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# THE DISTRIBUTION AND ACCUMULATION OF CHROMIUM IN THE WATER, SEDIMENT AND MACROPHYTES OF SKADAR LAKE

**Vlatko Kastratović[1]*, Željko Jaćimović[2], Miljan Bigović[1], Dijana Đurović[3], Slađana Krivokapić[1]**

[1]*Faculty of Natural Sciences and Mathematics, University of Montenegro, Džordža Vašingtona bb, 81000 Podgorica, Montenegro,*
[2]*Faculty of Metallurgy and Technology, University of Montenegro, Podgorica, Montenegro,*
[3]*Institute of Public Health of Montenegro, Podgorica, Montenegro*
*****Corresponding author; E-mail: vlatkok@ac.me

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**ABSTRACT.** The aquatic macrophytes *Phragmites australis* (Cav.) Trin. ex Steud., *Ceratophyllum demersum* L., and *Lemna minor* L. were used as bioindicator plant species in order to define contamination level by Cr in Skadar lake (Montenegro). Plants, water and sediments were tested for the content of Cr at six locations around Lake Skadar during four periods in 2011. The content of Cr in the examined sediment was in the range of 35.6-127 mg/kg dry weight. The largest proportion of detected Cr (50.6%) was associated with the oxidizable phase in the form of organic complexes. The concentration of Cr in the studied macrophytes declined in the following order: *C. demersum* > *P. australis* > *L. minor*. The highest average content of Cr was detected in the leaf of *C. demersum* (11.4 mg/kg) in April.

**Keywords:** Lake Skadar, chromium, *P. australis, C. demersum, L. minor*

## INTRODUCTION

It is known that metals are natural ingredients of fresh water ecosystems where they are found in relatively low concentrations (BABOVIĆ *et al.*, 2010). The main sources of microelements, i.e. heavy metals in natural waters, are related to natural processes and anthropogenic impact. Their common property is that even at relatively low concentrations their effects are toxic and therefore they fall under the category of very dangerous environmental pollutants (KASTORI and MAKSIMOVIC, 2006).

The fact that heavy metals are present in the environment does not mean that they are available for adoption by living organisms or for incorporation by them. The determination of the total amount of heavy metals in the sediment is insufficient to assess their environmental impact on aquatic ecosystems. Some of the metals are incorporated into the crystal lattice of minerals or firmly attached to other substrates of sediment and thus does not represent a threat to biota. What is much more important is the information on the amount of bioavailable (mobile) metals that are only available to the living world in the lake. A simulation of the real situation in the lake environment that leads to the increased mobility of metals can be made by their extraction from sediment by using various means of extraction.

Aquatic plants absorb metals from environment and concentrate them into their tissues. In this way, the metal entered into the trophic chain (OUTRIDGE and NOLLER, 1991; TREMP and KOHLER, 1995). According to KOVACS *et al.*, (1984) aquatic plants may accumulate considerable amounts of heavy metals in their tissues (perhaps 10-$10^6$ times higher than those in nearby environment).

In natural waters, Cr concentrations are low except in locations that are loaded by waste containing Cr. The concentration of dissolved Cr in unpolluted lakes and rivers usually varies between 1 and 2 μg/$dm^3$. Higher values (5 to 50 μg/$dm^3$) are found in some large rivers that flow through industrial areas. This is usually related to the discharge of waste water from large industrial facilities (AGBABA *et al.*, 2008). Due to the presence of anthropogenic sources of pollution, increases in the level of Cr in the sediment have been found. The enrichment of sediments is correlated with the influx of ash from different sources – the combustion of oil, coal and wood, noting that the filtration devices on chimneys significantly contributed to the reduction of Cr content in the sediments (VESELINOVIĆ *et al.*, 1995). The solubility of Cr in the water present in the soil is less than other potentially toxic metals, thus explaining the low level of its adoption by the plants. The accumulation of Cr by plants can reduce growth, induce chlorosis in young leaves, reduce pigment content, alter enzymatic function, damage root cells and cause ultrastructural modifications to the chloroplast and cell membrane (CHOUDHURY and PANDA, 2005). Chromium toxicity in plants depends on its valence state (OLIVEIRA, 2012). Chromium (VI) is toxic to plants and does not play any role in plant metabolism. Contrarily to Cr(VI), Cr(III) at low concentrations (0.05–1 mg/$dm^3$) was found to promote growth and increase yield, but it is not considered essential to plants (PERALTA-VIDEA *et al.*, 2009; PAIVA *et al*., 2009). Chromium (III) is found to be less soluble in water and less toxic than Cr (VI) and is required in very low concentrations as an inorganic nutrient for some plants (SCHIAVON *et al*., 2008 ). The normal range of Cr in plants is 0.03-14 mg/kg (BOWEN, 1979) and the critical concentration is 15-30 mg/kg (KABATA-PENDIAS and PENDIAS, 1992).

The determination of metal content by sediment fractions, then the estimation of their bioavailability, the analysis of relevant ratios of metal content in the sediment, water and macrophytes and its distribution in different plant tissues, may all indicate the possible pathways of absorption, distribution and potential for bioaccumulation.

Our research aims to find the Cr content per fractions of sediments, and estimate bioavailability, including by analysing content ratios in the sediment, in water and macrophytes and plant tissue distibution, which can indicate possible absorption or distribution mechanisms and ability to bioaccumulate Cr.

## MATERIALS AND METHODS

The aquatic macrophytes used as indicator species in this study *Phragmites australis* (Cav.) Trin. ex Steud., *Ceratophyllum demersum* L., and *Lemna minor* L. were taken from six locations around Lake Skadar, Montenegro. The locations were: 1- Raduš, 2- right estuary of the Morača River, 3- estuary of the Morača River, 4- Plavnica, 5- Crni Žar and 6- Rijeka Crnojevića (Figure 1). The samples were collected during four separate periods in 2011. The samples of *P. australis* and *C. demersum* were collected four times during 2011 at the six locations. The samples of *L. minor* were collected in two time periods, in August and October, from four locations. Samples of sediment and water were taken at the same time and from the same places as plant material.

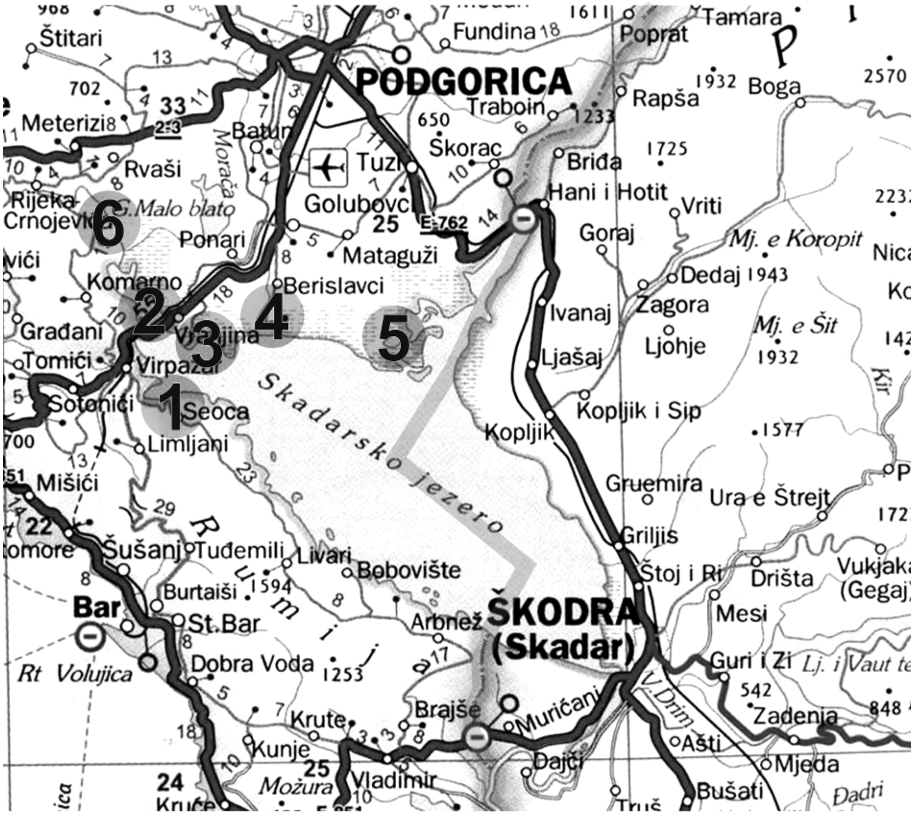


Figure 1. Location of the sampling stations around Lake Skadar

The plant material was separated in the laboratory into the root, stem and leaf of *P. australis*, the stem and leaf of *C. demersum* and the root and leaf of *L. minor* in order to determine bioaccumulation degree of each organ. The plant material then was dried at 75$^{o}$C over a 48 hour period. The samples were ground to a fine powder and homogenized. The samples were mineralized to avoid the influence of the matrix. The prepared plant samples, approximately 0.5 g (± 0.0001 g), were mineralized in a Milestone Microwave Ethos 1, with a mixture of $HNO_3$ and $H_2O_2$ (3:1).

Sediment samples, approximately 500g, were air dried and then dried at 75$^{o}$C over 48 hours. The dried sediment samples were chopped in an agate mortar and sieved through a sieve <1.5 mm. The sediment samples, approximately 0.5 g (± 0.0001 g), were mineralized with a mixture of HCl : $HNO_3$ (3:1) in a microwave furnace, a Milestone Microwave Ethos 1 (USEPA, 2007). The water samples were filtered through a 0.45 μm Millipore filter and stored in 1L plastic bottles with the addition of 2 mL of $HNO_3$.

In order to determine the distribution of the Cr in the sediment we applied a modified BCR (the Community Bureau of Reference of the European Union) sequential extraction procedure of the sample sediment (PUEYO *et al.*, 2003).

Mobile forms of Cr were determined using five different extracting solutions: cation-exchangeable ($NH_4Cl$, $CaCl_2$, $CH_3COONa$), organic acid (oxalic acid) and complexing reagent (EDTA).

Determining the concentration of Cr in the samples of water, sediments and plants was conducted by ICP-OES technique on a “Spectro Arcos” device.

The capacity of plants to absorb and accumulate metals from the growth media was evaluated by their bio-concentration factor (BCF). The BCF was calculated as the ratio of the concentrations of metals in parts of the plant and the water: BCF = $[\text{Metal}]_{\text{part of plant}}/[\text{Metal}]_{\text{water}}$ (HAWKER and CONNELL, 1991). The ability of plants to transport metals from their roots to their shoot was assessed by translocation ability (TA). Translocation ability is calculated as the ratio of the concentrations of metals in a part of a plant and its root: $TA=[Metal]_{part\ of\ the\ plant}/[Metal]_{root}$, and for *C. demersum* as the ratio of metal concentrations in the leaf and the stem: $TA=[Metal]_{leaf}/[Metal]_{stem}$ (DENG *et al.*, 2004).

## RESULTS AND DISCUSSION

Low Cr contents were recorded in the water. At four of the six sampled sites the content of Cr was below the detection limit of the instrument ($LOD_{Cr}$ = 0.002 mg/dm$^3$). At the locations of Plavnica and Rijeka Crnojevića, there was no seasonal variation in Cr content and the readings amounted to 0.003 and 0.002 mg/dm$^3$, respectively.

The concentration values of Cr in the sediments are given in Table 1. During the research period (April-October) there were no recorded statistically significant temporal variations in the concentration of metals in the sediment, but there were spatial variations. The highest concentrations of Cr were recorded in the sediment samples from Plavnica, and the lowest ones were recorded in the sediments from Raduša and Rijeka Crnojevića.

Table 1. Minimum - maximum and average concentrations ± standard deviation (S.D.)* of Cr in sediment (mg/kg)

| Location | Raduš | Right mouth | Left mouth | Plavnica | Crni Žar | Rijeka Crnojevića |
|---|---|---|---|---|---|---|
| Sediment | 35.6-44.6<br>40.7±3.93 | 73.1-76.3<br>75.0±1.34 | 68.9-74.5<br>71.2±2.38 | 117-127<br>123±4.54 | 54.6-62.6<br>57.9±3.46 | 40.4-45.5<br>42.6±2.11 |

*n=4

The mean values of the concentrations of Cr (mg/kg) as a result of the sequential extraction of sediments are shown in Table 2:

Table 2. Distribution of Cr (mg/kg) in fractions of sediments of Lake Skadar:

| Fractions | I | II | III | IV |
|---|---|---|---|---|
| **Min.-max.** | 0-1.28 | 0.10-3.06 | 11.8-91.4 | 15.4-57.4 |
| **Mean value** | 0.24 | 1.14 | 35.2 | 31.9 |

I - Removable and easy mobile; II - Reductable; III - Oxidable; IV - Residual

The highest amount of Cr is in the oxidable (organic) fraction, at a percent by weight of 50.6%, relative to the total content. Chromium follows the following trend of distribution by fractions: III > IV > II > I. In the changeable, easy soluble fraction there is an inconsiderable amount of Cr (0.35%) and this might be considered immobilized metal.

Table 3 shows the mean values of the total and extractable Cr content (minimum and maximum values and mean values) in the sediment for five different extraction mechanisms:

Table 3. Concentration of Cr (mg/kg) extracted by various solvents

| | Total Cr | 0.1 M $NH_4Cl$ (pH 9) | 0.1 M $CaCl_2$ (pH 7) | 0.1 M $CH_3COONa$ (pH 9) | 0.1M $H_2C_2O_4$ (pH 2) | 0.1 M EDTA (pH 7) |
|---|---|---|---|---|---|---|
| **Min.-max.** | 35.6-127 | 0.02-0.22 | nd | 0-0.20 | 2.98-16.4 | 0.29-3.36 |
| **Mean value** | 68.4 | 0.08 | | 0.08 | 7.91 | 1.20 |

nd - not detected

The order of solvents with a descending share of extracted Cr, compared to the total content, was as follows: $H_2C_2O_4$ (11.6%) > EDTA (1.75%) > $NH_4Cl$ (0.11%), $CH_3COONa$ (0.11%) > $CaCl_2$ (0%).

Acidic conditions (0.1 M $H_2C_2O_4$, pH=2) have shown a higher potential for the extraction of Cr. Chromium showed a 6.6 times higher rate of extractability in oxalic acids than in EDTA. The generation of the Cr (III) complex of EDTA at room temperature is very slow, in spite of its high stability constant (pKa = 23).

The results of the metal content in individual parts of the examined macrophytes across the seasons are given in Table 4:

Table 4. Seasonal changes in concentrations of Cr (mg/kg of dry matter) in parts of investigated plants; minimum and maximum value and the mean value of concentration ± standard deviation

| **Plant** | **Part of plant** | **Min.-max. Mean value±S.D.** | | | |
|---|---|---|---|---|---|
| | | **April** | **June** | **August** | **October** |
| ***P. australis*** | **root** | 1.25-4.73<br>3.32±1.21 **a**[*] | 3.27-19.1<br>10.3±6.31 **a** | 4.00-12.4<br>7.98±2.80 **a** | 2.37-10.7<br>5.90±2.32 **a** |
| | **stem** | 0.49-1.77<br>1.05±0.52 **b** | 1.28-6.16<br>2.68±1.76 **b** | 0.80-3.75<br>2.09±1.22 **b** | 0.40-3.20<br>1.26±1.03 **b** |
| | **leaf** | 0.21-0.38<br>0.28±0.06 **b** | 0.28-0.88<br>0.48±0.22 **b** | 0.41-1.09<br>0.66±0.24 **b** | 0.70-1.99<br>1.24±0.43 **b** |
| ***C. demersum*** | **stem** | 3.55-8.81<br>6.32±1.85 **b** | 1.49-4.43<br>3.21±1.32 **bcd** | 1.00-2.86<br>2.12±0.78 **cd** | 0.89-2.98<br>1.61±0.74 **d** |
| | **leaf** | 4.27-18.6<br>11.4±4.97 **a** | 3.14-9.00<br>5.78±2.07 **bc** | 2.29-8.19<br>4.62±2.09 **bcd** | 2.08-7.28<br>3.92±1.88 **bcd** |
| ***L. minor*** | **root** | | | 0.79-3.08<br>1.60±1.01 | 2.18-4.58<br>3.31±1.04 |
| | **leaf** | | | 1.18-1.78<br>1.36±0.28 | 1.57-5.32<br>2.81±1.73 |

n=6

[*] Means indicated by different letters within each row are significantly different at $p < 0.05$.

Figure 2 presents the average seasonal changes in the bioconcentration factor (BCF) of the examined macrophytes for Cr.

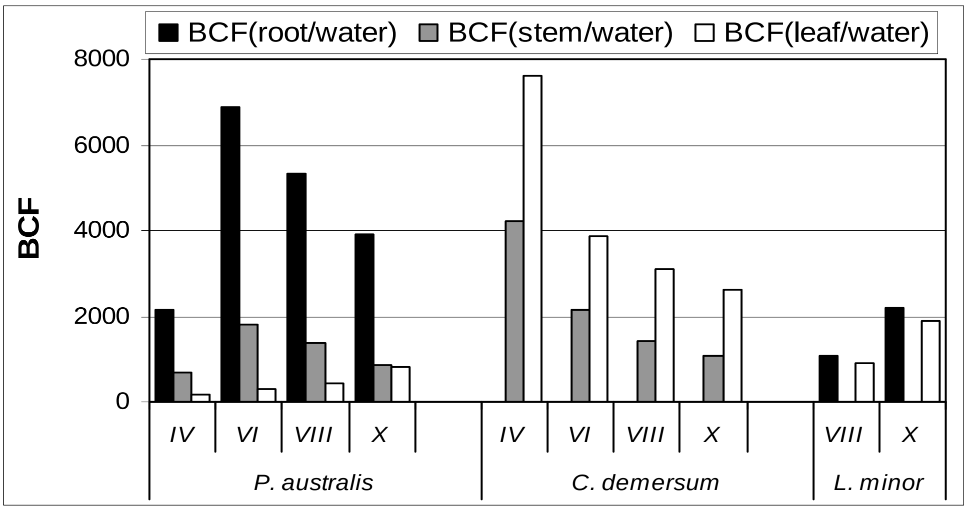


Figure 2. Seasonal changes in bioconcentration factors (BCF) for Cr in the examined macrophytes

Regardless of the sampling period, Cr in the parts of *P. australis* follows the trend of concentration: root > stem > leaf.

The concentrations of Cr in some parts of *C. demersum* differ significantly from their concentrations in the water and sediment, and follow the trend: sediment > *C. demersum*$_{\text{leaf}}$ > *C. demersum*$_{\text{stem}}$ > water. A higher content of Cr is recorded in the leaf of *C. demersum* at all locations and in all seasons. It is noteworthy that in submerged macrophytes translocation is not definitive, since in addition to the translocation from the stem, the leaf contains metals absorbed from water.

The concentration of Cr in some parts of *L. minor* decreases in the following order: sediment > *L. minor*$_{\text{root}}$ > *L. minor*$_{\text{leaf}}$ > water. The highest amount of Cr was found in the root sampled from the right bank of the mouth of the Morača River and in the leaf from the left bank of the mouth of the Morača River. Looking at the seasons, almost twice as much content is recorded in October than in August, in both the root and the leaf. Higher values for the leaf and root were recorded in October than in August. The average seasonal values of metal are higher in the root than in the leaf.

Values for the translocation ability of Cr are shown in Table 5:

Table 5. Seasonal changes in the translocation ability (TA) of macrophytes tested for Cr

| **Macrophytes** | | **April** | **June** | **August** | **October** |
|---|---|---|---|---|---|
| ***P. australis*** | **stem/root** | 0.24 | 0.22 | 0.19 | 0.16 |
| | **leaf/root** | 0.08 | 0.04 | 0.08 | 0.19 |
| | **leaf/stem** | 0.34 | 0.18 | 0.41 | 1.19 |
| ***C. demersum*** | **leaf/stem** | 1.52 | 1.78 | 1.97 | 2.27 |
| ***L. minor*** | **leaf/root** | | | 0.81 | 0.68 |

**Chromium distribution in sediment**

Chromium had an average content of 68.4 mg/kg of dry sediment at the six studied locations around Lake Skadar, and is in the range of 35.6 to 127 mg/kg (Table 1). The highest concentration was observed in the sediments taken from the site at Plavnica. Chromium content in the easily available fraction either was not recorded or an insignificant amount of bioavailable Cr was recorded in all locations. According to PETROVIĆ (1981), high concentrations of Cr in the sediment are of geological origin and are not related to anthropogenic pollution. The largest amount of Cr in the sediments of Lake Skadar was in the residual fraction (51.3%) (Figure 2) followed by the oxidized fraction (46.7%), as a result of the stronger adsorption ability of Cr into organic matter than the Fe and Mn-oxyhidroxides. The content of Cr in fraction I is very low, only 0.7% of its total content, which reduced its potential toxicity as a pollutant in Lake Skadar.

DAVIDSON *et al.* (1998) found the highest content of Cr in the residual fraction. Additionally, FERNANDEZ *et al.,* (11) recorded the highest concentration of Cr in the fourth fraction with a lower content of oxidized material and found that it was significantly lower in the reducible fraction of the sediment.

**Bioaccumulation of Cr in macrophytes**

***Phragmites australis*** **(Cav.) Trin. ex Steud**

The results of this study show that most of Cr taken up by plants, was found in the roots (Table 4). The highest root and stem accumulation was recorded in June, and then the rate decreased. Chromium concentration in the leaves of *P. australis* increased slightly during the entire investigation period.

BRAGATO *et al.* (2009) examined the accumulation of four metals (Cu, Zn, Ni and Cr) in *P. australis* in experimentally constructed wetlands which receive water from the River Po in Italy. Chromium was found to be accumulated in the stems and rhizome of *P. australis* mostly in July, and then the amount decreased and became constant from August to December.

In addition to lower accumulation, Cr also showed low mobility. In this study, the average ratio of accumulation in the root/aboveground part ranged from 4.10 (April) to 25.8 (June). During the growing season, the translocation ratio decreased indicating the limited transportation of toxic metals from roots to shoots. Some authors (KÄHKÖNEN *et al.*, 1997; BALDANTONI *et al.*, 2004; VYMAZAL *et al.*, 2007) indicate a 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 for this element from roots to the green parts of the plant. The relatively low concentration of Cr in foliar tissues during the whole sampling period is probably the result of the plant needing to prevent the pollution of the photosynthetic apparatus, as suggested by other authors (LANDBERG and GREGER, 1996; STOLTZ and GREGER, 2002; BRAGATO *et al.*, 2006).

***Ceratophyllum demersum* L.**

Significant seasonal variations in concentrations of Cr have been observed (Table 4). The highest concentrations were recorded in the stem and in the leaf during April, which then decreased until October. Chromium content ranged from 0.89 to 8.81 mg/kg (average 3.32 mg/kg) in the stems while in the leaves it ranged from 2.08 to 18.6 mg/kg (average 6.44). The bio-concentration degree of Cr in relation to the sediment in *C. demersum* is low. However, there is a noticeable translocation within the plant, with an average ratio leaf/stem of 1.85 (Table 5), depending on locations and sampling periods.

The results of RAI *et al.* (1995) have showed that *C. demersum*, under laboratory conditions, reduces the level of Cr in water contaminated by effluents from various industrial sources. Reduction varies from a concentration of 4.866 μM to below 2 μM. Their results indicated the possibility of removing Cr from diluted waste water using this plant. Higher concentrations of Cr in the leaves (1.03-2.71 mg/kg) compared to the stems of *C. demersum* were found by POURKHABBAZ *et al.* (2011). In the paper written by OSMOLOVSKAYA and KURYLENKO (2005) the Cr content was 16.5 times higher in the tissues of *C. demersum* originating from polluted lake ecosystems when compared to unpolluted ones.

***Lemna minor* L.**

Chromium has a low content in the parts of *L. minor*. The mean value of Cr during the research period in the root was 2.46 mg/kg, while in the leaf it is 2.09 mg/kg. The content of Cr in the leaf in October (5.32 mg/kg) significantly stands out from the other results for Cr. Higher values of Cr in the roots and in the leaves were recorded in October than in August. The bioaccumulation capacity of *L. minor* for Cr was higher in the roots comparing to the leaves. The bioaccumulation ability of *L. minor* for Cr is moderate as has been demonstrated by other authors (ZAYED, 1998). ATER *et al.*, (2006) observed the effectiveness of *L. minor* in the phytoremediation of Cr, especially for lower levels of contamination of the aquatic environment.

**Correlation analysis of Cr in macrophytes and the sediment**

Table 6 shows the values of Pearson's correlation coefficients (r) between the content of Cr in the surrounding environment (the sediment) and various parts of the macrophytes.

On the basis of the presented results, the following macrophytes of their individual parts can be proposed as bioindicators to assess the Cr load of the surrounding environment (sediment): *P. australis* (the stem), *C. demersum* (the stem and the leaf at the beginning and end of the growing season) and *L. minor* (the root and the leaf at the end of the growing season).

Table 6. Correlation coefficients, between the sediment and certain parts of the macrophytes

| **Metal** | | **Month** | ***P. australis*** | | | ***C. demersum*** | | ***L. minor*** | |
|---|---|---|---|---|---|---|---|---|---|
| | | | **root** | **stem** | **leaf** | **stem** | **leaf** | **root** | **leaf** |
| **Cr** | **Sediment** | **IV** | 0.45 | -0.36 | 0.65 | 0.65 | 0.58 | | |
| | | **VI** | 0.81 | 0.79 | -0.19 | 0.31 | 0.30 | | |
| | | **VIII** | -0.20 | 0.81 | -0.25 | 0.33 | 0.91 | -0.03 | -0.15 |
| | | **X** | -0.13 | 0.85 | -0.79 | 0.99 | 0.88 | 0.51 | 0.91 |

$p \leq 0.05$

Most of previous studies have shown insignificant correlation between the concentration of metals absorbed by macrophytes and concentrations in sediments (NOURI *et al.*, 2009). KELLER *et al.* (1998) consider that the absorption of the metal, either by roots or leaves, is not in linear correlation with increasing metal content in the surrounding environment. The lack of correlations, in most cases, indicate that these relationships do not dominate in determining the metal content adopted by the plant. The accumulation of metals in the tissues of macrophytes, in addition to their content in the sediment, depends on the interaction and variation of many other factors.

In contrary to this paper, SZYMANOWSKA *et al.* (1999) found a high positive correlation between the concentration of Cr in *P. australis* and *C. demersum* and the surrounding sediments. Unlike the tests in this paper, BRAGATO *et al.* (2006) found that the concentration of Cr in the incoming water and in the sediment was not correlated with the content in *P. australis*.

## CONCLUSIONS

Chromium is an immobilized metal, because the greater of its part is incorporated the crystal lattice of minerals (residual fraction). A small amount of this metal (as seen in relation to the total content) is in the removable, easy soluble fraction.
Bioavailable amounts of Cr originate from the exchangeable and smaller part of the organic fraction of the sediments.

Chromium concentrations in plant tissues depended on sampling season. In *P. australis* concentrations decreased from June to the end of the growing season, while in *L. minor* they increase from August to October. The highest Cr content during the tested season was determined in the leaves of *C. demersum* in April (11.4 mg/kg), and the lowest was in the same month in the leaves of *P. australis*. The highest average concentration of Cr for the tested season was in the root of *P. australis*. The amount of accumulated Cr decreased on average during the season within the plants in the following order: *C. demersum*> *P. australis*> *L. minor*.

The average value of the bioaccumulation factor calculated for the entire plant was identical for *P. australis* and *C. demersum*, whereas in *L. minor* the value was significantly less.

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