BIODIVERSITAS
Volume 19, Number 1, January 2018
Pages: 296-302

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190140

Bioconcentration of heavy metals in aquatic macrophytes of South Urals region lakes

TATYANA G. KRUPNOVA[1,♥], IRINA V. MASHKOVA[1], ANASTASIYA M. KOSTRYUKOVA[1], NIKITA O. EGOROV[1], SVETLANA V. GAVRILKINA[2]
[1]Department of Chemistry, South Ural State University, 76 Lenin Prospect, 454080 Chelyabinsk, Russia. Tel./Fax. +7-351-2679517,
[♥]email: krupnovatg@susu.ru
[2]Ilmen State Reserve, UrB RAS, Miass, Russia

Manuscript received: 8 November 2017. Revision accepted: 20 January 2018.

Abstract. *Krupnova TG, Mashkova IV, Kostryukova AM, Egorov NO, Gavrilkina SV. 2018. Bioconcentration of heavy metals in aquatic macrophytes of South Urals region lakes. Biodiversitas 19: 296-302.* This paper studies bioconcentration of heavy metals in macrophytes. High concentration of heavy metal compounds in the lakes of South Ural is natural. Moreover, some of the South Ural lakes are polluted by heavy metals that get into the water together with partly treated sewage of ferrous and non-ferrous industries as well as mining. The paper analyzes similarities and differences of macrophyte communities in six lakes: Bolshoye Miassovo, Bolshoy Ishkul, Bolshoy Tatkul, Argayash, Savelkul, Baraus. In our research, we determined species diversity for each lake. *Potamogeton lucens* L. and *Lemna minor* L. were found in all the studied lakes, and the concentrations of heavy metals were studied in their leaves. Such metals as Fe, Mn, Cu, Zn were found in macrophytes. We obtained a metal ratio Mn <Fe<Cu<Zn in plant leaves. High accumulation of Fe, Cu, Zn was observed in Lemna while Mn accumulates equally. Metals bioconcentration factors (BCFs) were calculated

Keywords: aquatic macrophytes, bioaccumulation, heavy metals, bioconcentration factor (BCF)

INTRODUCTION

Heavy metal contamination in aquatic environments is a serious environmental problem. Anthropic activities are source of heavy metals in aquatic systems (Prasad 2004). Trace elements can be accumulated in aquatic plants (Kabata-Pendias and Pendias 2001). It is known that the aquatic macrophytes are good bioindicators of heavy metal in lake systems (Zhou et al. 2008). There are a number of studies that focus on this issue. The results of studies suggested that *Myriophyllum aquaticum* (Vell.) Verdc. (Harguinteguy et al. 2013, 2016), *Potamogeton pusillus* L. (Harguinteguy et al. 2016), *Stuckenia filiformis* (Pers.) Börner (Harguinteguy et al. 2014), *Scirpus tripueter* L. and *Cyperus malaccensis* Lam. (Zhang et al. 2010), *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. (Peng et al. 2008) are good bioindicators of heavy metal in rivers. Ipomoea aquatic could be used as biomonitors of sedimentary metal contamination for the Beung Boraphet reservoir (Dummee et al. 2012). *Myriophyllum spicatum* L. was investigated for its ability to accumulate nutrients, and heavy metals from contaminated watercourses of Egypt (Galal and Shehata 2014) The aquatic macrophytes play a very significant role in removing the different metals from the ambient environments. They probably play a major role in reducing the effect of high concentration of heavy metals (Vardanyan and Ingole 2006, Keskinkan et al. 2004). Jackson reviews paradigms of metal accumulation in rooted aquatic vascular plants (Jackson 1998).

The aquatic macrophytes can use to remove heavy metals from the contaminated water. For example, it was shown that wastewater treatment can be used plants such as *Potamogeton pectinatus* L. and *P. malaianus* (Peng et al. 2008), *Pistia stratiotes* L., *Spirodela polyrrhiza* W. Koch and *Eichhornia crassipes* (Mart.) Solms (Mishra and Tripathi 2008), *M. aquaticum*, *Ludwigia palustris* (L.) Elliot. and *Mentha aquatica* L. (Kamal et al. 2004). *Lemna gibba* L. can be successfully used for metals (Cd, Cu, and Zn) removal (Khellaf and Zerdaoui 2009; Megateli et al. 2009, Drost et al. 2007). Moreover, *Lemna minor* L. is a good bioindicator species (Horvat et al. 2007). Studies involving plants and multielemental waters are very rare because of the difficulty in explaining interactions of the combined toxicities. Regardless of the complexity in interpretation, *Lemna* bioassay can be efficiently used to assess combined effects of multimetal treated electroplating wastewater's samples (Horvat et al. 2007). Three aquatic plants *E. crassipes*, *L. minor* and *S. polyrhhiza,* were used in laboratory for the removal of heavy metals from the coal mining effluent (Mishra et al. 2008). Aquatic plants (*Potamogeton natans* L.) can be used to enhance the performance of constructed wetland systems for stormwater treatment (Fritioff and Greger 2006). The uptake of heavy metals, As, and Sb by aquatic plants-fluvial horsetail, platyphyllous cattail, etc.-growing in industrial collection ponds of metal mining industry in the Kemerovo region, Russia, was studied. Cu, Pb, Cd, Zn, As, and Sb are the major pollutants in these plant habitats (Hozhina et al. 2001).

Plants exposed to high concentrations of heavy metals should respond in order to avoid the deleterious effects of heavy metal toxicity at the structural, physiological and molecular levels (Oveka and Takac 2014). Nevertheless, the physiological changes observed in plants at high metal concentrations and accumulations, did not represent a risk in relation to their survival. This is shown in the example of *M. aquaticum* and *Egeria densa* (Planch.) Casp. (Harguinteguy et al. 2015).

High concentration of heavy metal compounds in the lakes of east piedmont limnological region of South Ural is natural. It is said to be connected with the region geology. In Lake Bolshoye Miassovo the average concentrations were: Cu-0.02 $mg \cdot L^{-1}$, Zn-0.03 $mg \cdot L^{-1}$, Pb-0.01 $mg \cdot L^{-1}$, Mg-0.05 $mg \cdot L^{-1}$, Sr-0.58 $mg \cdot L^{-1}$ (Gavrilkina et al. 2000). Large concentration of heavy metals is considered as a natural background (Rogozin 2003). Moreover, some of the South Ural lakes are polluted by heavy metals that get into the water together with partly treated sewage of ferrous and non-ferrous industries as well as mining.

Bioconcentration factor (BCF) is widely used to assess of heavy metal bioaccumulation (Parkerton et al. 2008). The aim of this paper was to investigate bioaccumulation of heavy metals in macrophate from the lakes of South Ural. Our research seeks to address the following issues: (1) to study macrophyte species composition of the six South Urals region lakes, (2) to determine the heavy metal concentrations in chosen macrophyte species and (3) to evaluate heavy metals bioconcentration factors (BCFs).

MATERIALS AND METHODS

Study area

The studied waterbodies are the part of Kisegach-Miassovo hydrological system forming an almost closed ring of several large and medium-sized lakes connected by small rivers and creeks (Figure 1).

Figure 1. Map of lakes of South Urals, Russia

Bolshoye Miassovo, Bolshoy Ishkul, Bolshoy Tatkul, Argayash, Savelkul, Baraus are located on the territory of the Ilmen State Reserve and may be considered conventionally undisturbed. The lakes of the Ilmen group are located in rows along the meridian-oriented mountain ranges in the low-mountain and piedmont zones at the height of 270-375 m above sea level. The lakes are of erosion-tectonic origin and in various stages of development. Thus, they may be characterized by complex bolson, considerable depth, angularity of the coastline, steep stony coasts. The lakes under study belong to small and middle in terms of surface they occupy and middle and deep in terms of depth. Their chemical composition refers them to the lakes of hydrocarbonate, calcium and magnesium water of different types (according to the classification of Alekin (1970). pH value changing with seasons varies in the range of 8.0-8.6 in epilimnion from May to September. This value, as well as the gas regime (oxygen, carbon dioxide), is closely related to the thermal regime, which in turn is in direct proportion to their depth. Low water salinity 0.1-0.3 $g{\cdot}L^{-1}$, the predominance of hydrocarbonate ions and rich microelement water composition are the characteristic features of the lakes (Gavrilkina et al. 2000).

Sample collection

We collected data of species diversity in 2005-2015 (Mashkova et al. 2015) and used data of other research works (Vejsberg 2007, Vejsberg 2014). We studied six lakes. In field research standard methods of ecological profiling were used (Katanskaya, 1981). In our research, we determined species diversity, water flora taxonomic structure for each lake. Ecological groups were identified according to the classification generally accepted in hydrobotanical literature which divides macrophytes into aquatic and semi-aquatic according to their interaction with the aquatic environment. We made flora lists of all the studied lakes in common (Krupnova et al. 2017).

For chemical analysis, we collected plants leaves from July to August in 2015-2016. At each study site, where individual plant species were found, plant leaves were collected in five replicates. Two species that were common for all study lakes were selected for analysis: *Potamogeton lucens* L. from lakes B. Miassovo (n=5), B. Ishkul (n=3), Argayash (n=3), B. Tatkul (n=3), Savelkul (n=4), Baraus (n=4) and *L. minor* from lakes B. Miassovo (n=4), B. Ishkul (n=3), Argayash (n=3), B. Tatkul (n=3), Savelkul (n=4), Baraus (n=3). Leaves from plants were carefully collected, washed thoroughly with the lake water, freed of all adhering materials, and transferred to the laboratory in clean plastic bags.

Water samples were taken from June to September in 2014-2016. A total of 5-12 comparable samples of water in different sites distributed around the perimeter of the lake were collected every study year.The sampling system PE-1110 was used for hydrochemical sampling. Water samples for metal analysis were collected into 2.0 dm^3 polymer dishes according to the National Standard (GOST R 51592-2000; GOST R 51593-2000). Sample preservation and storage was in accordance with the National Standard GOST 31861-2012.

Analytical determination of metal concentrations in macrophytes

To reduce individual differences, 5 plants of each species were sampled at each lake. Macrophytes were washed and dried at 60 ° C for 48 hours. The dried leaves of 5 plants were combined into one mixed sample, respectively. Mixed samples were ground in a mortar. The finely ground rock powder was compressed using a hydraulic press into a pellet.

XRF patterns were registered in the lab of Center for Nanotechnology at South Ural State University. Rigaku SuperMini200 XRF Spectrometer was used for XRF analysis. The Russian National State Standard Samples GSO 8923-2007 Standard sample of birch leaves, GSO 8922-2007 The standard sample of a mixture of herbs, OSO 10-150-2008 seaweed (kelp) and GSO 8921-2007 *Elodea canadensis* Michx. were used. The relative standard results deviation was not more than 5%.

Analytical determination of metal concentrations in water

Water samples were digested with concentrated HNO_3 acid as described by APHA (1998). The concentrations of heavy metals (Cu, Zn, Mn, and Fe) in the water samples were determined using Analyst 400 (Perkin-Elmer) atomic absorption spectrometer with a flame atomization mode. A standard metal solution was used to prepare the standard curve according to GOSTR 51309-99. All the metal concentrations were measured in the lab of the South-Ural Common Use Center of the Ilmen State Reserve UrB RAS.

Data processing

The total weight of each heavy metal can be calculated from the XRF-results, and the concentration data (μg/g) used in this study is the heavy metal weight divided by the dry weight (DW) of the macrophyte samples. Microsoft Excel 2013 and SPSS 24.0 software were used to organize and analyze the data. Differences in heavy metal concentration among the species and the lakes were analyzed using ANOVA with post-hoc comparisons made using Fisher's least significant difference (LSD).

We analyzed the data using a special program module "GRAPHS" (Nowakowski 2004).

BCF values in this study were calculated as reported by Gobas et al. (2009) where bioconcentration factor (BCF) is defined as the ratio of the steady-state metal ions concentrations in the plant vs the concentration in water:

$$BCF = \frac{C_{macrophyte}(mg \bullet kg^{-1})}{C_{water}(mg \bullet L^{-1})}.$$

Where:
BCF = Bioconcentration factor

RESULTS AND DISCUSSION

Macrophyte species composition

The studied lakes vegetation in syntaxon terms is rather diverse with vegetation being well developed in shallow waters. It is often rare in open spaces, goes a narrow broken line along the coasts and has a pattern character. Macrophytes of small lakes (Argayash, Savelkul, Baraus) are distributed more evenly, and their distribution zones occupy a relatively larger area of the water area. Formed communities occupy the bottom to the depth of 3.0-4.0 in average, some species of *Fontinalis antipyretica* Hedw. and charophytes are met to the depth of 5 m.

During our research, we registered about100 macrophyte (Krupnova et al. 2017) species of which 63 (63%) is water plants, and 37 (37%) are coastal. The list includes 8 species of charophytes belonging to 3 genera, 2 species of moss, 1 species of Equisetophyta and ferny and 88 species of Magnoliophyta of 44 genera, 29 families. The difference between our data and reference data in terms of macrophyte taxonomic composition refers only to Magnoliophyta and some updating of the species composition of Argayash, B. Miassovo, B. Tatkul. A smaller number of genera and species is registered without considering woody plants (willows and birches). The species proportion of some families is different, e.g., Potamogeton, Cyperaceae, cereal, buckwheat, Labiatae, Rosaceae, and Ranunculaceae.

The discrepancies between our data and reference data are likely to be connected with the aim of our work, that was not to make a comprehensive re-inventory of macrophytes. So, we may assume that some species were not covered. Moreover, most of the species that were not described in terms of neo-botany are registered in the reference literature as rare or singular.

Besides, the aim of our research was to study lake ecosystems, so numerous rivers, streams, flows, and bogs were not considered. That explains the change in proportion of aquatic and semi-aquatic plants for the benefit of the latter. However, given the fact that the reference data is rather old (more than 15 years) the change in macrophyte species composition of many lakes under study may be explained by the influence of various factors, apparently, to a larger extent of natural origin. Macrophyte habitats may have been reduced due to the waterbodies aging and overgrowing.

According to the research macrophyte species composition of the studied lakes is different. B. Miassovo is remarkable in species abundance (95 species). Many species absent in other lakes are found here, e.g., charophytes, water moss (*F. antipyretica*), some *Potamogeton* species, several hygrophyte species growing on floating bogs. Macrophyte list of B. Miassovo has 95% of species of the total list (Krupnova et al. 2017).

The lakes under study are stated to be different in taxonomic diversity of aquatic flora. According to Veisberg (2014) the representatives of Najadaceae, Zannichelliaceae, Calliergonaceae families, rare for the South Ural, are found in B. Miassovo. Potamogetonaceae, Characeae are also rich in composition. It may be explained by the fact that during natural eutrophication in the process of overgrowing flora is becoming poor mainly due to the habitat reduction of aquatic plants. It is higher, for example, in B. Miassovo for some charophyte species, *Nymphaea candida* J. Presl, *Nuphar pumila* (Timm) D.C., *P. lucens*, *Potamogeton perfoliatus* L., *Persicaria amphibian* (L.) Gray, *Schoenoplectus lacustris* (L.) Palla and others. Most of these species prefer solid soil and wandering water. However, quantitative analysis considering each species frequency of occurrence showed a fairly high similarity of waterbodies flora diversity. The dendrogram constructed on the basis of the Jacquard species similarity index is shown in Figure 2.

Potamogeton lucens and *L. minor* were found in all the studied lakes These species were chosen for studying heavy metal concentrations.

Heavy metals content in plants

Elemental composition of macrophyte was studied. Such metals as Mn, Fe, Cu, and Zn are found in macrophytes of all the studied lakes. The heavy metal concentrations (mean and standard deviation) are shown in Table 1.

Table 1. Mean values (±standard errors), $mg \cdot kg^{-1}$DW, for all heavy metal concentrations in macrophytes

Metal	Lakes					
	B. Miassovo	**B. Ishkul**	**Argayash**	**B. Tatkul**	**Savelkul**	**Baraus**
P. lucens						
Mn	721±198a	970±87a	760±208a	721±98a	708±201a	690±126a
Fe	639±120a	514±87a	520±125a	490±110a	556±180a	523±145a
Cu	36±5a	42±6a	48±5a	46±6a	37±6a	41±7a
Zn	30±5a	32±4a	36±6a	38±6a	27±6a	29±5a
L. minor						
Mn	912±185a	710±71a	809±114a	712±125a	703±135a	676±130a
Fe	402±68b	390±96b	380±198b	289±90b	321±85b	368±112b
Cu	26±4b	28±5b	34±4b	34±5b	29±4b	27±4b
Zn	16±5b	22±6b	28±5b	26±6a	17±4b	11±7b

Note: *P. lucens* from lakes B. Miassovo (n=5), B. Ishkul (n=3), Argayash (n=3), B. Tatkul (n=3), Savelkul (n=4), Baraus (n=4). *L. minor* from lakes B. Miassovo (n=4), B. Ishkul (n=3), Argayash (n=3), B. Tatkul (n=3), Savelkul (n=4), Baraus (n=3). Different letters indicate significant differences among the species according to Fisher's LSD ($p<0.05$)

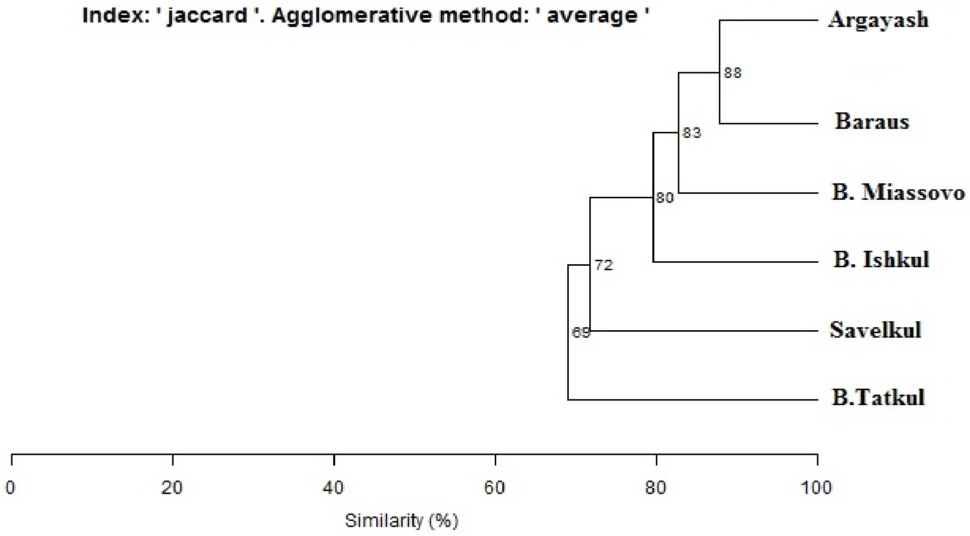

Figure 2. Dendrogram of the similarity of the lakes studied by species of macrophytes

We obtained a metal ratio Mn <Fe<Zn<Cu in plant leaves. Mn content in the organs of plants usually takes values from 10 to 25 mg/kg (Parzych et al. 2016) which covers the physiological needs of most plants. Very high levels of this element were found in *P. lucens* (690-970 $mg \cdot kg^{-1}$DW) and *L. minor* (676-912 $mg \cdot kg^{-1}$DW). These concentrations are reported as toxic to most plants (Bonanno and Lo Giudice 2010, Bonanno 2013). But study lakes are not polluted. Probably the increased Mn content in organs of aquatic plants indicates a positive influence of these macrophytes on purification of waters and bottom sediments from manganese compounds in relation to physiological demand and can be a genetic feature (Parzych et al. 2016).

Iron concentration in leaves remained at 289-639 $mg \cdot kg^{-1}$DW. This metal is required as essential microelement for the plant metabolism (Kumari and Tripathi 2015). Generally, Cu (26-48 $mg \cdot kg^{-1}$DW) and Zn (11-38 $mg \cdot kg^{-1}$DW) concentrations in leaves of macrophytes studied were within the range of natural content in aquatic plants (Parzych et al. 2016).

High accumulation of Fe, Cu, Zn is observed in *L. minor* while Mn accumulates equally (Table 1). The differences may be associated with the different types of plants rooting. *L. minor* is free-floating, unrooted macrophyte, *P. lucens* is a rooted water plant.

Bioaccumulation of metals

The heavy metal concentrations in lakes water are shown in Table 2. Cu was characterized by the highest variability of concentration within the research area. Waters of lakes catchments were rich in dissolved iron and manganese. In the lake B. Miassovo discovered iron-manganese nodules which are differed from the same formations from freshwater lakes higher contents of Mn and an extremely high rate of Fe (Gavrilkina et al. 2000).

The macrophytes showed diversified accumulation properties in relation to Mn, Fe, Cu and Zn present in water (Table 3). The highest bioaccumulation was found in the case of Mn. The BCFs of Fe and Cu were statistically equal. It is known that the majority of iron and manganese are biologically accumulated by plankton in Lake B. Miassovo (Gavrilkina et al. 2000). A high degree of accumulation of Mn and Fe can be explained by the widespread detection of oxides of these elements in the upper layer of bottom sediments and iron-manganese nodules (Gavrilkina et al. 2000).

In the Lake B. Ishkul, the high content of Cu (1.02 $mg \cdot L^{-1}$) was detected. This is due to its greatest proximity to the zone of the winds Kyshtym copper-smelting plant. But the high Cu content in the macrophytes was not observed (see Table 1). The lowest content of Cu (0.003 $mg \cdot L^{-1}$) but statistically significant decrease in the content of copper in plants is not found was in the water of the Lake Savelkul but statistically significant decrease of the Cu content in plants was not found (see Table 1). We excluded these lakes for calculating mean values of BCFs. Bioaccumulation of Zn was slightly lower.

In conclusion, the composition of macrophyte ecological groups in its relation to aquatic factor is one of the most important indicators characterizing waterbody biotope diversity. So, local flora of the waterbodies under study has some differences in spite of the lakes being equal in surface and belonging to the single hydrological system. Bolshoye Miassovo has a higher level of taxonomic diversity and variety of ecological forms of plants. It is explained by a higher diversity of its biotopes which is the result of the complex formation of its coasts and a variety of soil.

Table 2. Mean values (±standard errors), mg·L^{-1}, for all heavy metal concentrations in water of lakes . Miassovo (n=58), B. Ishkul (n=78), Argayash (n=14), B. Tatkul (n=16), Savelkul (n=18), Baraus (n=11)

Metal	Lakes					
	B. Miassovo	B. Ishkul	Argayash	B. Tatkul	Savelkul	Baraus
Mn	0.0151±0.0001	0.0728± 0,0002	0.0945 ±0.0007	0.1622±0.0014	0.0347±0.0041	0.0251±0.0002
Fe	0.0206± 0.0009	0.0310± 0.0005	0.1073±0.0014	0.0888±0.0009	0.0300± 0,0002	0.0250±0.0004
Cu	0.00148±0.00012	1.020± 0.003	0.00400± 0.00005	0.0021±0.0003	0,00027±0.00001	0.0070±0.0002
Zn	0.00414±0.000011	0.00792±0.00004	0.00415± 0.00012	0.00515±0.00013	0.00380±0.00014	0.00251±0.00011

Table 3. Bioconcentration factor (BCF) of heavy metals in plants from study lakes

Metal	Lakes							
	B. Miassovo	B. Ishkul	Argayash	B. Tatkul	Savelkul	Baraus	Mean values	Standard errors
Potamogeton lucens								
Mn	47748	13324	8042	4445	20403	27490	20242a	15845
Fe	31019	16580	4846	5518	18533	20920	16236b	9904
Cu	24000	41*	12000	21904	123333*	5857	15940b	8519
Zn	7317	4050	8571	7307	7105	14500	8141c	3458
Lemna minor								
Mn	60397	9752	8560	4389	20259	26932	21714a	20697
Fe	19514	12580	3541	3254	10700	14720	10718b	6388
Cu	17333	27*	8500	16190	96667*	3857	11470b	6414
Zn	3902	2784	6667	5000	4474	5500	4721c	1338

Note: *Excluded values when calculating the average. Different letters indicate significant differences among the heavy metals according to Fisher's LSD ($p<0.05$)

Leaves' elemental composition of *P. lucens* and *L. minor* was studied, such metals as Mn, Fe, Cu, and Zn are found in macrophytes of all the studied lakes. Statistically significant differences were found in the content of Fe, Cu, and Zn in the leaves of *P. lucens* and *L. minor*. Bioconcentration factors could be arranged into the following sequences: Mn>Fe=Cu>Zn.

ACKNOWLEDGEMENTS

The work was supported by Act 211 Government of the Russian Federation, contract No. 02. A 03.21.0011.

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