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Original scientific paper

Bioaccumulation and translocation of heavy metals by *Ceratophyllum demersum* from the Skadar Lake, Montenegro

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Abstract: Lacustrine systems are very complex water systems in terms of the transport of and interaction with heavy metals. Primarily due to its high variability and current chemical parameters, the tissue of macrophytes is a more plausible bio-indicator of the load level of metals within lake ecosystems than are water or sediment analyses. The macrophyte, *Ceratophyllum demersum*, sampled from the Skadar Lake in Montenegro was used as a bio-indicator. Sediments, water and plants were examined for their contents of ten metals in four different periods of 2011. The concentrations of the metals followed the trend: sediment > leaf *C. demersum* > stem *C. demersum* > water. There were differences in the sequences of the metal content in the plant compared to the sequences of their bioaccumulation ability. These differences suggest a different capacity of macrophytes for different metals. The accumulation of Mn was several times higher than the accumulation of the other analyzed metals. The highest ratio of leaf/stem concentrations was recorded for Mn (2.19) and the lowest was for Pb (1.04). The highest contents of Cd, Co, Cr, Pb, V and Sr were found in the tissues of *C. demersum* at the beginning of the growing season, whereas Ni, Zn, Cu and Mn were found at the end of the vegetative phase.

Keywords: *Ceratophyllum demersum*; Lake Skadar; heavy metals; bioaccumulation; translocation.

INTRODUCTION

Due to their toxicity, resistance to bio-degradation and long duration in the environment while entering the food chain, heavy metals are considered to be

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some of the most serious potential pollutants of aquatic ecosystems.[1] Therefore, the behavior of metals in sediment, their adoption by the plant and further fate of the plant tissues, although extremely complex, is undoubtedly of great importance for research in the environment. There is no single theoretical model by which the metal content of plants could be predicted from its content in a nearby sediment or water. Likewise, no pattern existed by which plants acquired metals and transported them through their tissues.

The application of plant organisms for research of heavy metal pollution in a lake environment has a number of advantages over standard methods for the detection of metals in water by chemical analysis. Thus, metal concentrations in water are often below the detection limit of the employed instrument whereas the concentrations of metals in plants are much higher and allow for the determination of the available biological and cellular metals in aqueous medium.[2–4]

When choosing the type of macrophyte for either biomonitoring or phytoremediation, it is necessary to reach a compromise between certain conditions:[5]

– it is essential that macrophytes constantly accumulate and tolerate large amounts of metal without harmful effects on their growth and development;

– it must be widespread, but being linked to one place, it could be a real representative of a given area;

– it must be easily accessible for the collection, identification and handling;

– its lifetime must be long enough for it to become a bioaccumulation phenomenon;

– there must be sufficient disposable tissue for chemical analysis;

– it must tolerate physical and chemical changes in the environment.

The achieved level of precision and accuracy of instrumental analytical methods today, and the training and experience of the analysts keeps the errors in the determination of the contents of the metals to a minimum. The greatest source of inaccuracies could be the sampling of the materials; hence, it is especially important to ensure that the sampled material is truly representative.

The preparation of the samples for analysis must be consistent with the accepted standards in order that the results are comparable with those from other geographic areas.

Ceratophyllum demersum is a perennial submerged, free-floating aquatic rootless plant, which grows in stagnant or slow-moving waters. The buds grow in the winter at the bottom of lake water and form a new plant in the spring.

C. demersum can be used as a measure of the lake pollution, as its tissue may contain toxic metals, such as Cd and Pb.[6] It could also be used to remove low concentrations of heavy metals from aquatic ecosystems.[7]

There are a small number of studies on the trace metals concentrations in plant species of Lake Skadar, and particularly insufficient data on their concentration in different parts of aquatic macrophytes and their seasonal variations.[8]

In the present study, the content of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn, Sr and V) in sediment, water and different organs of *C. demersum* collected from Lake Skadar, Montenegro, was investigated during different periods in 2011.

The aim of this study was to determine the ways and means of metal adoption within *C. demersum* tissue, as well as the differences and degree bioaccumulation in dependence on the metal, plant part, location and season.

EXPERIMENTAL

Geographical details about the study area are given in the Supplementary material to this paper.

Sample collection

Samples of *C. demersum* were collected four times by hand from six locations every 60––70 days from early April to late October in 2011 (Fig. S-1 of the Supplementary material). Places of maximum coverage and density were selected for examination of the macrophyte sample. Aiming at repeating the results from each site, 3–4 whole, healthy plants of similar size, shape and weight were sampled at each site separately within an area of about 25 m^2. The plant material was labeled, packed in polyethylene bags and transferred to the laboratory in the shortest possible time. Water and sediment samples were also taken from the same place as the plant material. Sediment sampling was conducted using an Eckman dredge to a depth of 0–20 cm. Stones and coarse plant material were mechanically removed. The sediment samples were placed in plastic boxes, carefully labeled and transferred to the laboratory for further analysis. Water samples were collected from the depth of 0.5–1 m using 1.5 L PET bottles. The samples were stored in a refrigerator (5±2 °C).

Sample analyses

The sampled plant material was first washed with tap water, and then twice with deionized water and gently dried with a paper towel. The samples of the *C. demersum* plants were separated into stem and leaf to determine bioaccumulation diversity of the plant organs. The plant material was then dried at 75 ° C for 48 h. The samples were ground into a fine powder and homogenized in an electrical mill (Büchi-Mixer B-400). The samples were mineralized to avoid the influence of the matrix. 0.5 g of prepared samples were approximately measured with an accuracy of ±0.0001 g and mineralized using a Milestone Microwave Ethos model 1600, with a mixture of HNO_3 and H_2O_2 (3:1). Mineralization was realized in two stages: pretreatment at a power of 300 W for a period of six min, followed by five minutes of microwave digestion at a power of 500 W. After digestion, the solutions were diluted with deionized water to a final volume of 50.0 cm^3.

The plant samples were ready for analysis and dry ashing. The measured plant material (0.5±0.0001 g) was heated in a porcelain crucible on hot plate to ash while care was taken not to ignite the sample. Then it was placed in a muffle furnace and the temperature was gradually increased to 550 °C, at which temperature, the samples were calcined for 5 h. The carbonized plant material was transferred with 2 M HCl to a 50.0 cm^3 volumetric flask. Prior to analysis, the samples were kept in plastic bottles. The analytical accuracy of the measured concentrations of the metals in the plants was tested using a tealeaf reference material (INCT–TL-1). The reproducibility of the results was within 8 % of the certified values.

The sediment samples were dried in air and then in an oven at 75 ° C for 48 h. The dried sediment samples were ground in an agate mortar and sieved through a 1.5 mm sieve. Approximately, 0.5 g (±0.0001 g) of each sample mineralized under pressure and high temperature and microwave digested with a mixture of HCl:HNO_3 (3:1). After digestion, the solutions were diluted with 2 M HNO_3 to a final volume of 100 cm^3.[9] The analytical accuracy was determined using a certified standard reference material of the National Institute of Standards and Technology (USA) for trace elements in lake sediment (SRM 2709). The recoveries were within 10 % of the certified values.

Water samples were filtered through a 0.45 μm Millipore filter and stored in plastic 1L bottles to which 2 mL of 70 % super pure nitric acid was added.

All parts of the plant samples, sediments and water were prepared in triplicate and their average value was assessed. Blank solutions were added to the series of samples and measured after every tenth sample determination. The concentrations of the heavy metals (Cd, Cu, Co, Cr, Mn, Ni, Pb, Zn, V and Sr) were determined by the ICP-OES technique using a Spectro Arcos instrument.

Statistical analysis

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

The ability of plants to absorb and accumulate metals from the aqueous growth media was evaluated using the bio-concentration factor (*BCF*). The *BCF* value is calculated as the ratio of the concentrations of metals in the plant and the associated water:

$$BCF = \frac{\left[\text{Metal}\right]_{\text{part of plant}}}{\left[\text{Metal}\right]_{\text{water}}}$$

Higher BCF values imply greater phyto-accumulation ability of the plant.

The possibility of plants to transport metals from the stems to the leaves was estimated using the translocation ability (*TA*). Translocation ability was calculated as the ratio of the concentrations of metals in leaves and stems:

$$TA = \frac{\left[\text{Metal}\right]_{\text{leaf}}}{\left[\text{Metal}\right]_{\text{stem}}}$$

A higher *TA* value means higher translocation ability.

RESULTS

The minimum and maximum values of the metal content in the lake water during the research period in the studied locations, as well as the mean value with the standard deviation results are presented in Table S-I of the Supplementary material to this paper.

There was no temporal variation in the mean concentrations of metals at the 95 % level of confidence. There were considerable spatial variations in the results, reflected in the high standard deviation. Low concentrations of metals were registered in the water samples. Strontium has the highest concentration, whereas Cd, Co and Pb were below the LOD (lower limit of detection) at all sampling locations. Chromium was below the LOD at one location and Ni at three of the six sampling locations. Low concentrations of metals naturally occur in fresh water. The levels in sediments and organisms are much higher because of their concentration through natural processes. Even in relatively pristine areas, metal contents can vary between different water systems because of variations in the sediment characteristics and organic matter concentration. The amounts of metals in macrophytes (and other organisms) and the "total" metal content in the sediment cannot be estimated based on their content in the surrounding water.

The results of the determination of the average metal content in the sediment are given in Table S-II. Temporal and spatial changes in metal concentrations (ppm d.w.) in the parts of *C. demersum* are given in Table S-III. The seasonal minimum and maximum concentrations in the individual parts of *C. demersum* are given in Table S-IV. The Tables S-II–S-IV can be found in the Supplementary material to this paper. There was no significant seasonal variation in the results of the metal content in the sediments, as opposed to the variations found in the organs of *C. demersum*.

The concentrations of metals in the individual parts of *C. demersum* were significantly different from their concentrations in water and sediment and followed the trend: sediment > leaf *C. demersum* > stem *C. demersum* > water, except for Mn (stem and leaf) and Zn (leaf, October), when a higher concentration was found than in the sediment.

Concentrations of the metals in the stem and in the leaf of *C. demersum* followed the trend: Mn > Zn > Sr > Cu, Ni > Pb > Cr > Co > V > Cd. A similar sequence of metal contents in the tissues of *C. demersum* were reported previously.[10–14]

The concentrations of Co and Cr did not show spatial variation in the results (see Tables S-III and S-IV). Significantly higher values of the concentration of Mn, Ni and Zn were recorded at the locations Raduš, Plavnica and the right estuary of the Morača compared to the other three sites (Table S-III). The concentration of cadmium was significantly higher in the tissues of *C. demersum* sampled from Crnojevića River, Cu from the left estuary of the Morača, Pb from the right estuary of the Morača and V from Crni Žar. Strontium had significantly lower scores from the sites Crni Žar and Crnojevića River (Table S-III).

The bioaccumulation capacity of *C. demersum* was shown through the bioaccumulation factors (Fig. 1), which shows that the concentration of the studied metals in the stem and leaf decreased as follows:

– for the stem ($BCF_{\text{stem/water}}$): Mn > Zn > Ni > Cr > Cu > Co > Pb > Sr > > V > Cd;

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

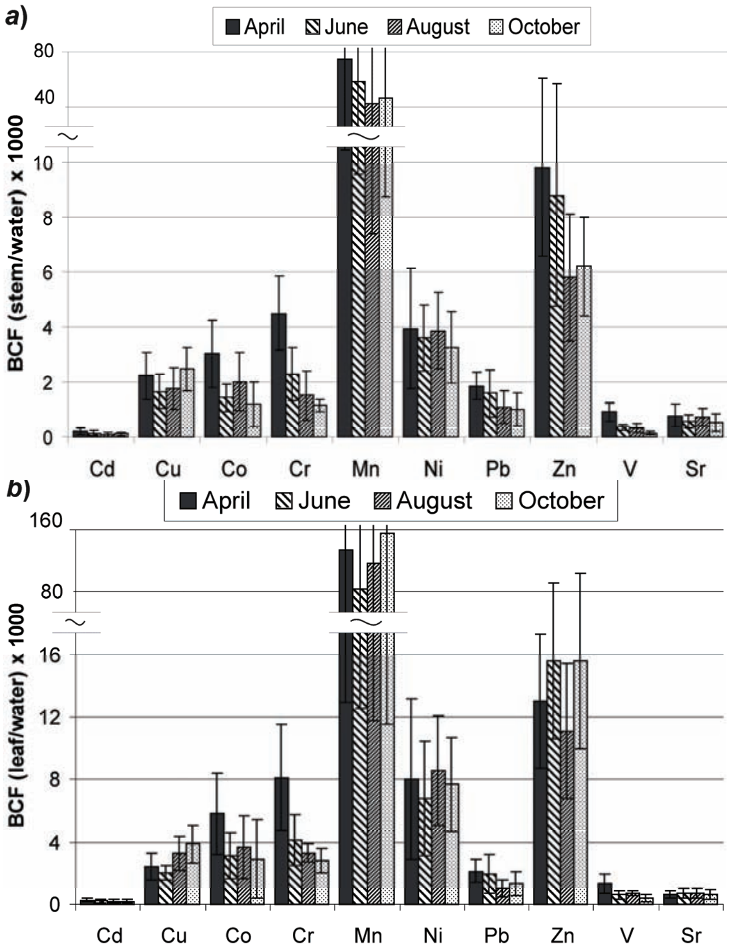

Fig. 1. Seasonal changes of bioconcentration factor (BCF): a) stem and b) leaf of *Ceratophyllum demersum*.

Differences in the sequences of the metal contents in the plant, the sequences of their bioaccumulation capacity and their numerical relationships could be seen to a certain extent. These differences suggest a different bioaccumulation capacity of macrophytes for different metals. The plant accumulates certain metals irrespective of their concentrations in water and sediment, which is obviously a characteristic provided by its capacity for each individual element.

The orders of the intensity of bioaccumulation of the examined metals in the stem and leaf were different. These differences cannot be interpreted solely by differences in metal translocation through the plant because, most probably, the bioaccumulation capacities of the leaves for individual metals directly from the water are different.

The bioaccumulation capacity of *C. demersum* for Mn was several times higher than those for the other metals (Fig. 1).

The contents of all investigated metals in the leaf of *C. demersum* were higher than in the stem at all sites and during all seasons (Tables S-III and S-IV), except Pb (at three locations during the season in August) and Sr (at three locations during the season in April). The largest ratio leaf/stem concentration of 7.14 was recorded for Cd in the sample from the right estuary of the Morača in August, whereas the lowest ratio of 0.42 was found for Pb in the sample from the left estuary of the Morača in August. The seasonal values of the trans-located capabilities are given in Table I, from which it can be seen that the translocation ability decreases in the following order: Mn > Ni > Co > Cr > V > Zn > Cd > Cu > Sr > Pb. It is important to note that with a submersed macrophyte, translocation is not uniquely determined, since in addition to translocation from the stem, the leaf certainly contains metals absorbed directly from the water.

TABLE I. Seasonal changes of translocation ability (*TA*)

Metal	Sample	April	June	August	October
Cd	Leaf/stem	1.43	1.33	1.96	1.09
Cu	Leaf/stem	1.03	1.14	1.82	1.35
Co	Leaf/stem	1.78	2.17	1.64	2.13
Cr	Leaf/stem	1.52	1.78	1.96	2.27
Mn	Leaf/stem	1.47	1.47	2.50	3.33
Ni	Leaf/stem	2.00	1.89	2.22	2.22
Pb	Leaf/stem	1.11	1.03	0.82	1.22
Zn	Leaf/stem	1.19	1.78	1.75	2.38
V	Leaf/stem	1.39	1.59	2.22	2.22
Sr	Leaf/stem	0.86	1.37	1.05	1.26

DISCUSSION

Cadmium

Of the studied metals, cadmium was present in the lowest amount in the tissues of *C. demersum* in the stem from 0.03 to 0.35 ppm (mean annual value of 0.13 ppm) and from 0.05 to 0.42 ppm (mean value 0.21 ppm) in the leaf. The concentration of Cd in the stems and leaves decreased during the season (Tables S-III and S-IV).

Pourkhabbaz *et al.*[13] found higher concentrations of Cd in the leaf of *C. demersum* from the Anzali Wetland, Iran, 0.94–1.26 ppm. Borišev *et al.*[10] did not find Cd in the tissues of *C. demersum* sampled from four sites on the River Jegricka (Serbia), while Bilyk[15] found Cd within the limits of detection in the plant of Vyrlytsa Lake (Ukraine). On the contrary, Babovic *et al*.[12] recorded 9.69 ppm throughout the whole plant from the Fish Pond Ečka, Serbia.

Bio-concentration factor (*BFC*) for Cd was the highest in April, 1.16, which is given as the ratio of the Cd concentration in the whole plant and sediment, as compared to the water >420. Assia *et al*.[16] found a BCF_{Cd} of 600, as the ratio of Cd content in *C. demersum* (Bahr in the El Bakar drain, Egypt) in relation to water. They also found that the value of the *BFC* tended to decline with increasing metal concentration in the water.

Out of 16 tested macrophytes from around Wroclaw, Poland, Samecki-Cymerman and Kempers[17] found the highest concentration of Cd in the tissue of *C. demersum*, 0.65 ppm. Foroughi *et al*.[18] claimed that *C. demersum* collected from the Zayanderood River (Iran) had a greater ability for the removal of Cd (even Pb and Ni) with respect to Fe, Mn and Zn. In their experiments with prolonged plant contact with wastewater, opposing results were obtained for the absorption of some metals.

Copper

The minimum and maximum values of Cu in the stem of *C. demersum* for all seasons were in the range 6.48–24.5 ppm (Tables S-III and S-IV) with an average annual concentration of 14.2 ppm, while in the leaves, the range was 9.85–34.5 ppm (mean value 19.5 ppm). The variations during different seasons in the Cu concentration in the stems and leaves were the smallest compared to the other studied metals (Table S-III). The largest leaf/stem ratio concentration of 1.82 was recorded in August. The maximum value of the bio-concentration factor (for leaf) in relation to water was found in the October sample and it amounted to 3900. In April, the Cu content in the whole plant was similar to that in the sediment. Contrary to this, in August and October, the content was higher in the plant (Tables S-II and S-III). These values mostly refer to accumulation capabilities of *C. demersum* for Cu, and the plant is recommended as bio-indicator of Cu, regardless of its low content in the water.

El Sarraf[19] found two-times higher concentration of Cu in the leaves of *C. demersum* than in the stems. Pourkhabbaz *et al*.[13] also found a higher Cu content in the leaves of *C. demersum* in the range of 19.9–40.0 ppm. Osmolovskaya and Kurylenko[20] detected 10.8 times more copper in *C. demersum* from contaminated compared to uncontaminated areas from five inland ponds within the city of Saint-Petersburg.

Shaltout *et al*.[21] observed the seasonal changes of Cu in tissues of *C. demersum* from the Nile Delta, Egypt. The concentrations increased from winter, when the concentration was the lowest, rose during the spring and reached the maximum in summer, and thereafter decreased. Zuccarini and Campus[22] indicated *C. demersum* as one of the macrophytes with greatest tolerance to Cu. Therefore, they recommended it as a valid bio-indicator for medium to high levels of copper in fresh water.

Cobalt

Highest mean concentration of Co, 5.82 ppm, was observed in April in the leaf of *C. demersum* and it was statistically significantly different from the concentration of Co in the leaf and in the stem in the other months (Table S-III). The mean concentration of Co in the stem was 1.91 (0.69–5.78 ppm) and in the leaf it was 3.88 ppm (1.48–7.63 ppm) (Table S-IV). In addition to the concentration, the highest bio-concentration of Co was recorded in April in the stem and in the leaf, which then declined until October (Fig. 1).

Pajević *et al*.[23] recorded 5–8 ppm Co in *C. demersum* from the littoral zone of Danube–Tisza–Danube canal system, while Bilyk *et al*.[15] found the amount of Co was lower than the detection limit. Samecka-Cymerman and Kempers[17] found a mean value of 7.2 ppm of Co in the tissues of *C. demersum*.

Al-Rekabi[24] observed the monthly changes of the Co concentrations in the tissues of *C. demersum* from southern Iraq; the concentrations increased from January to June, decreased until August and September, then increased again until October and November, when they reached the maximum, and eventually decreased until December. Compared to other seasons, the greatest bio-concentration was observed during the summer season.

Chromium

Significant seasonal variations in concentrations of Cr have been observed (Table S-IV). Highest concentrations were recorded in the stem and in the leaf during April having declined until October. Cr content ranges from 0.89 to 8.81 ppm (average 3.32 ppm) in the stems while in the leaves it ranges from 2.08 to 18.6 ppm (average 6.44). Bio-concentration of Cr in relation to sediment in *C. demersum* is the smallest out of the studied elements. However, there is a noticeable translocation of the average ratio leaf/stem 1.85 (Table I), compared to locations and sampling periods.

The results of Rai *et al.*[25] have showed that *C. demersum*, under laboratory conditions, reduces the level of Cr from water contaminated by effluents from various industrial sources. Reduction varies from the concentration of 4.866 μM to below the 2 μM. Their results indicate the possibility of removing Cr from diluted wastewater by this plant.

Higher concentrations of Cr in the leaves (1.03–2.71 ppm) compared to the stems of *C. demersum* were found by Pourkhabbaz *et al*.[13]

Osmolovskaya and Kurylenko[20] reported that the Cr content was 16.5 times higher in the tissues of *C. demersum* from polluted lake ecosystems compared to unpolluted ones.

Manganese

Manganese was the most abundant metal found in the tissues of *C. demersum* (Tables S-III and S-IV). The mean concentration of Mn in the stem ranged from 275 to 1189 ppm (mean annual value 557 ppm) whereas in the leaf it was 539 to 1984 ppm (mean value 1218 ppm). Spatial and temporal variations in the results were evidenced (Tables S-III and S-IV). The Mn content in the stem declined throughout the study period, while that of the leaf decreased from April to June and then increased, reaching a maximum at the end of the vegetative cycle.

According to a number of authors,[11,15,20,26] together with Fe in the tissues of *C. demersum*, Mn had the highest content of metals and/or maximum bioaccumulation capacity. The Mn concentrations reached values of up to 1000 ppm, the bio-concentration factor reached 2000 and the accumulation of Mn was higher in the leaf of *C. demersum* in relation to the stem.

El-Sarraf[19] claimed that the content of Mn in the leaf was more than two times higher than in the stem. Shaltout *et al*.[21] followed the seasonal variations of Mn in *C. demersum*. The Mn content was the lowest in the winter and then increased during flowering and ripening (spring and summer). According to the authors, unlike Cu and Pb, the Mn concentrations increased in the tissues of *C. demersum* after summer and reached its maximum in autumn, during the maximum vegetative stage.

Nickel

The contents of Ni in the stem of *C. demersum* were significantly different from the contents in the leaf (Table S-III). The Ni concentrations ranged from 3.68 to 14.9 ppm in the stem during the research period (the mean concentration was 7.35 ppm) and in the leaf the Ni content ranged from 6.48 to 28.5 ppm (the mean concentration was 15.5 ppm) (Table S-IV). The contents of Ni in the leaves and stems were relatively uniform throughout the sampling period. After Mn and Zn, nickel showed the highest bioaccumulation ability (Fig. 1) and after Mn, it exhibited the highest translocation ability (Table I), as compared to other investigated metals.

Samecki and Kempers[17] found 36.1 ppm Ni in the tissues of *C. demersum*. Pajević *et al*.[23] determined 5–12 ppm and Babovic *et al*.[12] evidenced 23.6 ppm in the tissues of *C. demersum*.

Al-Rakabi[24] observed the seasonal changes in the concentration of Ni in the tissues of *C. demersum*. The concentration of Ni was constant from January to March, then grew until May and June, declined until July and remained balanced until September, again increasing until October and November, and then decreased until December.

Chorom *et al*.[27] recorded decreases in the biomass of *C. demersum* when grown under laboratory conditions with 1–6 ppm Ni in the water, whereby, death of the plant did not occur despite the phytotoxic levels of Ni. The percentage entering into the plant from the contaminated media was 42–53 %. According to the authors, the highly efficient removal of Ni and the high accumulation capacity make *C. demersum* an excellent choice for phytoremediation of Ni.

Lead

There were no significant variations in the results for Pb during the research period (Tables S-III and S-IV). A slightly higher content of Pb was recorded in the leaves of *C. demersum*, 3.18–16.8 ppm (the mean annual value was 8.04 ppm) while in the stems, it was 2.74–12.7 ppm (the mean annual value was 6.92 ppm) (Table S-IV). The concentration of Pb in the stem decreased from April to the end of the vegetative cycle, while that of the leaf fell from April to August and then increases very slightly until October. Lead had the lowest ratio of leaf/ /stem of 1.02 (Table I) of the investigated metals, probably because of the limited translocation of toxic metals through the plant.

Keskinkan *et al*.[28] found 44.8 ppm in the tissues of *C. demersum*, grown under laboratory conditions in dilute solutions of Pb. Rai *et al*.[25] reported that more than 70 % of Pb was removed by *C. demersum* from industrial waste. Pourkhabbaz *et al*.[13] found a higher content of Pb (7.49–11.88 ppm) in the leaves of *C. demersum* compared to that in the stems.

Shaltout *et al*.[21] investigated the seasonal variation of Pb in the tissues of *C. demersum*. Their findings are to a certain degree different from the conclusions of this study. The content of Pb, similarly to Cu, increased from winter, when its concentration was lowest, during spring and summer, when it reached its maximum and then decreased until winter. Fawzy *et al*.[14] also monitored the spatial and temporal variation of metals in 6 macrophytes, of which *C. demersum* absorbed Pb the most. The maximum concentration of Pb, 31.55 ppm, was observed in the leaves in the winter. Therefore, the authors proposed *C. demersum* as ideal for phytoremediation of Pb.

Zinc

The concentration of Zn in the stem of *C. demersum* decreased from April, when it was the highest, and thereafter to the end of the growing season, it remained almost constant (Table S-III). The content of Zn in the leaves from increased April until the beginning of the growing season, fell until the end of the growing season and then grew to the end of the vegetative phase. The seasonal concentrations of Zn in the stems ranged from 16.7 to 75.6 ppm (the mean value was 38.7 ppm), and in the leaves from 25.6 to 114 ppm (the mean value was 69.2 ppm, Table S-IV). Out of the ten studied metals, after Mn, Zn had the highest potential for bioaccumulation and was the metal with the highest concentration in the tissues of *C. demersum* (Fig. 1). However, many other authors report the highest content of Zn being lower than the concentration of Mn.

Borišev *et al*.[10] found 20.6 ppm Zn in the tissues of *C. demersum* and Babovic *et al*.[12] found 106 ppm. Pourkhabbaz *et al*.[13] observed a higher content of Zn in the stem of *C. demersum*, in the range 19.89 to 40.01 ppm. Osmolovskaya and Kurylenko[20] reported a 5.3 times higher Zn concentration in the tissues of *C. demersum* from contaminated compared to uncontaminated areas.

Fawzy *et al*.[14] noticed no significant seasonal differences in the concentration of Zn in *C. demersum*. They found a higher content of Zn in the leaves of the plants, which was supported by El-Sarraf[19] who found that the Zn content of the leaves was much higher.

Vanadium

The concentration of V depended on both the season and the sampling location (Table S-III). The average seasonal value in the stems of *C. demersum* ranged from 0.34 to 6.87 ppm (the annual average concentration was 1.74 ppm) and it varied from 0.70 to 8.81 ppm (the mean annual concentration was 3.06 ppm) in the leaves (Table S-IV).

The content of V in the stems fell from April to October while its concentration in the leaves kept increasing until June, during the peak biomass production when it decreased until the end of the vegetative period of growth. After Cd, the stem of *C. demersum* and after Cd and Sr, the leaves of *C. demersum* showed the lowest bioaccumulation of V (Fig. 1).

The major anthropogenic sources of V are the products of oil and coal combustion. It is widely used as a catalyst in the production of plastics, although officially there is no such production in the area of Lake Skadar.

Correa *et al*.[29] registered the highest concentrations of V in *C. demersum*, from Ranco Bay, Lago Maggiore (Northern Italy), during the summer (19.5 ppm) with a reduced concentration in winter (6.1 ppm). Ravera *et al*.[30] found that in 8 macrophytes, the concentration of V ranged from 6 to 27 ppm and the content in *C. demersum* was 17 ppm. Zubcov *et al*.[31] recorded V contents in the range 0.5––4.4 ppm in *C. demersum* from the Dniester River ecosystem (Moldava). In 2008 and 2009, Fagbote and Olanipekun[32] found 0.29 and 0.36 ppm V in the Agbabu Bitumen Deposit Area (Nigeria) during the dry season, while in the rainy season of the same years, the values were slightly higher ranging from 0.36 to 0.48 ppm.

Strontium

Seasonal changes in the concentration of Sr in *C. demersum* from Lake Skadar were not observed, nor were they observed in the separate organs (stem and leaf, Table S-IV). Likewise, no spatial variation of the Sr content was noticed (Table S-III). A significantly lower content of Sr was observed only in the samples of stems taken from the Crnojevića River site, and a significantly higher content in the leaves taken from the left mouth of the Morača River (Table S-III). Strontium was, after Mn and Zn, the most abundant metal in the tissues of *C. demersum*. After Pb, Sr exhibited the lowest translocation ability from the stem to the leaf of *C. demersum* (Table I). Mean seasonal values in the stem are within the range from 10.8 to 35.1 ppm (mean value 22.3 ppm) and in the leaf from 12.1 to 37.4 ppm (mean value 23.5 ppm, Table S-IV).

At the same location, Stanković *et al.*[6] determined 141.7 ppm Sr in the tissues of *C. demersum* of the Lake Provala (Serbia) in 1996, and in 1998, they found 380.3 ppm Sr. These data indicate the reflection of changes in environmental conditions on aquatic plants. They also reported higher Sr contents in submerged as compared to emerged macrophytes.

Abdelmalik and El-Shinawy[33] asserted that *C. demersum*, of the Ismailia canal (Egypt), is a favorable biological indicator for radioisotopes of Sr in the concentration range from 0.5 to 10 μCi/L, while the contamination period lasted for 16 days. Strontium-89 uptake increased with increasing initial concentration of radionuclides in the water. The maximum intake was reached after 1–4 days.

CONCLUSIONS

Concentrations of metals in different parts of *C. demersum* were significantly different from their concentrations in water and sediment following the trend: sediment > leaf *C. demersum* > stem *C. demersum* > water.

Concentrations of metals in the stem and in the leaf followed the declining trend: Mn > Zn > Sr > Cu, Ni > Pb > Cr > Co > V > Cd. The bioaccumulation ability (*BCF*) decreased in the following order: Mn > Zn > Ni > Cr > Cu, Co > > Pb > Sr,V > Cd. Except for a few exceptions, there were difference in the sequence of the metal content in the plant compared to the sequences in their bioaccumulation capacity and their numerical relationships. These differences suggested a different capacity of macrophytes for different metals. Plants accumulate certain metals irrespective of the concentrations of the metals in water and sediment, which obviously are their characteristics determined by their capacity for individual elements. The ability of *C. demersum* to bioaccumulate Mn was, on average, several times higher annually than to bioaccumulate the other investigated metals.

The highest contents of Cd, Co, Cr, Pb, V and Sr were found in the tissues of *C. demersum* at the beginning of the growing season; the highest amounts of Ni and Zn were found during the growing season, while most Cu and Mn were accumulated at the end of the vegetative phase. Regarding the Sr content in the tissues of *C. demersum*, there is no statistically significant difference throughout the year, while Cr, Mn and V exhibited the highest temporal variation. The results did not show spatial variation for Co and Cr. Mn, Ni and Zn showed the greatest variation in relation to the sampling site. The content of Mn was higher in the organs of *C. demersum* from the following locations: the right estuary of the Morača, Raduš and Plavnica compared to the other three sites; Ni from the right and left estuary of the Morača and Raduš; Zn from the right estuary of the Morača, River Crnojevića and the left estuary of the Morača. In the waters of the River Morača flow into the wastewater of the capital Podgorica and the Aluminum Plant. Raduš is known as an area that is rich in fish. In recent years, the construction and renovation of old fishermen's houses had intensified. In River Crnojevića, there is a fish processing plant. In the last few years, Plavnica has been exposed to a large number of tourists and fishermen.

The highest translocation from the stem to the leaf was for Mn and Co and the lowest for Pb. Rootless submerged macrophytes absorb metals from water by the stem and leaf. Apart from the absorbed metals from water, the leaf contains a certain quantity of metals transported from the stem.

Due to its ability to accumulate metals, its large coverage of the lakes and the availability of *C. demersum* throughout the year, which makes it easily collectible, this plant is highly recommended for bio-monitoring studies within which trace metal contamination of the lake could be assessed.

SUPPLEMENTARY MATERIAL

Information about study area and collected data are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

БИОАКУМУЛАЦИЈА И ТРАНСЛОКАЦИЈА МЕТАЛА У ОРГАНИМА *Ceratophyllum demersum* ИЗ СКАДАРСКОГ ЈЕЗЕРА, ЦРНА ГОРА

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

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Језерски системи су врло сложени водени системи када је реч о транспорту и интеракцији тешких метала. Ткива макрофита, пре свега због високе варијабилности и тренутног карактера хемијских параметара су погоднији биоиндикатори степена оптерећења језерског еко-система металима од анализе воде или седимента. Као биоиндикаторска врста коришћена је макрофита *Ceratophyllum demersum*, узоркована из Скадарског језера, Црна Гора. Седименти, вода и биљка испитивани су на садржај десет метала у четири различита периода током 2011. године. Концентрације метала следе тренд: седимент > лист *C. demersum* > стабло *C. demersum* > вода. Постоји разлика у секвенци садржаја метала у биљци од секвенце њихове биоакумулационе способности (BCF). Та разлика указује на различит капацитет макрофите за поједине метале. BCF за Mn је неколико редова величине већи у односу на друге метале. Највећи нађени однос концентрација лист/стабло забиљежен је за Mn (2,19) а најмањи за Pb (1,02). Највећи садржаји

Cd, Co, Cr, Pb, V и Sr нађени су у ткивима *C. demersum* на почетку сезоне раста; Ni и Zn у току вегетативне фазе, а Cu и Mn на њеном крају.

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