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

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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-Pendas and Pendas 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 (Khellaaf and Zerdouini 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. polyrrhiza, 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 Miasovo the average concentrations were: Cu-0.02 mg·L⁻¹, Zn-0.03 mg·L⁻¹, Pb-0.01 mg·L⁻¹, Mg-0.05 mg·L⁻¹, Sr-0.58 mg·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-Miasovo 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](image-url)
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·L⁻¹, the predominance of hydrocarbonate ions and rich microelement water composition are the characteristic features of the lakes (Gavrulkina 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 dm³ 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 HNO₃ 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:

\[
\text{BCF} = \frac{C_{\text{macrophyte}}(\text{mg} \cdot \text{kg}^{-1})}{C_{\text{water}}(\text{mg} \cdot \text{L}^{-1})}.
\]

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 about 100 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, Zannicheliaceae, 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 Jacqard 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 kg⁻¹DW, for all heavy metal concentrations in macrophytes

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

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
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)

<table>
<thead>
<tr>
<th>Metal</th>
<th>Lakes</th>
<th>B. Miassovo</th>
<th>B. Ishkul</th>
<th>Argayash</th>
<th>B. Tatkul</th>
<th>Savelkul</th>
<th>Baraus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn</td>
<td>0.0151±0.0001</td>
<td>0.0728±0.0002</td>
<td>0.0945±0.0007</td>
<td>0.1622±0.0014</td>
<td>0.0347±0.0041</td>
<td>0.0251±0.0002</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>0.0206±0.0009</td>
<td>0.0310±0.0005</td>
<td>0.1073±0.0014</td>
<td>0.0888±0.0009</td>
<td>0.0300±0.0002</td>
<td>0.0250±0.0004</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>0.0014±0.00012</td>
<td>1.00±0.003</td>
<td>0.0040±0.0005</td>
<td>0.0021±0.0009</td>
<td>0.00027±0.0001</td>
<td>0.0070±0.0002</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>0.0041±0.00001</td>
<td>0.0079±0.0004</td>
<td>0.00415±0.00012</td>
<td>0.00515±0.00013</td>
<td>0.00380±0.00014</td>
<td>0.00251±0.00011</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Metal</th>
<th>Lakes</th>
<th>B. Miassovo</th>
<th>B. Ishkul</th>
<th>Argayash</th>
<th>B. Tatkul</th>
<th>Savelkul</th>
<th>Baraus</th>
</tr>
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<tbody>
<tr>
<td>Potamogeton lucens</td>
<td></td>
<td>47748</td>
<td>13324</td>
<td>8042</td>
<td>4445</td>
<td>20403</td>
<td>27490</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>31019</td>
<td>16580</td>
<td>4846</td>
<td>5518</td>
<td>18533</td>
<td>20920</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>24000</td>
<td>12000</td>
<td>12904</td>
<td>12333*</td>
<td>5857</td>
<td>15940b</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
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<td>4050</td>
<td>8571</td>
<td>7307</td>
<td>7105</td>
<td>14500</td>
</tr>
<tr>
<td>Lemna minor</td>
<td></td>
<td>60397</td>
<td>9752</td>
<td>8560</td>
<td>4389</td>
<td>20259</td>
<td>26932</td>
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<td>Fe</td>
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<td></td>
<td>Cu</td>
<td>17333</td>
<td>27*</td>
<td>8500</td>
<td>16190</td>
<td>96667*</td>
<td>3857</td>
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<td></td>
<td>Zn</td>
<td>3902</td>
<td>2784</td>
<td>6667</td>
<td>5000</td>
<td>4474</td>
<td>5500</td>
</tr>
</tbody>
</table>

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

ACKNOWLEDGEMENTS

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