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THE DISTRIBUTION OF ZINC IN THE WATER, AQUATIC MACROPHYTES AND SEDIMENT OF LAKE SKADAR

SUMMARY

The use of aquatic plants as indicators of water ecosystems is based on their ability to absorb pollutants (heavy metals, chemical substances, etc.). The aim of this study is to determine the zinc content in sediment, water and plants in Lake Skadar, Montenegro, and monitor the distribution and adoption in the roots, stems and leaves of plants. The aquatic macrophytes *Phragmites australis*, *Ceratophyllum demersum* and *Lemna minor* were used as bioindicator plant species to define the contamination level of Zn. The Zn content of sediment, water and plants was tested at six locations around Lake Skadar in each season. The Zn content in the examined sediment was 47.6–135 mg kg^{-1} dry weight. The largest proportion of Zn based on the total amount of the sediment is incorporated into the crystal lattice of minerals (the residual fraction). In descending order, the highest amounts of Zn concentration in the studied macrophytes were as follows: *L. minor* > *C. demersum* > *P. australis*. The highest average content of Zn was detected in the root of *L. minor* (97.8 mg kg^{-1}) in October.

Keywords: Lake Skadar, zinc, *Phragmites australis, Ceratophyllum demersum, Lemna minor*

INTRODUCTION

Aquatic macrophytes are taxonomically close to earth/based plants, but they live in a completely different environment. Their entire cycle ends within a certain aquatic ecosystem, with little potential to avoid pollution, especially if they live in standing water where there is no significant dilution effect. Under such conditions, macrophytes participate in the process of the transfer of toxins by interacting with the atmosphere, water and sediment, and often in real conditions, also with suspended matter, detrius, and the like. Aquatic macrophytes are often in contact with potential pollutants across their whole body. Being similar to all primary producers, macrophytes react to changes in the quality of the environment in which they live (water/sediment) and are good

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bioindicators of surface water condition. The chemical analysis of aquatic plants leads not only to an understanding of the current situation but also to the evaluation of the trends in environmental changes in time and space (Kastratović et. al., 2015).

Zinc is an essential and useful element for plants, mainly as a part of various metalloenzymes. In most aquatic ecosystems, Zn^{2+} can be toxic for organisms. The phytotoxicity of Zn is manifested by morphological deformations: low levels of growth, small leaves and underdeveloped root systems (Jablanović et. al. 2003). The plant requirement for Zn is in the range of 10-50 mg kg^{-1} (Larcher, 2003). The normal range is 1-400 mg kg^{-1}, while the critical range is 100-400 mg kg^{-1} of Zn (Kabata-Pendias and Pendias, 1992).

Most of the zinc in the sediments is in a soluble form, bound to oxides of iron and manganese, carbonates and organic substances, while about 30% is insoluble. The proportion of zinc in the sediment depends mainly on the pH and the redox potential. If the ambience of the environment is anoxic, zinc is in an insoluble, bio-unavailable form, $Zn(OH)_2$. In an acidic environment and in oxidizing conditions, the content of the soluble zinc forms increases, Zn^{+2} and $Zn(OH)^+$. Zinc can be released into the water body due to the disturbance of the sediment, which leads to its oxidation and mobilization (Zoumis et. al., 2001).

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

All metal concentrations in natural vegetation vary from species to species (Popoviciu et. al., 2017). 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 plants, water and sediment samples were examined for their zinc content over four different periods of the year.

MATERIAL AND METHODS

Study area

Lake Skadar (19°03'-19°30’E, 42°03-42°21’N) is the largest lake in the Balkan Peninsula. It is located at the border between Montenegro and Albania. Two-thirds of the lake is in Montenegro. During the summer, Lake Skadar has a surface of 370 km^2, while in the winter the area is 540 km^2. The water level also varies seasonally from 4. 7 to 9. 8 m above sea level (Milošević et al., 2017). The lake is 44 km long and 13 km wide.

Sediment samples from Lake Skadar were collected from 6 locations around the lake: 1-Raduš (42° 13' 26, 85" N; 19° 09' 54, 44" E), 2- left estuary of Morača (42° 15' 55, 80" N; 19° 08' 31, 49" E), 3- right esturay of Morača (42° 16' 50, 18" N; 19° 07' 38, 92" E), 4- Plavnica (42° 16' 17, 48" N; 19° 12' 1, 01" E), 5-

Crni Žar (42°17' 49,30" N; 19°22' 23,75"E) i 6- River Crnojevića (42°21' 6,03"N; 19°02' 23,05"E).

Sampling collection

The samples of *P. australis* and *C. demersum* were collected four times during the season, from the beginning of April to the end of October, from six locations. Samples of sediment and water were taken at the same time and from the same places, where the plant material was collected.

Sediment sampling was conducted using an Eckman dredge to a depth of 0–20 cm. 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 (at 5 ± 2 °C).

Preparation of the samples for chemical analysis

Macrophytes

The sampled plant material in the laboratory was first washed with tap water, and then twice with deionized water. The plant parts were cut with stainless scissors, into the roots, stems and leaves of the macrophytes to determine the bioaccumulation diversity of the plant organs. The plant material was then dried at 75°C for 48 hours, and subsequently ground into a fine powder and homogenized. In order to avoid the influence of the matrix, the samples were mineralized. An amount of approximately 0. 5 g (± 0. 0001 g) of the prepared samples was measured and mineralized in a Milestone Microwave Ethos 1, with the mixture of HNO_3 and H_2O_2 (in a ratio of 5 ml : 2 ml). After digestion the solutions were diluted using deionized water to their final volume of 25.0 mL.

Sediment

The sediment samples were dried in air, and in a dryer at a temperature of 75°C for 48 hours. The dried sediment samples were ground in an agate mortar and sieved through a sieve <1. 5 mm. Approximately 0. 5 g (± 0. 0001 g) of the sample under pressure and high temperature was then mineralized with the mixture of $HCl:HNO_3:HF$ (in ratio of 6 ml : 2 ml : 1 ml). After mineralization, the solutions were diluted with deionized water to their final volume of 25. 0 mL.

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

Water

The water samples were filtered through a Millipore filter of 0. 45 μm and stored in plastic bottles of 1L by adding 2 mL of HNO_3.

All the samples of the plants parts, sediments and water were prepared three times and their average value was analyzed. In each batch of ten samples, a blank solution was measured. The concentration of Zn was determined using the ICP-OES technique on a Spectro Arcos instrument.

RESULTS AND DISCUSSION

Table 1. The seasonal minimum and maximum concentrations of zinc in the water (mg dm^{-3}) and the sediment (mg kg^{-1}) and the mean concentration ± standard deviation

Metal		April	June	August	October
Zn	Water	0. 002-0. 008 0. 005±0. 002	0. 002-0. 007 0. 005±0. 002	0. 003-0. 008 0. 005±0. 002	0. 003-0. 008 0. 005±0. 002
	Sediment	47. 6-117 75. 4±24. 0	56. 1-135 79. 1±29. 7	59. 1-128 76. 2±26. 1	53. 2-108 73. 8±19. 7

Table 2. The distribution of Zn (mg kg^{-1}) in fractions of sediments of Lake Skadar:
I- Removable and easy mobile; II- Reductabile; III- Oxidabile; IV- Residuale

Metal	Fraction	I	II	III	IV
Zn	Min. -max. Mean	0.06-0.54 0.22	11.6-37.4 22.7	19.9-32.6 25.2	12.6-59.7 28.0

Table 3. Seasonal changes in Zn content (mg kg^{-1} dry matter) in some parts of *Phragmites australis;* Min. and max. concentrations and the mean concentration ± standard deviation

Part of plant	Minimum - maximum concentrations Mean concentration ± standard deviation			
	April	June	August	October
root	18.4-40.2 28.4±8.33 a(c)	25. 3-49. 1 37.5±9.15 a(b)	36.3-72. 9 52.1±15.8 a(a)	21. 1-79. 4 45. 8±21. 6 a(ab)
stem	5.34-22.5 14.8±5.68 b(b)	17.1-29.1 24.4±4.21 b(a)	13.0-31.7 22.0±7. 67 b(a)	8.28-33.4 16.3±9.68 b(b)
leaf	12.8-31.7 22.4±6.86 ab(b)	13.1-44.8 29.0±11.1 ab(a)	17.4-48.4 30.7±11.1 b(a)	9.42-48.3 25.2±12.9 ab(a)

* The values of individual metals with the same first letter(s) are not significantly different at p = 0. 05 in the column (i. e., between the different parts of the plant);
* * The values in individual parts of the plant with the same letter(s) in parentheses are not significantly different at p = 0. 05 in the row (i. e., between seasons)

The significant translocation of Zn from the root to the above-ground organs was observed. The average seasonal root/stem ratio is 2.39 and root/leaf 1. 61. Schierup and Larsen (1981) find a higher concentration of Zn in the above-ground parts as opposed to other investigated metals, which mainly remain behind in the root and rhizomes. Babović et al. (2010) find the folowing relationships: rhizome/stem = 1.42 and rhizome/leaf = 1.32. Baldantoni et al. (2004) reported the ratio of root/leaves as 4.2 and root/shoots as 1.6. Świerk and Szpakowska (2011) indicate the low mobility of Zn, with a rhizome/leaves ratio of 3.7.

Table 4. Seasonal changes in Zn content (mg kg^{-1} dry matter) in some parts of *Ceratophyllum demersum;* Min. and max. concentrations and the mean concentration ± standard deviation

Part of plant	Minimum - maximum concentrations Mean concentration ± standard deviation			
	April	June	August	October
root	18. 4-40. 2 28. 4±8. 33 a(c)	25. 3-49. 1 37. 5±9. 15 a(b)	36. 3-72. 9 52. 1±15. 8 a(a)	21. 1-79. 4 45. 8±21. 6 a(ab)
stem	5. 34-22. 5 14. 8±5. 68 b(b)	17. 1-29. 1 24. 4±4. 21 b(a)	13. 0-31. 7 22. 0±7. 67 b(a)	8. 28-33. 4 16. 3±9. 68 b(b)
leaf	12. 8-31. 7 22. 4±6. 86 ab(b)	13. 1-44. 8 29. 0±11. 1 ab(a)	17. 4-48. 4 30. 7±11. 1 b(a)	9. 42-48. 3 25. 2±12. 9ab(a)

[*] The values of individual metals with the same letter(s) do not differ significantly at p≤0. 05

Table 5. Seasonal changes in Zn content (mg kg^{-1} dry matter) in some parts of *Lemna minor;* Min. and max. concentrations and the mean concentration ± standard deviation

Metal		Part of plant	August	October
Zn	Min. -max. Average ± S. D.	root	59. 9-115 78. 6±25. 2	72. 1-115 97. 8±18. 6
	Min. -max. Average ± S. D.	leaf	41. 3-82. 5 58. 6±18. 2	48. 9-109 83. 2±27. 7

The distribution of Zn in the sediment

Zinc is present in the examined sediments of Lake Skadar in the range of 47. 6 to 135 mg kg^{-1} of dry sediment with an average value of 76. 1 mg kg^{-1}. The highest concentrations of Zn were observed in the sediment from River Crnojevica, where there is a canned fish factory with a small production capacity. The total content of Zn at the other sites is relatively uniform. In the short terms, the movement of heavy metals in soil is very slow (Dumitrel *et. al.,* 2017). It can be said that there is a slight amount of Zn in the exchangeable and acid soluble fraction of the sediment, with a maximum of 0. 54 mg kg^{-1}, or 0. 92 % expressed in percentage. Zinc is almost evenly deployed, with a few exceptions, to the II, III and IV fraction sediments of Lake Skadar. There is no higher variation between the sampling sites with regard to the share of Zn in individual phases of the sediment.

In their study Gao et al. (2010) detected zinc in all four phases of the sediment, with the highest concentration in the residual fraction, which is similar to the results of this study. Zinc is released from the sediment as a result of the microbiological oxidation of organic substrates and remobilized by co-precipitation and/or adsorption on the hydrous ferric oxides (Jones and Turki, 1997). The mobilization of Zn from the sediment to the water occurred under anoxic conditions and soon stopped when the water phase became oxic (Petersen *et. al.,* 1995).

The bioaccumulation of zinc in macrophytes

Phragmites australis

According to the present study, the roots of *P. australis* actively adsorbed Zn, and contained the most Zn during the whole sampling period.

Schierup and Larsen (1981) report maximum increase in Zn in the leaves and stem during the growing season, and that after that, the zinc concentration is reduced. Bragato *et al.* (2009) show almost similar concentrations of Zn in the root, leaves and stem of *P. australis*, from July to October. The zinc concentration in the leaves is 2-3 times higher than in the stem and the rhizome in December.

The distribution of the metal in some parts of the plant is the result of differences in the amount and rate of metal input, primarily by the root pressure and its release into the environment mainly through the transpiration of the leaves (Lasat, 2000; Ravera, 2001). Some metals are accumulated in roots, probably because of some physiological barriers for the transport of toxic elements in traces. The metals essential for metabolic needs are easily transported to the above-ground parts of the plant.

Ceratophyllum demersum

The seasonal concentrations of Zn in the stems ranged from 16. 7 to 75. 6 mg kg^{-1} (the mean value was 38. 7 mg kg^{-1}), and in the leaves from 25. 6 to 114 mg kg^{-1} (the mean value was 69. 2 mg kg^{-1}). Borišev *et al.* (2006) found 20. 6 mg kg^{-1} Zn in the tissues of *C. demersum* and Babović *et al.* (2010) found 106 mg kg^{-1}. Pourkhabbaz *et al.* (2011) observed a higher content of Zn in the stem of *C. demersum*, in the range of 19. 89 to 40. 01 mg kg^{-1}. Fawzy *et al.* (2012) 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 also supported by El-Sarraf (1995) who found that the Zn content of the leaves was much higher.

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. The content of Zn in the leaves from increased from April until the beginning of the growing season, subsequently fell until the end of the growing season and then grew once more to the end of the vegetative phase.

Osmolovskaya and Kurylenko (2005) reported a 5. 3 times higher Zn concentration in the tissues of *C. demersum* from contaminated compared to uncontaminated areas. The content of Zn was slightly higher than *P. australis*, which is also the conclusion of our study.

Lemna minor

The mean seasonal concentration of Zn in the root is 88. 2 mg kg^{-1}, and 70. 9 mg kg^{-1} in the leaf. As was the case with Pb, Jamnická *et al.* (2006) found a lower value of Zn in the tissues of *L. minor*, 14. 68 mg kg^{-1}, and Iram *et al.* (2012) a higher value, of between 35 and 213 mg kg^{-1} of Zn (and a mean of 106 mg kg^{-1}), compared to the concentration present in the *L. minor* from Lake Skadar. The concentration of Zn in the roots and in the leaf at all locations was higher in October than in August. Significantly higher spatial dispersion results than seasonal results are clear in terms of Zn content. The highest differences were observed in the zinc content of the leaves between the two periods of research.

Khellaf and Zerdaoui (2009) report that *L. minor* can survive in a medium containing of 15 mg dm^{-3} Zn. Although biomass and plant growth rates were reduced at these concentrations, the authors point out that *L. minor* can be a good candidate for the treatment of wastewater contaminated with Zn. Radić *et al.* (2010) determined that, due to the high potential of the bioaccumulation of Zn, *L. minor* has the potential for the phytoremediation of water bodies contaminated with low levels of Zn.

Comparative analysis of Zn accumulation in the examined macrophytes

Table 6. Temporal and spatial mean content of Zn (mg kg^{-1}) for the research season in the organs of *Phragmites australis, Ceratophyllum demersum* and *Lemna minor*

Phragmites australis			*Ceratophyllum demersum*		*Lemna minor*	
Root	stem	leaf	stem	leaf	root	leaf
41.0	19.4	26.8	38.7	69.2	88.2	70.9

Table 7.Mean annual values of BCF for Zn in the organs of *Phragmites australis, Ceratophyllum demersum* and *Phragmites australis, Ceratophyllum demersum* and *Lemna minor* (whole plants) in relation to the sediment

	Phragmites australis	*Ceratophyllum demersum*	*Lemna minor*
Zn	1.19	1.49	2.13

The concentration of Zn in the examined macrophytes decreases in the following order: *L.minor* > *C.demersum* > *P.australis*. The bioaccumulative ability decreases in the same order.The highes value of Zn content was recorded in the root of *L.minor* (97.8 mg kg^{-1}) in October and the lowest in the stem of *P.australis* in April and October (14.8 and 16.3 mg kg^{-1} respectively) (Figure 1). They are different seasonal variations in the concentration of Zn in the tissues of the macrophytes.In *P .australis*, the concentrations in all parts of the plant rise from April to August and then fall until to October.The parts of plant the *L.minor* follow the same trend, that is, they show an increase in concentration from August to October, both in the root and in the leaf. The content of Zn in both plants was higher in the root, and the smaller part is transferred to other organs.In the case of *C. demersum*, most of the metal content is transferred to the leaf, where the zinc concentration increases from April to June, decreases until August and then rises again until the end of the growth season.

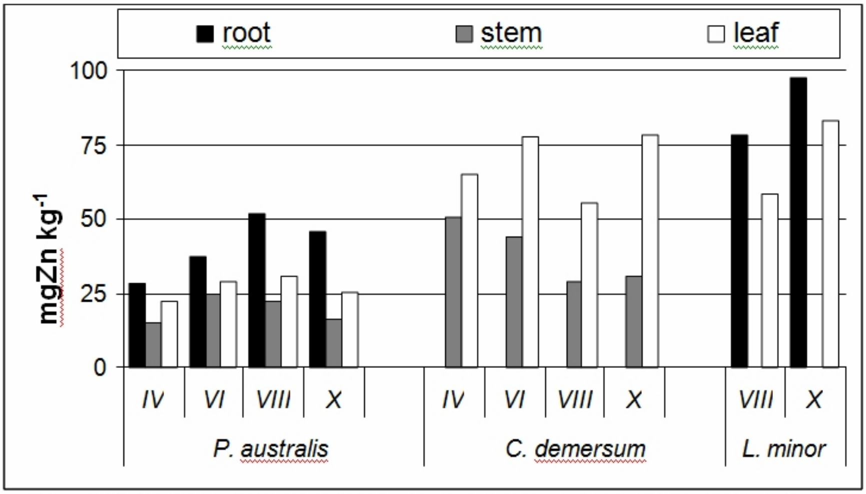

Figure 1. Seasonal changes in Zn concentration in the individual organs of *Phragmites australis*, *Ceratophyllum demersum* and *Lemna minor*

CONCLUSIONS

Zinc is present in the examined sediments of Lake Skadar in the range of 47.6 to 135 mg kg^{-1} of dry sediment with an average value of 76.1 mg kg^{-1}. The highest concentrations of Zn were observed in the sediment from River Crnojevica.

There is a slight amount of Zn in the exchangeable and acid soluble fraction of the sediment, with a maximum 0.54 mg kg^{-1}, or 0.92 % expressed in percentage.Zinc is, with a few exceptions, almost evenly deployed to the II, III and IV fraction sediments of Lake Skadar. There is no significant variation between the sampling sites with regard to the share of Zn in the individual phases of the sediment.

The concentration of Zn in the examined macrophytes decreases in the following order: *L. minor* > *C. demersum* > *P. australis.*The highest value of Zn content was recorded in the root of L.minor (97.8 mg kg^{-1}) in October and the lowest in the stem of *P.australis* in April and October (14.8 and 16.3 mg kg^{-1} respectively).

According to the present study, the roots of *P. australis* actively adsorbed Zn, and contained the most Zn during the whole sampling period.A significant translocation of Zn from the root to the above-ground organs was observed.The concentration of Zn in the stem of *C. demersum* decreased from April, when it was highest, and thereafter to the end of the growing season, it remained almost constant.The content of Zn in the leaves from increased from April until the beginning of the growing season, subsequently fell until the end of the growing season and then grew to the end of the vegetative phase.The concentration of Zn in the roots and in the leaf of *L. minor* at all locations is higher in October than in August. Significantly higher spatial dispersion results than seasonal results are clear for Zn content in all parts of *L. minor*.

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