conditions of Lake Skadar, *L. minor* shows a greater degree of bioaccumulation for Cu than it does for Cr.

Manganese, Mn

This was the most abundant examined metal in the tissues of *L. minor*. Its presence was 30-40 times higher than that of Zn, which is the second ranked metal by concentration. The average value of the content of Mn in the root is 3427 mg/kg and 2225 mg/kg in leaves. IRAM *et al.* (9) recorded significantly lower Mn, from 591 to 1873 mg/kg (average 1055 mg/kg), in the whole plant of *L. minor*. In addition, lower values are found by BRANKOVIĆ *et al.* (4) in the tissues of *L. minor*, 1740 mg/kg.

Manganese has shown a significantly higher bioaccumulation ability (several tens of times higher) compared to the other metals in the tissues of *L. minor*. With one exception, in all measurements, the content of Mn is two to three times higher in the root than in the leaf. After Cd, it shows the lowest level of translocation from the root to the leaf.

Nickel, Ni

The average value of the content of Ni in the root of *L. minor*, during the research period, is 5.11 mg/kg and in the leaf, the value is 9.46 mg/kg. Similar values were found by Iram *et al.* (9) in the whole plant *L. minor* 1-15 mg/kg Ni (with a mean value of 5 mg/kg).

Apart from a few exceptions, any significant spatial and temporal variation of results was not noticed. After Mn and Zn, Ni has the highest bioaccumulation in the various parts of *L. minor*. The mobility of Ni in plants varies between species, from mobile in some plants (TIFFIN, 1971; THIESEN and BLINCOE, 1988) to immobile in others (SAJWAN *et al.*, 1998). In our studies of *L. minor*, Ni showed a medium translocation ratio from root to leaf compared to the other tested metals.

Lead, Pb

Our results showed that the average amount of Pb in the investigation period is 7.75 mg/kg in the root, and 7.09 mg/kg in the leaf. JAMNIČKA *et al.* (10) found a lower value, 2.71 mg/kg Pb in the tissues of *L. minor*, whereas IRAM *et al.* (9) observed a higher value, 8-23 mg/kg of Pb (average 12 mg/kg), in comparison to the results for *L. minor* from Skadar Lake.

After Sr, Pb had the highest leaf/root concentration ratio. This significantly increased translocation ability compared to other metals is mainly due to the increased mobility of Pb in the plant, but there is probably also a foliar adsorption of metal in the period of intense tourist activity on the lake, which involves the use of small boats and boats with leaded gasoline. The metal accumulated in the leaves may be once again released into the environment by secretions from leaves.

Zinc, Zn

Zinc is the most abundant metal after Mn in the tissues of *L. minor*. The mean seasonal concentration of Zn in the root is 88.2 mg/kg, and 70.9 mg/kg in the leaf. As was the case with Pb, JAMNIČKA *et al.* (10) found a lower value of Zn in the tissues of *L. minor*, 14.68 mg/kg, and IRAM *et al.* (9) a higher value, 35 to 213 mg/kg of Zn (mean 106 mg/kg), compared to the concentration present in the *L. minor* from Skadar Lake. The concentration of Zn in the roots and in the leaf at all locations is higher in October than in August, with one

exception. After Mn, *L. minor* also showed the highest bioaccumulation capacity for Zn. Metals which are necessary for metabolism can be more easily absorbed from the surrounding environment and transported to the green parts of the plants (LASAT, 2000).

Vanadium, V

The content of V in the root of *L. minor* is on average 3.70 mg/kg and in the leaf 2.25 mg/kg. Out of the tested elements, this content is slightly higher than that of Cr and Cd. After Sr and Cd, it had the lowest bioaccumulation and after Mn and Cd, it has the lowest translocation from root to leaf. In August, around the area of Plavnica, a higher content of V was measured in the leaf, and in all other determinations, a higher content is recorded in the root. With few exceptions, the V content in the root and leaf of *L. minor* was higher in October than in August. There is a tendency for many elements to show their highest concentration at the end of the growing season, due to continuous accumulation during the growing season. A content of V that is almost twice as high was recorded in the parts of *L. minor* at the mouth of the Morača locations, in relation to Plavnica and Rijeka Crnojevića.

Strontium, Sr

After Mn and Zn, strontium is the third metal most commonly present in the parts of *L. minor*. The mean seasonal value in the root of *L. minor* is 32.9 mg/kg, while in leaf it is 31.6 mg/kg. The narrow interval of the values found and/or the low value of the coefficient of variation (most frequently around 70%) suggests that there is no temporal and spatial variation in the results for Sr in the parts of *L. minor*. There were no difference in the content of Sr between the roots and leaves. Strontium has the highest value of the translocation coefficient (the highest ratio of leaf/root), meaning it is the most mobile metal of those investigated through the parts of *L. minor*. Out of the investigated metals, Sr had the highest concentration in the water, which would mean that it is the most available metal. However, after Cd, it showed the lowest bioaccumulation capacity in *L. minor*.

Correlation analysis of the metal content in macrophytes and the sediment

Table 5 shows the values of the Pearson's correlation coefficient (r) between the metal content in the environment (sediment) and in individual parts of the macrophytes.

		Cd	Cu	Со	Cr	Mn	Ni	Pb	Zn	V	Sr
August	root	-0.45	-0.05	0.94	-0.03	0.07	0.48	-0.32	-0.49	-0.31	0.47
	leaf	0.64	-0.06	0.66	-0.15	0.54	0.34	-0.84	0.92	-0.42	0.50
October	root	-0.38	0.37	0.84	0.51	0.28	0.77	-0.38	0.08	-0.24	0.83
	leaf	0.84	0.26	0.52	0.91	0.63	0.25	0.99	-0.03	0.08	0.88

Table 5. - Correlation coefficients, r, ($p \le 0.05$) between the sediment and certain parts of *L. minor*.

Based on these results, it is obvious that there are specific and unique ways by which macrophytes accumulate and concentrate various metals in their tissues. The positive correlation between metal content in the sediment and *L. minor* occurs depending on the plant organ and the sampling period. Five (Cd, Cr, Mn, Pb, Sr) of the ten investigated metals showed a significant positive correlation between the ration of the content in the sediment and the content in the leaf in October. Based on results in Table 5, *L. minor* can be proposed as a bioindicator, in order to estimate the presence of metals in the surrounding environment (sediment) for all the studied metals except Cu and V.

CONCLUSION

The floating macrophyte *L. minor* accumulated more metals from its surrounding in water through its completely submerged roots, when compared to the leaf that floats on the water surface. In addition to the uptake from the water, metals in the leaf of *L. minor* can also be found due to translocation from the roots and deposition from the air.

Manganese in the root and leaf and Zn in root of *L. minor* show a higher content than in the sediment. The concentration of the other investigated metals decreases in the following order: sediment > root > leaf > water. For all the investigated metals, higher concentrations were observed during October in both the root and in the leaf. The investigated metals followed, in descending order of concentrations in both the root and the leaf of *L. minor*: Mn > Zn > Sr > Cu > Ni > Pb > Co > V > Cr > Cd while the decreasing order of bioaccumulation ability was as follows: Mn > Zn > Ni > Co > Pb > Cu > Cr > V > Sr > Cd. The difference in the order of the metal content in a plant compared to the sequence of their bioaccumulation ability shows the different bioaccumulation capacity of *L. minor* for certain metals, regardless of their concentration in the environment. The greatest mobility through the plant was observed for Sr and Pb and the least for Mn, Cu and V.

Higher metal contents were recorded at locations to the left and right of the estuary of the Morača River where it flows into Skadar Lake, in comparison to those recorded at the other locations, Plavnica and Rijeka Crnojevića. The Morača River carries various impurities which are accumulated along its flow.

The investigated macrophyte *L. minor* can be used as an indicator plant for understanding the situation in the environment (water, sediment) because it can constantly accumulate metals during the growing season; it tolerates large amounts of metal without adverse impact on its growth and development; it can be tied to one location, so it is a real representative of the area; it is easily available for collection, identification and handling; the specimens of the plants are similar in size and age and it is easy to select a representative sample. In our paper, we found a correlation between the content of the studied metals in plants and sediments, except for the content of Cu and V. The disadvantage of this macrophyte is that it does not have a long enough lifetime to fully exhibit the phenomenon of bioaccumulation.

References:

- ALBERS, P.H., CAMARDESE, M.B. (1993): Effects of acidification on metal accumulation by aquatic plants and invertebrates. 1. Constructed wetlands. *Environmental Toxicology and Chem*istry 12: 959-967.
- [2] ATER, M., ALI, N.A., KASMI, H. (2006): Tolerance and accumulation of copper and chromium in two duckweed species: *Lemna minor* L. and *Lemna gibba* L. *Journal of Water Science* **19**: 57-67.
- [3] AYENI, O.O., NDAKIDEMI, P.A., SNYMAN, R.G., ODENDAAL, S. (2010): Chemical, biological and physiological indicators of metal pollution in wetlands (Review). *Scientific Research and Essays* **5**: 1938-1949.
- [4] BRANKOVIĆ, S., PAVLOVIĆ-MURATSPAHIĆ, D., TOPUZOVIĆ, M., GLIŠIĆ, R., STANKOVIĆ M. (2010): Concentration of Some Heavy Metals in Aquatic Macrophytes in the Reservoirs Near City Kragujevac (Serbia). *Biotechnology & Biotechnological Equipment* 24: 223-227.

- [5] DENG, H., YE, Z.H., WONG, M.H. (2004): Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. *Environmental Pollution* **132**: 29-40.
- [6] DENG, H., YE, Z.H., WONG, M.H. (2009): Lead, zinc and iron (Fe(II)) tolerances in wetland plants and relation to root anatomy and spatial pattern of ROL. *Environmental and Experimental Botany* **65**: 353-362.
- [7] ELLES, M.P., BLAYLOCK, M.J., HUANG, J.W., GUSSMAN, C.D. (2000): Plants as a natural source of concentrated mineral nutritional supplements. *Food Chemistry* **71**: 181-188.
- [8] HAWKER, D., CONNELL, D. (1991): An evaluation between bioconcentration factor and aqueous solubility. *Chemosphere* 23: 231-241.
- [9] IRAM, S., AHMAD, I., RIAZ, Y., ZAHARA, A. (2012): Treatment of Wastewater by Lemna minor. Pakistan Journal of Science 44: 553-557.
- [10] JAMNICKÁ, G., HRIVNÁK, R., OŤAHEĽOVÁ, H., SKORŠEPA, M., VALACHOVIČ, M. (2006): Heavy metals content in aquatic plant species from some aquatic biotopes in Slovakia, In: Proceedings 36th International Conference of IAD. Austrian Committee Danube Research/IAD, Vienna, p. 366-370.
- [11] JASTRZĘBSKA, M., CWYNAR, P., POLECHOŃSKI, R., SKWARA, T. (2010): The Content of Heavy Metals (Cu, Ni, Cd, Pb, Zn) in Common Reed (*Phragmites australis*) and Floating Pondweed (*Potamogeton natans*). *Polish Journal of Environmental Studies* 19: 243-246.
- [12] KASTRATOVIĆ, V. (2013): Aquatic macrophytes of Skadar Lake as bioaccumulators of heavy metals-role in monitoring water system and the possibility of remediation. PhD Thesis, Faculty of Natural Sciences and Mathematics, University of Montenegro, Podgorica, 150 pp. [in Serbian].
- [13] KASTRATOVIĆ, V., KRIVOKAPIĆ, S., ĐUROVIĆ, D., BLAGOJEVIĆ, N. (2013): Seasonal changes in metal accumulation and distribution in the organs of *Phragmites australis* (common reed) from Skadar Lake, Montenegro. *Journal of the Serbian Chemical Society* 78: 1241-1258
- [14] LASAT, M.M. (2010): Phytoextraction of metals from contaminated soil: A review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *Journal of Hazardous Substance Research* 2: 1-25.
- [15] MARCHAND, L., MENCH, M., JACOB, D.L., OTTE, M.L. (2010): Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: A review. *Environmental Pollution* 158: 3447-3461.
- [16] MATTHEWS, D.J., MORAN, B.M., OTTE, M.L. (2005): Screening the wetland plant species Alisma plantago-aquatica, Carex rostrata, and Phalaris arundinacea for innate tolerance to zinc and comparison with Eriophorum angustifolium and Festuca rubra Merlin. Environmental Pollution 134: 343-351.
- [17] PAVLOVIĆ, S., PAVLOVIĆ, D., TOPUZOVIĆ, M. (2005): Comparative Analysis of Heavy Metal Content in Aquatic Macrophytes in the Reservoirs Gruža, Bubanj and Memorial Park. *Kragujevac Journal of Science* 27: 147-156.
- [18] PRASAD, M.N., GREGER, M., & ARAVIND, P. (2006): Biogeochemical cycling of trace elements by aquatic and wetland plants: Relevance to phytoremediation. In Taylor & Francis Group, LLC (Ed.), *Trace elements in the environment* pp. 451.

- [19] SAJWAN, K.S., ORNES, W.H., YOUNGBLOOD, T.V., ALVA, A.K. (1996): Uptake of soil applied cadmium, nickel and selenium by bush beans. *Water, Air and Soil Pollution* **91**: 209-217.
- [20] THIESEN, M.O., BLINCOE, C. (1988): Isolation and partial characterization of nickel complexes in higher plants. *Biological Trace Element Research* **16**: 239-251.
- [21] TIFFIN, L.O. (1971): Translocation of nickel xylem exudate of plants. *Plant Physiology* **48**: 273-277.
- [22] USEPA Method 3051a. Microwave assisted acid digestion of sediments, sludges, soils and, Revision 1, 2007.
- [23] ZAYED, A. (1998): Phytoaccumulation of trace elements by wetland plants Duckweed. *Journal of Environmental Quality* 27: 715-721.
- [24] ZHOU, Q., ZHANG, J., FU, J., SHI, J., JIANG, G. (2008): Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. *Analytica chimica acta* 606: 135-150.