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Acta Bot. Croat. 75 (2), 236–243, 2016
DOI: 10.1515/botcro-2016-0031

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Aquatic plant *Trapa natans* L. as bioindicator of trace metal contamination in a freshwater lake (Skadar Lake, Montenegro)

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Abstract – Skadar Lake is the largest shallow lake in southeastern Europe. It is located within a national park, and is included in the Ramsar List of international important wetlands, so its preservation and protection from pollution is very important. The aim of this study was to investigate bioaccumulation of the ecotoxic metals Cd, Pb and Cr from sediments of Skadar Lake in the aquatic macrophyte *Trapa natans* L. Samples of sediment and plants were collected at nine locations covering all major water inputs to the lake as well as locations where contamination could be expected. The obtained results indicate that sediments from the Skadar Lake are only locally contaminated with Cd (0.03–1.18 mg kg^{-1}), generally contaminated with Cr (15.8–180 mg kg^{-1}), the concentrations of both elements frequently exceeding sediment quality guidelines, while concentrations of Pb were low (2.7–17.4 mg kg^{-1}). The highest bioaccumulation of all metals from sediment to *Trapa natans* L. was observed in the root, with accumulation efficiency decreasing in the order Cd > Cr > Pb. Translocation from root to stem was also higher for Cd than for Cr and Pb, while the translocation from stem to leaf was comparable for all three metals. From the three investigated metals Cd showed the highest mobility. The results indicate that *Trapa natans* L. may be a very promising bioindicator of trace metal contamination in Skadar Lake.

Key words: bioaccumulation, bioindicator, cadmium, chromium, lead, sediment, Skadar Lake, *Trapa natans* L.

Introduction

Metals are introduced into aquatic systems from both natural and anthropogenic sources and today contamination with metals is a widespread problem due to increasing industrialization and urban development. Upon introduction to the aquatic environment metals are deposited in the bottom sediments and from there may be transferred to plants and the aquatic food chain (Vardanyan and Ingole 2006, Rai 2009, Mazey et al. 2010).

Some aquatic plants have the ability to accumulate metals and other contaminants and can be used as bioindicators of environmental contamination (Whitton and Kelly 1995, Cardwell et al. 2002, Kumar et al. 2006). Plants that can both accumulate metals and have high tolerance to them are important in phytoremediation strategies of contaminated aquatic systems (Clemens et al. 2002, Wang at al. 2002, Marchand et al. 2010).

Uptake from sediment and distribution of metals in plants depends on many factors: the chemical characteristics of the metal, the plant species used and the environmental conditions (Baldantoni et al. 2004, Kumar et al. 2006). The availability of trace metals in sediment is related to their chemical forms in pore water and their affinity for particulate matter, which depends on different factors such as pH, redox potential or organic matter content (Guilzzoni 1991). Some of the sediment phases (exchangeable cations, organic phases, carbonates) contain metals in a form that can be easily released into the pore water and thus made available for uptake by plants. Aquatic macrophytes differ both in their capacity to take up metals in root and in the proportion of metals transferred to the above-ground parts (Baldantoni et al. 2004, Vardanyan and Ingole 2006, Mazey and Germ 2009).

Trapa natans L. is an aquatic, floating macrophyte, typical for natural wetlands. *Trapa natans* L. is also known to live within a wide range of nutrient levels and metal concentrations (Rai et al. 1996, Rai and Sinha 2001, Kumar at al. 2002, Sweta et al. 2015). As it floats on the water surface, it is exposed, in addition to uptake of contaminants

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from sediment, to their uptake from water and the atmosphere. The large biomass of this species and its ability to accumulate metals makes it suitable for the monitoring and even for the phytoremediation of contaminated aquatic ecosystems (Kumar at al. 2002, Sweta et al. 2015).

Skadar Lake is the largest shallow lake in southeastern Europe. It is a national park, included in the Ramsar List of international important wetlands, so its preservation and protection from pollution is very important. However, intensive industrial and urban development in the region exposed Skadar Lake to anthropogenic pollution by organic and inorganic contaminants, including metals (Stešević et al. 2007). The largest tributaries of the Skadar Lake are the Morača River and the Crnojevića River which flow through industrial and urban settlements and transport pollutants to the lake. Previous studies demonstrated that lake sediments are contaminated by metals, mostly Ni and Cr (Stešević et al. 2007, Vemić et al. 2014). Uptake of metals from water and sediment into two types of aquatic macrophytes from the Skadar Lake (*Phragmites communis, Ceratophyllum demersum)* was also studied (Kastratović et al. 2013, 2014).

The aim of this work was to investigate the bioaccumulation of the ecotoxic metals Cd, Pb and Cr from sediments of the Skadar Lake in the aquatic macrophyte *Trapa natans* L. Cadmium and lead are important as they are on the EU priority list of pollutants that should be regularly monitored, while chromium is especially important for the Skadar Lake as it was suggested that elevated concentrations of this metal caused toxic effects to the aquatic plants from the lake Stešević et al. (2007). The study includes determination of the level of these three metals in lake sediments, study of their bioaccumulation from sediment to the root of *Trapa natans* L., and their further transfer to the above-parts (stem and leaf) of the plant. The main goal of this study is to evaluate if *Trapa natans* L. could be a good bioindicator of contamination of the Skadar Lake with Cd, Pb and Cr, thus providing a tool for monitoring these metals in the future.

Materials and methods

Sample collection

Sediment and plant materials were collected from May to June 2012 at nine locations in the Skadar Lake (Fig. 1). Description of sampling locations and their positions are given in On-line Suppl. Tab. 1. Sampling locations cover all major water inputs to the lake (Morača River, Crnojevića River, Raduš underwater spring) as well as locations where potential local contamination with metals (like small villages or ports) can be expected. Possible anthropogenic input of metals into the ecosystem of the Skadar Lake comes from industries located in the vicinity of the lake as well as from the use of agricultural fertilizers and pesticides. In the small town of Reka Crnojevića, which lies on the river from which its name derives, untreated industrial (fish processing plant) and municipal wastewaters are discharged into the Crnojevića River.

After a preliminary survey of areas where the plant *Trapa natans* L. can be found in sufficient abundancy, 3–4 complete healthy plants of similar size, shape and weight were sampled at each sampling location, over an area of about 25 m^2. Plants were collected by hand, packed in polyethylene bags and transferred to the laboratory.

Fig. 1. Map of Skadar Lake with sampling locations. T1 – inflow of the Morača River, T2 – small lake at the right branch of the Morača River, T3 – Kamenik, T4 – Milovića bay, T5 – underwater spring Raduš, T6 – inflow of the Morača River, T7 – inflow of the Plavnica River, T8 – Crnojevića River near the small town of Reka Crnojevića, T9 – the village Karuč.

Sediment samples were taken from the same place as the plant material. Sediment samples (1 kg) were taken using an Ecman-type dredge and the layer of 0–20 cm was collected.

Metal analysis

In the laboratory plant material was washed thoroughly with deionized water to remove detritus and periphyton. Samples of plants were divided into roots, stems and leaves and dried at 75 °C for 48 hours. Dry samples were ground into a fine powder and homogenized in an electric blender. Prepared samples (0.5 g) were mineralized with a Milestone Microwave Ethos 1, with a mixture of HNO_3 and H_2O_2 (3:1). After digestion, the solution was diluted with deionized water to a final volume of 50 mL.

Sediment samples were first dried in air and then in an oven at 75 °C for 48 hours. Dry sediment samples were ground in an agate mortar and sieved through a 1.5 mm sieve. Approximately 0.5 g of the sample was mineralized by microwave digestion with a mixture of $HCl:HNO_3$ (3:1). After mineralization, solutions were diluted with 2 M HNO_3 to a final volume of 100 mL.

Concentrations of metals in plant (Cd, Pb, Cr) and sediment (Cd, Pb, Cr, Fe) samples were determined by inductively coupled plasma optic emission spectroscopy (ICP-OES) technique on a Spectro Acros instrument. Working standards for measurements of elements were prepared from

Sigma Aldrich solutions of 1000 mg dm^{-3} each. The reliability of the analytical method was evaluated by analysis of certified standard reference materials NCS DC73348 (Bush Branches and Leaves) and NCS DC70312 (Tibet sediment) from the China National Analysis Center for Iron and Steel, Beijing. All results are expressed on a dry weight basis.

Calculation of bioconcentration factor and translocation ability

Transfer of metals from sediment to plant (metal phytoavailability) was estimated by the bioconcentration factor from root to sediment ($BCF = Metal_{root}/Metal_{sediment}$). Higher BCF implies greater phytoaccumulation ability. Transfer of metals within the plant (from root to stem and from stem to leaf) was estimated by the translocation ability (TA), which was calculated as the ratio of concentration of metal between the individual parts of the plant, from lower to the upper part of the plant ($TA = Metal_{root\ or\ stem}/Metal_{stem\ or\ leaf}$). A higher TA means a smaller translocation ability.

Statistical analysis

Experimental data were analyzed using the statistical software program Statistica 7.1. (StatSoft Inc., 2006). Since the data did not show a normal distribution, the statistically significant differences between groups were tested using the nonparametric Kruskal Wallis test ($p < 0.05$), followed by the post hoc Tukey test ($p < 0.05$).

Results

Distribution of metals in sediments and plants

Distributions of Cd, Pb, Cr and Fe concentrations in sediment at nine investigated locations are presented in Fig. 2. Concentrations of Cd (Fig. 2A) showed the greatest variations (from 0.03 to 1.18 mg kg^{-1}) and were the highest at locations T8 and T9. Locations T3 and T4 demonstrated medium Cd concentrations (from 0.5 to 0.7 mg kg^{-1}), while at the remaining locations the Cd level was below 0.2 mg kg^{-1}. Concentrations of Pb (Fig. 2B) varied from 2.7 to 17.4 mg kg^{-1} and Pb distribution in sediment showed some similarities with Cd distribution, as the highest Pb values were found at locations T9, T3 and T4 and the lowest at locations T2 and T7. Concentrations of Cr (Fig. 2C) were also quite variable (15.8 to 180 mg kg^{-1}), but showed a very different distribution to those of Cd and Pb, with the highest concentration at location T1 and the lowest at location T8, while remaining locations showed medium Cr levels. Concentrations of Fe (Fig. 2D) varied between 9.1 and 51 g kg^{-1}, covering the whole range between typical Fe contents in limestone and shale (15 and 48 g kg^{-1}, respectively, Wedepohl 2004). This indicates that the abundance of the fine sediment fraction (rich in Fe) at investigated locations is very variable, being the lowest at locations T8 and T2 and the highest at location T3.

Distributions of Cd, Pb and Cr concentrations in individual parts of *Trapa natans* L. at nine investigated locations are presented in Fig. 3. Concentrations of Cd in different parts of the plants varied between 0.03 and 1.05 mg kg^{-1}. At all locations the Cd concentration was highest in the root, but differences between concentrations in root, stem and leaf were not large, except at location T8 and partly T9, where the highest Cd levels in the plant were observed. For Pb, concentrations were in the range from 0.03 to 3.68 mg kg^{-1} and the difference in concentrations between root and the upper parts of the plants was much larger than for Cd at most locations. The highest concentrations of Pb in the plant were obtained in the root at locations T9 and T8, as in Cd, while Pb concentrations in stem and leaf were low at all locations. Distributions of Cd and Pb in the plant (especially in the root) were similar to the distribution of these elements in sediment, as the highest concentrations in both media were obtained at locations T8 and T9. Concentrations of Cr varied between 0.37 and 15.8 mg kg^{-1} and were also the highest in the root, showing, as for Pb, a large

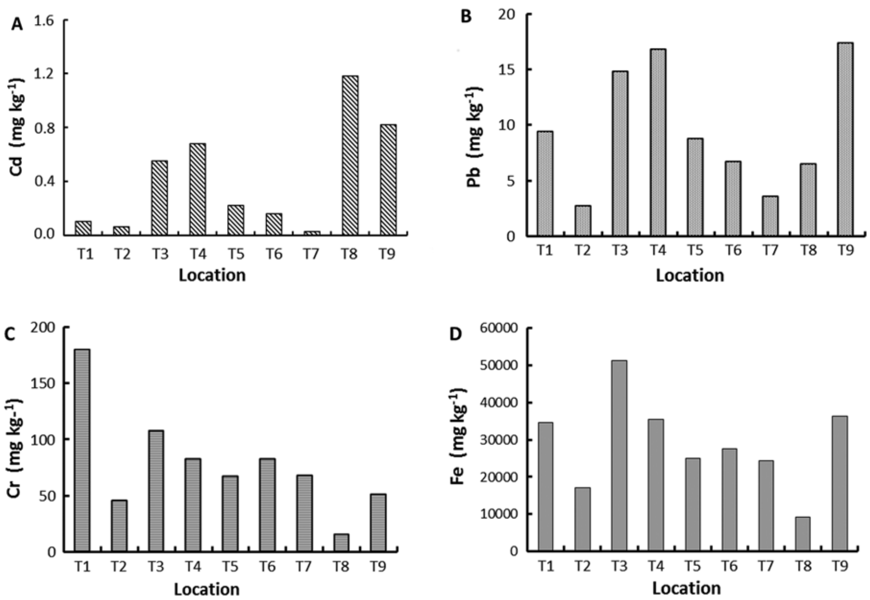

Fig. 2. Concentration of Cd (A), Pb (B), Cr (C) and Fe (D) in sediment samples from different locations (see Fig. 1 for explanation).

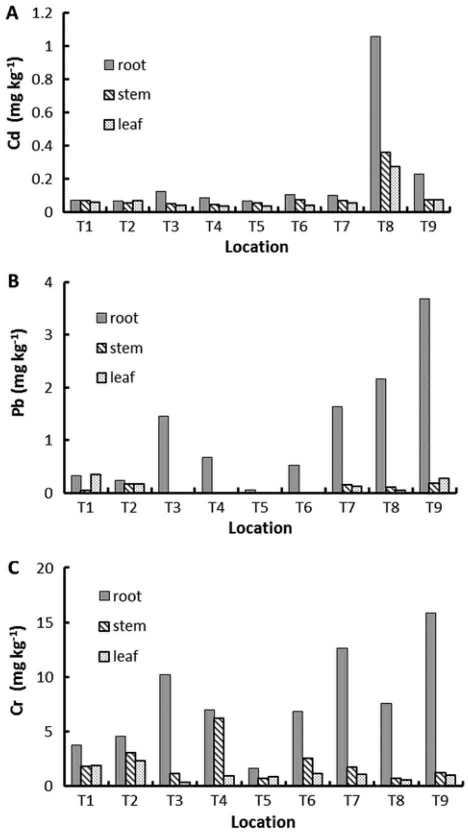

Fig. 3. Concentrations of Cd (A), Pb (B) and Cr (C) in *Trapa natans* parts sampled from different locations (see Fig. 1 for explanation).

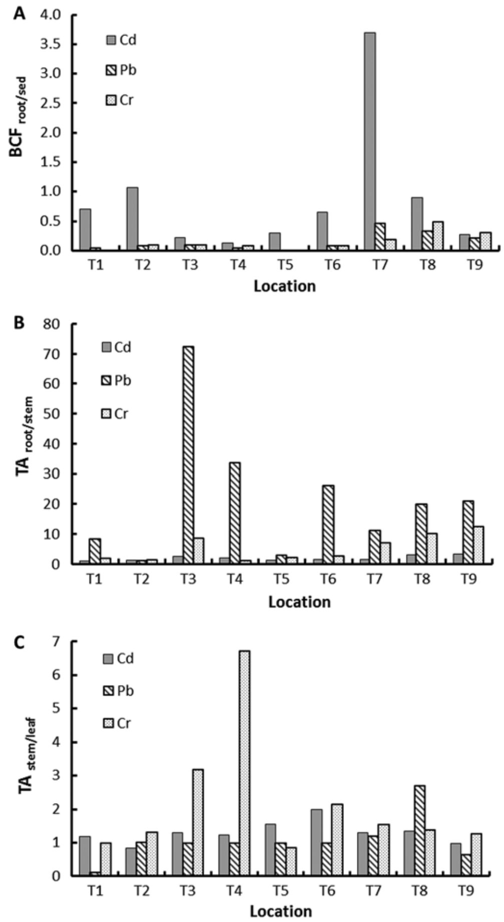

Fig. 4. Bioconcentration factors (BCF root/sed) (A) and translocation ability (TA root/stem (B) and TA stem/leaf (C)) for Cd, Pb and Cr at different sampling locations (see Fig. 1 for explanation).

difference between levels in root and the upper parts of the plant. However, Cr distribution in the root was very different from distribution in sediment, as at location T1, where the highest level of Cr was observed in sediment, while the concentration in the root was rather low.

Transfer of metals in the sediment/plant system

Root/sediment bioconcentration factors of metals at nine investigated locations are presented in Fig. 4A, whereas translocation abilities of metals between root/stem and stem/leaf compartments are presented in Figs. 4B and C. Calculated BCFs indicated a much higher uptake from sediment to root for Cd (BCF = 0.1–3.7) than for Pb and Cr, which demonstrated similar mobility (BCF = 0.01–0.5 and 0.02–0.5, respectively). The highest transfer from sediment to root for all three metals was observed at locations T7, T8 and T9 and for Cd also at location T2. At remaining locations BCF for Cd was lower than 0.7 and for Pb and Cr lower than 0.1. Translocation ability from root to stem was also highest for Cd ($TA_{root/stem}$ = 1.0–3.2) and showed a decreasing trend from Cr ($TA_{root/stem}$ = 1.1–12.6) to Pb ($TA_{root/stem}$ = 1.3–72). The highest values of $TA_{root/stem}$ for all three metals were observed at locations T3, T8 and T9 and for Pb also at locations T4 and T6. Translocation ability from stem to leaf demonstrated much lower variations both among the three metals and among the different locations. Except two higher $TA_{stem/leaf}$ values for Cr at locations T3 and T4 all other $TA_{stem/leaf}$ values were lower than 2.

Discussion

Evaluation of sediment contamination by Cd, Pb and Cr

In addition to the metals of natural origin, sediments may also accumulate elements from anthropogenic sources. Concentration of elements of natural origin is usually a function of the abundance of the fine sediment fraction, as this fraction has the highest ability to adsorb and bind trace elements. Thus, normalization of trace elements concentrations to some of the main components of the fine sediment fraction, such as Al or Fe, which are usually conservative and not affected by anthropogenic influence, may help to distinguish if elements are coming from natural or anthropogenic sources (Boes et al. 2011). The relationship between Fe and concentrations of Cd, Cr and Pb in investigated sediments is presented in Fig. 5. Elevated concentrations of Cd at locations T8, T9 and T4 suggest that some anthropogenic source of Cd exists at these locations. The highest level of Cd is obtained at location T8 (Figs. 2 and 5), which is probably related to the discharge of untreated industrial and municipal waste waters of the town of Reka Crnojevića placed downstream from this location. Lead showed elevated concentrations at the same locations as Cd (Figs. 2 and 5), suggesting identical contamination source for both metals, but the extent of contamination was lower for Pb, especially at location T8. Both distribution of Cr in sediment (Fig. 2) and its relationship to Fe (Fig. 5) indicate that Cr is transported to Skadar Lake by the Morača River. Previous investigations also demonstrated that Cr is one of the most significant pollutants in the Montenegrin part of the Skadar Lake and that the principal origin of Cr is waste waters from an aluminum processing plant located near Podgorica (Stešević et al. 2007).

Ranges of Cd, Pb and Cr concentrations obtained in this work were similar to previous measurements in the same area (On-line Suppl. Tab. 2). Comparison with data from other remote freshwater lakes in Europe (On-line Suppl. Tab. 2) indicates similar ranges of concentrations for Cd and Pb, but much higher levels of Cr in Skadar Lake than in the pristine Plitvice Lakes (Croatia). In order to evaluate possible ecotoxic effect of metal concentrations in sediments we compared the measured concentrations with the most frequently used sediment quality criteria for freshwater sediment (MacDonald at al. 2000), which define TEC (threshold level concentration) as a lower limit below which toxic effect is not probable, and PEC (probable effect concentration) as an upper limit above which toxicity to aquatic organisms can be expected (On-line Suppl. Tab. 2). According to such criteria, all Pb and most of Cd concentrations can be considered as non-toxic to aquatic organisms, as they were lower than TEC, except Cd level at location T8 which was higher than TEC, but lower than PEC. However, in the case of Cr, only concentration at location T8 (Crnojevića River) was lower than TEC; the majority of concentrations were between TEC and PEC, and at locations T1 and T3 they were even higher than PEC, indicating that some toxic effects of Cr may be expected. Stešević et al. (2007) indeed demonstrated that the content of metals in sediments from Skadar Lake inhibited growth of *Myriophyllum aquaticum* and attributed this toxic effect to the elevated Cr concentrations.

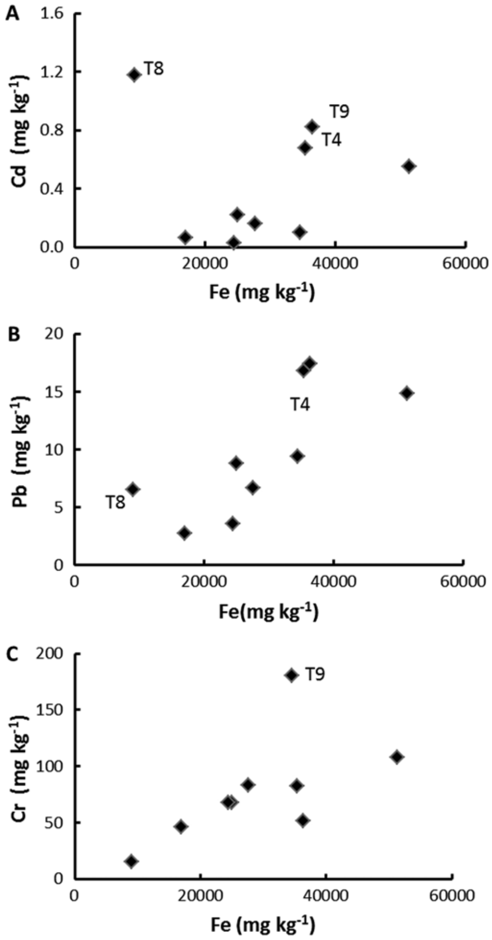

Fig. 5. Correlation of Cd, Pb and Cr with Fe concentration in sediment at different sampling locations (see Fig. 1 for explanation).

Bioaccumulation of Cd, Pb and Cr in the *Trapa natans* L.

Distribution of metals in aquatic plants depends primarily on the plant species, plant organs and the type of metal (Guilzzoni 1991). Some metals are accumulated mostly in the root, because of the existence of a physiological barrier to their transport into the above-ground parts of plants, while others can be easily transported to the branches (Kumar et al. 2006, Baldantoni et al. 2004). In our study the highest concentrations of all three investigated metals (Cd, Pb and Cr) were found in the root of the *Trapa natans* L. (Fig. 3) and for all metals the average concentration in the root was significantly higher ($p < 0.05$) than in the stem or leaf (Fig. 6). Furthermore, considering plants from all locations, variations in the concentration of Cd, Pb and Cr in the root were much larger than in the stem and leaf (Fig. 6). Slightly higher concentrations of Cd and Cr could be noticed in the stem, but they were not significantly higher than

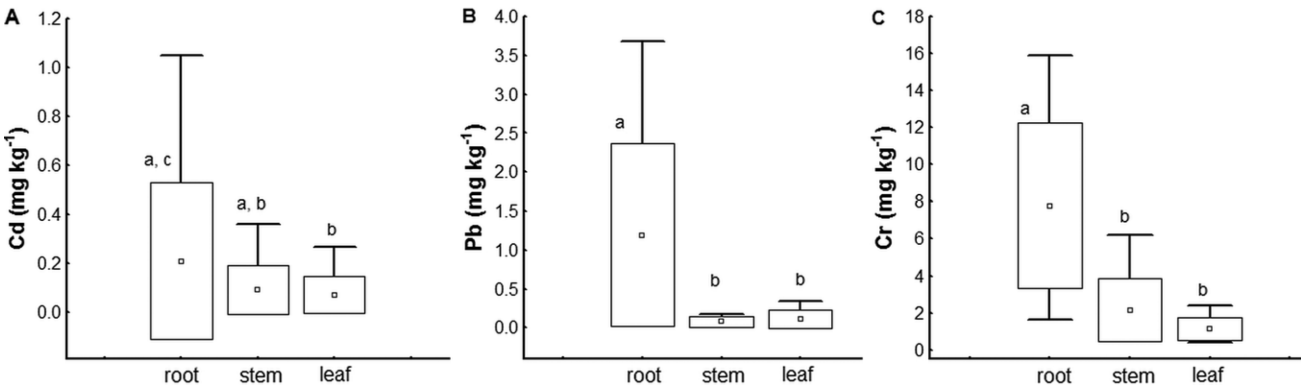

Fig. 6. Box-plot graphs of Cd (A), Pb (B) and Cr (C) concentrations in the individual parts of *Trapa natans* at nine sampling locations (box-plot boundaries indicate average value, standard deviations, and minimum and maximum value). The statistically significant differences among groups according to post hoc Tukey's test ($p < 0.05$) are indicated by different letters.

in the leaf (Fig. 6). It is interesting to note that the concentration of Pb was slightly higher in the leaf than in the stem (Fig. 6). This is due to higher Pb levels in the leaf at locations T1 and T9 (Fig. 3), which could be a consequence of Pb absorption from the atmosphere through the leaf surface (Schreck et al. 2012). Some other aquatic plants also demonstrated consistently higher metal concentrations in root than in stems or leaves (Cardwell et al. 2002, Baldantoni et al. 2004, Mazej and Germ 2009).

We further compared the concentrations of Cd, Cr and Pb in the parts of *Trapa natans* L. obtained in this work with the content of Cd and Pb in *Trapa natans* L. from some other water environments. We also compared concentrations for all three metals in *Trapa natans* L. with data for two other macrophytes from Skadar Lake (On-line Suppl. Tab. 2). The average concentration of Cd was lower in Skadar Lake than in other areas, but at location T8, which was contaminated with Cd (Fig. 3), concentrations of Cd in all plant parts were similar to those in areas contaminated with metals (Sawidis et al. 1995, Sweta et al. 2015). In all plant species from Skadar Lake accumulation of metals decreased in the order Cr > Pb > Cd, following the levels of these metals in lake sediments.

At all locations, concentrations of all three metals were higher in sediments than in the roots of the plant (Figs. 2 and 3), with the exception of Cd at location T7, where very low Cd concentration in sediment was observed. Bioaccumulation ability of metals from sediment to root, estimated by bioconcentration factor, $BCF_{root/sed}$ (Fig. 4A), varied greatly among themetals and decreased in the order Cd > Cr > Pb. The BCF values for Cd at all locations (Fig.7A) were significantly higher ($p < 0.05$) than for Pb and Cr (the average concentration for Cd was about 6 times higher), thus indicating much higher root uptake of Cd than Pb and Cr. The availability of trace metals for plants is related to their chemical forms in pore waters and to their availability in particulate matter (Guilzzoni 1991). Different factors such as pH, redox potential, organic matter content and microbial activity influence metal distribution between pore water and sediment particles and thus their availability to aquatic macrophytes (Guilzzoni 1991, Mazej and Germ 2009). Metals investigated in this study show different chemical behavior in sediment and affinity to the main sediment components, such as carbonates, Fe-Mn oxides, organic matter and aluminosilicates, and their mobility in sediment decrease in the order Cd > Pb > Cr (Filgueiras et al. 2004), which explains the much higher $BCF_{root/sed}$ for Cd than for Pb and Cr. The same order of bioavailability of these three elements was found in other aquatic plants in Skadar Lake (Kastratović et al. 2013, 2014) and also in the publications of other authors (Mazej and Germ 2009).

Translocation factors between root and stem (Fig. 4B) decreased in the order Pb > Cr > Cd and were the highest for Pb at all locations. However, due to the large variations for Pb values (Fig. 7B), a statistically significant difference ($p < 0.05$) between $TA_{root/stem}$ for various metals could be confirmed only between Cd and Cr. Very low values of $TA_{root/stem}$ for Cd (in average 10 times lower than for Pb), indicate that Cd is very mobile within the plant, and its translocation from root to stem, after its uptake from the sediment, is very effective. On the other hand, Pb is mostly retained in the root after its accumulation from sediment, while Cr shows medium mobility from root to stem. However, there was no significant difference in the translocation of the three investigated metals from stem to leaf (Fig. 7C), since the determined values of $TA_{stem/leaf}$ mostly varied between 1 and 2 (except for Cr at location T3 and T4 where slightly higher values are noticed, Fig. 3). This leads us to assume that metals translocated from root to stem are easily further transported to the leaves. Other studies on translocation of metals in macrophytes also demonstrated the relatively high mobility of Cd and the comparably low translocation of Cr and Pb, which was explained by the existence of the physiological barrier for the transport of Cr and Pb to the above ground parts of the plant (Baldantoni et al. 2004, Mazej and Germ 2009).

Trapa natans L. as biondicator of ecotoxic metals contamination

A comparison of Cd distribution in sediment and plant (Figs. 2 and 3) shows that, both in sediments and plants, the highest Cd concentrations were found at locations T8 and T9, thus showing that effective Cd accumulation occurs at these sites. Actually, taking into account data from all nine locations, significant correlation (Pearson; $r = 0.77$; $p < 0.05$) can be observed between the content of Cd in sedi-

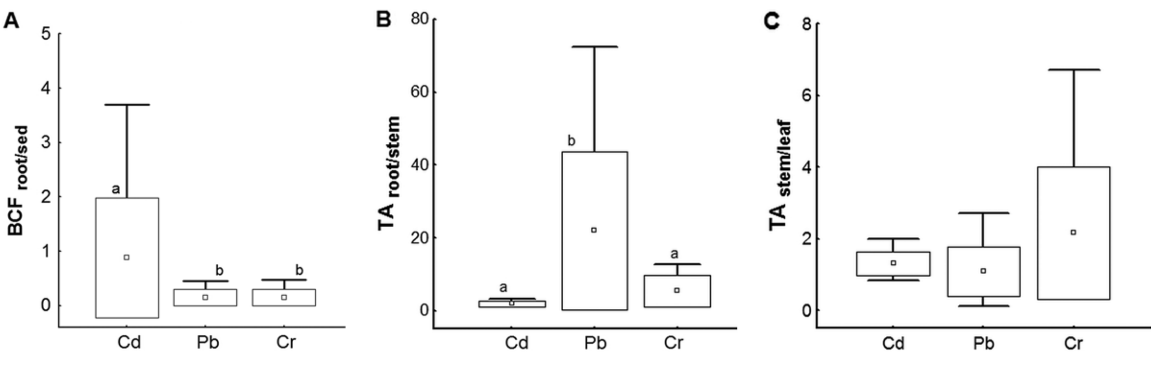

Fig.7. Box-plot graphs of $BCF_{root/sed}$ (A), $TA_{root/stem}$ (B) and $TA_{stem/leaf}$ (C) values for Cd, Pb and Cr at nine sampling locations (box-plot boundaries indicate average value, standard deviations, and minimum and maximum value). The statistically significant differences among groups according to post hoc Tukey's test ($p < 0.05$) are indicated by different letters.

ment and its concentration in the root (Fig. 8). It is evident that the highest concentration of Cd in sediment at location T8 is reflected in the significantly higher level of this metal in the root of the plant.

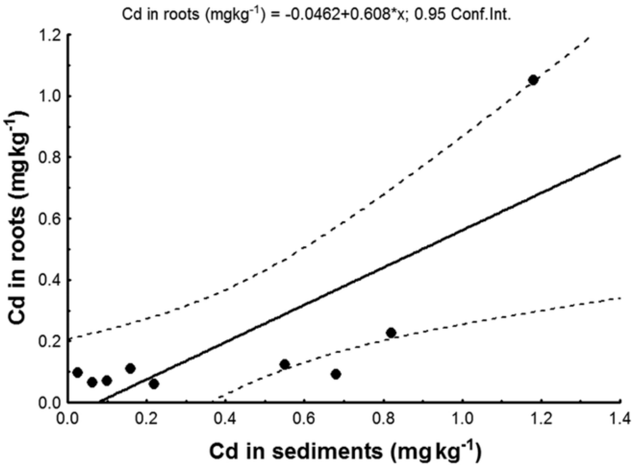

Fig. 8. Correlation between Cd content in sediment and in the root of the plant.

This leads us to assume that *Trapa natans* could be a potential biondicator for Cd contamination. Furthermore, low $TA_{root/stem}$ values for Cd at all locations indicate effective translocation of accumulated Cd to the above ground plant parts. High accumulation of Cd in *Trapa natans* was also demonstrated in ponds in industrial areas in India, and this plant was proposed as a suitable candidate for the phytoremediation of metals from aquatic ecosystems (Sweta et al. 2015). Regarding Pb and Cr, no correlation between content in sediment and root was established in Skadar Lake, and bioaccumulation to the plant was much lower than for Cd. However, in India, in ponds highly contaminated with metals (including Cr and Pb), where this plant is cultivated as a source of food, high accumulation of Cr and Pb in *Trapa natans* fruit was found, indicating that, in polluted areas, accumulation of these metals may also take place (Rai and Sinha 2001). If we compare the metal bioaccumulation ability of *Trapa natans* with two other plants which were studied in Skadar Lake (*Phragmites australis* – Kastratović et al. 2013 and *Ceratopgyllum demersum* – Kastratović et al. 2014) we can notice that the same order of metal bioaccumulation efficiency (Cd > Pb > Cr) from sediment was found for all three macrophytes. However, *Trapa natans* showed the highest bioconcentration factors from sediment to root for Cd and Cr, thus further indicating that it may be a promising biondicator for metal contamination in the Skadar Lake.

Acknowledgement

Financial supports from the Ministries of Science and Education of Croatia and Montenegro within the bilateral collaboration, as well as from the Croatian Science Foundation under the project »Transport and chemodynamics of trace elements in freshwater and coastal sedimentary systems« (HRZZ- 7555) are gratefully acknowledged.

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