BIOSCIENCE JOURNAL

EFFECT OF HEAVY METALS ON STEM ANATOMICAL CHARACTERISTICS OF *Trapa natans* L. FROM SKADAR LAKE (MONTENEGRO)

Dragana PETROVIĆ[1], Slađana KRIVOKAPIĆ[1], Goran ANAČKOV[2], Jadranka LUKOVIĆ[2]

[1] Department of Biology, Faculty of Natural Science and Mathematics, University of Montenegro, Podgorica, Montenegro.
[2] Department of Biology and Ecology, University of Novi Sad, Novi Sad, Serbia.

Corresponding author:
Dragana Petrović
Email: dragana.p@ucg.ac.me

How to cite: PETROVIĆ, D., et al. Effect of heavy metals on stem anatomical characteristics of *Trapa natans* l. from Skadar Lake (Montenegro). *Bioscience Journal*. 2021, **37**, e37083. https://doi.org/10.14393/BJ-v37n0a2021-54073

Abstract

This study aimed to analyze stem anatomical characteristics of *Trapa natans* from five Skadar Lake locations (L1 - Milovića bay, L2 – inflow of the Morača river, L3 - Kamenik, L4 - Grmožur, L5 - Lipovik) with different concentrations of Cu, Mn, Zn, Co and Pb, during the summer period of the year 2012. Cross sections of stem were made using cryotechnic procedure. For all analyzed quantitative anatomical parameters, the minimum values at the location L2 were recorded, with the presence of maximum content for all investigated metals in stem of *Trapa natans*. On the other hand, except for cobalt, at the location L4 the minimum concentration for all investigated metals were recorded. Plants collected from this location have the largest average values of the most measured anatomical parameters. The results of Discriminant Analysis showed that plants from different location could be clearly classified into three groups according to their stem quantitative anatomical characteristics which corresponds with heavy metals content. Our research also showed that there is no statistically significant correlation between the content of most investigated metals (Co, Cu, Pb and Zn) and the values of anatomical parameters. Statistically significant negative correlation was found between Mn content in stem and values of two anatomical characters (stem cross-sectional area and Mn content, $r = -0.88$; $p < 0.05$; number of hypodermal cell layers and Mn content, $r = -0.90$; $p < 0.05$).

Keywords: Anatomy. Heavy Metals. Stem. *Trapa natans* L.

1. Introduction

Metals in aquatic systems are available from different sources, natural and anthropogenic. Toxicity of some metals can be reduced by changing their chemical forms, but they forever remain in the environment (Rai 2009)

Aquatic plants have different ability to accumulate metals in their tissue. Accumulation and heavy metals distribution in the plant depends on the plant species, pH, bioavailability, temperature, dissolved oxygen and root secretion. Different heavy metals (Cu, Zn, Mn) are very important for plants, as they are involved as constituents of enzymes and other proteins in various metabolic processes. But in case that their concentration is above specific critical value, they can become very toxic, as they can cause various interactions at molecular and cellular level (Baldisserotto et al. 2007; Pandey et al. 2009; Buta et al. 2011).

Ability of some aquatic plants for metals and other contaminants accumulation can be used as bio-indicators of environmental contamination (Whitton and Kelly 1995; Cardwell et al. 2002; Kumar et al. 2006). Plants than can both accumulate metals and have high tolerance in phytoremediation strategies of contaminated aquatic systems (Clemens et al. 2002; Wang et al. 2002; Marchand et al. 2010).

The knowledge of biochemical, physiological and morpho-anatomic plants response, after they are exposed to heavy metals is of significant importance for the contaminated areas recovery (Ent et al. 2013).

Anatomical changes in root, leaves and stem can be caused by heavy metals. High accumulation and absorption of heavy metals in different plants roots results in different anatomical changes, like diameter of root, diameter of xylem vessels, central vein, size of pericycle, number of xylem arms (Kasim 2006; Farzadfar and Zarinkamar 2012). Al- Saadi et al. (2013) reported that anatomical analyses of plant Potamogeton stem after contaminated treatments revealed several changes in the plants stem (plants were exposed three weeks to Cu and Ag metal treatments). Stems underego changes in shape, size, cortical parenchyma cells arrangement and reduced in vascular bundles.

Trapa natans L. is floating macrophyte characteristic for natural wetlands. *Trapa natans* can live in wide range of nutrient levels and metal concentrations (Rai et al. 1996; Rai and Sinha 2001; Kumar at al. 2002; Sweta et al. 2015). It is exposed as it floats on the water surface, in addition to uptake of contaminants from sediment and water. This species ability for metals accumulation and large biomass is grateful for monitoring and also for the contaminated aquatic ecosystems phytoremediation (Kumar et al. 2002; Sweta et al. 2015).

Skadar Lake is national park and the largest shallow lake in southeastern Europe. It is included in the Ramsar List of international important wetlands and its preservation and protection from pollution is essential. However, intensive urban development and industrial in the region exposed Skadar lake to anthropogenic pollution by different contaminants. Previous studies shows that the lake sediments are contaminated by metals, mostly with Cr and Ni (Stešević et al. 2007; Vemić et al. 2014). Levels and distributions of metals in a system of sediment-water-macrophytes from the Skadar lake was also studied (Kastratović et al. 2013, 2014, 2017, 2018; Petrović et al. 2016).

The present study aimed to analyze the influence of some heavy metals (Cu, Mn, Zn, Co and Pb.) on stem anatomical features of *T. natans* L**.** from five Skadar Lake locations.

2. Material and Methods

Sampling collection

Plant and sediment materials were collected twice during 2012 (from May to July) from five locations in the Skadar Lake (Figure 1): L1- Milovića bay (42°26’22’’N: 19°10’77’’E), L2- Inflow of the Morača river (42° 27’ 70N; 19° 12’ 32’’ E), L3- Kamenik (42° 28’ 54’’ N; 19° 10’ 25’’ E), L4-Grmožur (42° 22’ 79N; 19° 12’ 94’’ E), L5-Lipovik (42° 35’ 81’’ N; 19° 03’ 99’’E). Sampling locations covers all major water inputs to the Skadar Lake (Morača River, Crnojevića River, Raduš underwater spring) as well as locations with potential metals contamination. Potential anthropogenic metals contribution into Skadar Lake ecosystem comes from industries located in the neighborhood of the lake as well as from the use of agricultural pesticides and fertilizers.

Based on preliminary survey of area where the plant *Trapa natans* can be found in abundancy, 3 or 4 complete healthy plants with similar size, shape and weight were sampled on each sampling location, over an area of approximately 25m^2. Plants were handpicked and placed in polyethylene bags and transported to the laboratory. Sediment samples as well were taken from the same locations as plant material. Sediment samples (~1kg) were collected using Ecman-type dredge and the layer of 0-20cm was taken.

Plant and sediments samples were prepared in triplicate and their average value was analyzed. Blank solutions were added to the series of samples and measured after every tenth sample determination. The heavy metals (Cu, Mn, Zn, Co and Pb) concentrations were determined by the ICP-OES technique using Spectro Arcos instrument.

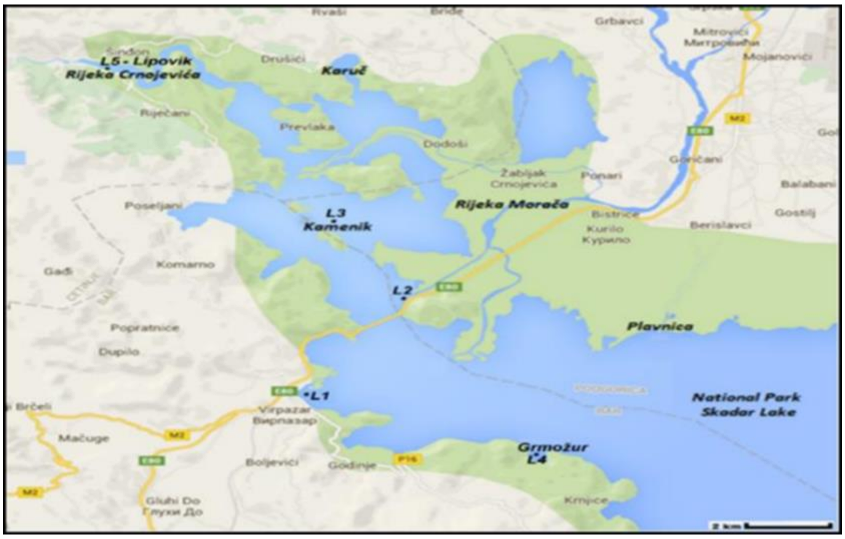

Figure 1. Map of Skadar Lake with sampling locations.

Metal analysis

Plant materials (stem) were washed systematically with deionized water to remove periphyton and detritus. Samples after drying were grinded into fine powder and homogenized in electrical mill. Samples (0.5 g) are mineralized by the 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 targeted volume of 50 mL.

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

Determination of metals (Cu, Mn, Zn, Co and Pb) content in stem samples was performed by inductively coupled plasma optic emission spectroscopy (ICP-OES) technique on the Spectro Acros instrument. Working standards for measurements of elements were prepared from of the Sigma Aldrich solutions of 1000 mg dm^{-3} each. 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 was used for evaluation of the reliability of analytical method. All results are expressed based on a dry weight basis. The recoveries were within 8 % of the certified values.

Stem anatomical analysis

The middle part from 10 plants of stems internodes, close to the leaf rosette, were fixed in 50% ethanol, after which cross-sections were obtained using Leica CM 1850 cryostat, at temperature of -20˚C, at cutting intervals of 40 µm.

The analysis covered 9 anatomical characters of the stem: stem cross-sectional area, thickness of hypodermis, number of hypodermal cell layers, % of cortex cavities, the surface of the central cylinder, the surface of the cylinder parenchyma, the cross-sectional area of the cylinder parenchyma cells, the cross-sectional area of individual vessels and number of vessels per cross section. Observations and measurements of stem parameters were performed using Carl Zeiss Imaging system Axio Vision Release 4.7.

Statistical analysis

Experimental data were processed with the Statistica 7.1. (StatSoft Inc., 2006) software program. The significance of differences in the measured anatomical parameters between the analysed groups of individuals collected at different sites was determined using Duncan's test ($p \leq 0.05$). The variation of the anatomical parameters of all samples of *T. natans* examined by Principal Components Analysis (PCA). Multivariate Discriminant Analysis (MDA) was done in order to check the intergroup variability of anatomical characters, where groups are referred to as analysed groups of individuals collected at different sites. The correlation between the content of metals in stem of *T. natans* and values of anatomical characters was assessed by Pearson's correlation coefficient. ($p < 0.05$).

3. Results and Discussion

Distribution of metals in sediment and stem of *Trapa natans*

Content of metals of natural origin is usually a function of the abundance of the fine sediment fraction, as this fraction has the highest ability to absorb and bind trace elements. In addition to the metals of natural origin, sediment may also accumulate elements from anthropogenic sources (Boes et al. 2011).

Distribution of Co, Cu, Mn, Pb and Zn contents in sediment at five investigated locations is presented in Figure 2. The highest concentration was registered for Mn (1228 mg kg^{-1}) at location L3, and the lowest for Co (1.7 mg kg^{-1}) at location L5. The metal distribution in the sediment decreases in the following order: Mn >Zn>Cu>Pb >Co.

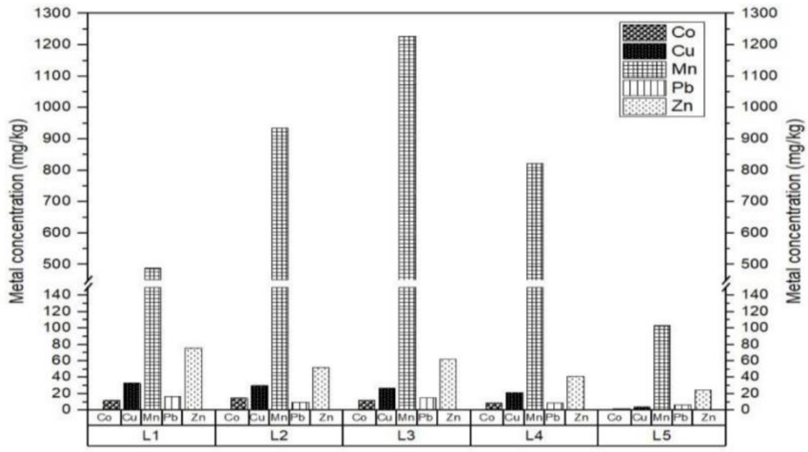

Figure 2. Content of metals (Co, Cu, Mn, Pb and Zn) in sediment sampled from different locations.

Content of metals in aquatic plants depends primarily on the plant species and plant organs. The availability of trace metals for plants is related to their chemical forms in pore waters and their availability in particulate matter. 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 (Guilizzoni 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 shoots. The certain plant species possess some of the specific mechanisms binding at the root level. A significant mechanism for the tolerance of plant accumulated with heavy metals is the ability to compartmentalize them into soluble and insoluble complexes in the cytoplasm and vacuole. In addition, some plant species manage to deposit certain amounts of heavy metals into the cell walls, primarily in the cells of the root tissue, thus preventing their further transport into the aboveground parts of plants (Baldantoni et al. 2004; Kumar et al. 2006).

The metal contents in the stem by the locations used in this research is given in the Figure 3. The maximum contents were recorded for Mn and Zn (116,2 $mgkg^{-1}$, 26,2 $mgkg^{-1}$) at locations L2, while the minimum content was registered for Pb (0,02 mgkg-1) at locations L4. We also registered a very high values of coefficient of variation especially for Pb (98.5%).

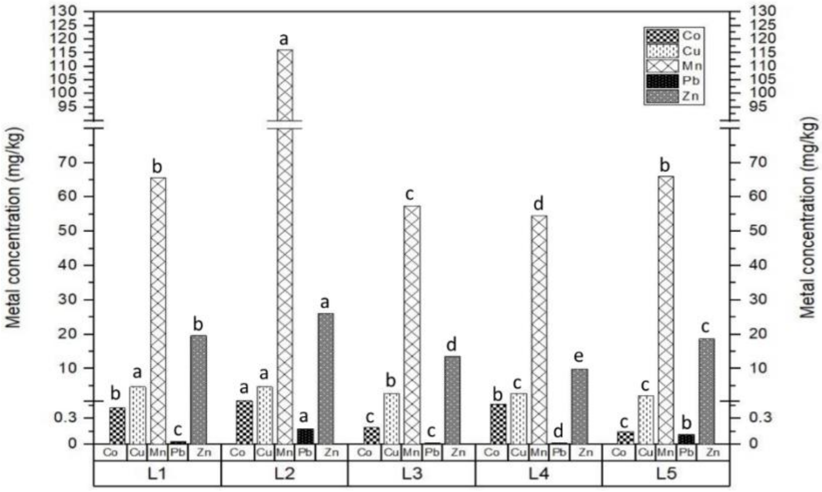

Figure 3. Content of metals (Co, Cu, Mn, Pb and Zn) in stem of *Trapa natans* sampled from different locations.

The metal distribution in the stem of *T. natans* decreases in the following order: Mn >Zn>Cu>Co >Pb. Of all the metals analyzed, the highest content was registered at location L2, while the minimum content for all the metals, with the exception of Co, was registered at location L4.

Anatomical measurements

For all analyzed anatomical parameters, with the exception for cortex cavities, the minimum values at the location L2 were recorded, with the presence of maximum content for all investigated metals. On the other hand, at the location L4 the minimum content for all investigated metals were recorded. Plants collected from this location have the largest average values of the most measured anatomical parameters (Figure 4).

Table 1. Stem anatomical characteristics in *Trapa natans* in five sampling locations (average ± SD and coefficient of variation – in percent, given in parantheses).

Characters	L1	L2	L3	L4	L5
Stem cross-sectional area (μm^2)	65418±292.4[b] (4. 47)	50039±1230.3[d] (2.45)	61008±1429.6[c] (2.34)	71235±696.7[a] (0.97)	67118±3059.4[b] (4.55)
Thickness of hypodermis (µm)	94±2.73[b] (2.90)	76±5.33[d] (7.02)	89±1.65[c] (1.87)	115±6.87[a] (5.95)	92±3.00[b] (3.25)
Number of hypodermal cell layers	7±0.483[a] (6.61)	7±0.31[a] (4.58)	7±0.421[a] (5.85)	7±0.42[a] (5.85)	7±0.421[a] (5.85)
Cortex cavities (%)	6.0±0.321[dc] (5.38)	8±0.46[a] (6.29)	7±0.400[ba] (5.37)	5±0.147[d] (2.91)	6.5±0.410[cb] (6.34)
The surface of the central cylinder (μm^2)	11920±740.8[b] (6.21)	8768±719.8[d] (8.20)	10089±381.3[c] (3.77)	14333±542.5[a] (3.78)	9962±436.5[c] (4.38)
The surface of the cylinder parenchyma (μm^2)	5870±306.5[b] (5.22)	4100±349.8[d] (8.53)	4924±301.7[c] (6.12)	7271±916.0[a] (12.5)	5455±426.0[b] (7.81)
The cross-sectional area of the cylinder parenchyma cells (μm^2)	216±12.51[a] (5.79)	150±14.9[d] (9.91)	178±7.01[c] (3.93)	200±23.7[b] (11.85)	189±10.20[bc] (5.39)
The cross-sectional area of individual vessels (μm^2)	586±69.80[b] (11.92)	467±83.2[c] (17.82)	539±59.44[bc] (11.02)	799±150.9[a] (18.08)	578±44.4[b] (7.68)
Number of vessels per cross-section	23±1.15[c] (4.97)	20±1.05[d] (5.37)	23±0.632[c] (2.77)	28±0.48[a] (1.47)	24±1.15[b] (4.77)

Different superscripts indicate that differences between localities according to Duncan´s test ($p \leq 0.05$).

Duncan's test showed that the plants from the locality Grmožur (L4) had a significantly higher stem cross-sectional area, thickness of hypodermis, number of hypodermal cell, the surface of the central cylinder, the surface of the cylinder parenchyma, the cross-sectional area of individual vessels, number of vessels per cross-section than the plant from the other four populations (Table 1).

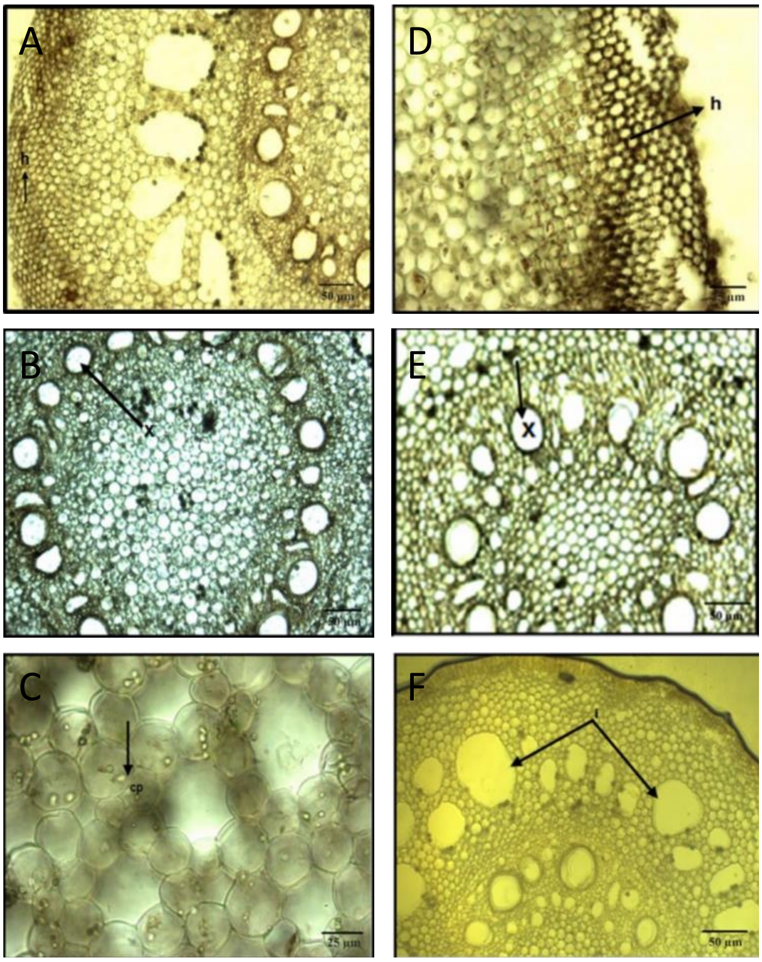

Figure 4. Stem cross-sections of *Trapa natans* from different locations. A – L4 location (hypodermis); B – L4 location (xylem); C – L4 location (cylinder parenchym); D – L2 location (hypodermis); E – L2 location (xylem); F – L2 location (cylinder parenchym). h-hypodermis; x-xylem; cp- cylinder parenchym cell; I-cortex cavity.

The variation of the anatomical parameters of the *T. natans* was examined by Principal Components Analysis (PCA). According to anatomical characters the first principal component accounted for 45.48% of total variation and the second component represented 22.71% (Figure 5). The cumulative contribution percentage of the first two PCS was 68.19%. The distribution of samples along the first axis is largely due to the following parameters: % of the central cylinder, % of the cylinder parenchyma, the cross-sectional area of individual vessels, the number of individual vessels and % of the cortex intercellular. The projection of the cases of the first two components showed that investigated populations could be clearly separated into three groups according to the variability of anatomical characters. The first group consists of specimens from samples from the inflow of the Morača river (L2), the second from the samples from the locality Grmožur (L4) and the third group consist of specimens from the samples from the Milović bay (L1), Kamenik (L3) and Lipovik(L5).

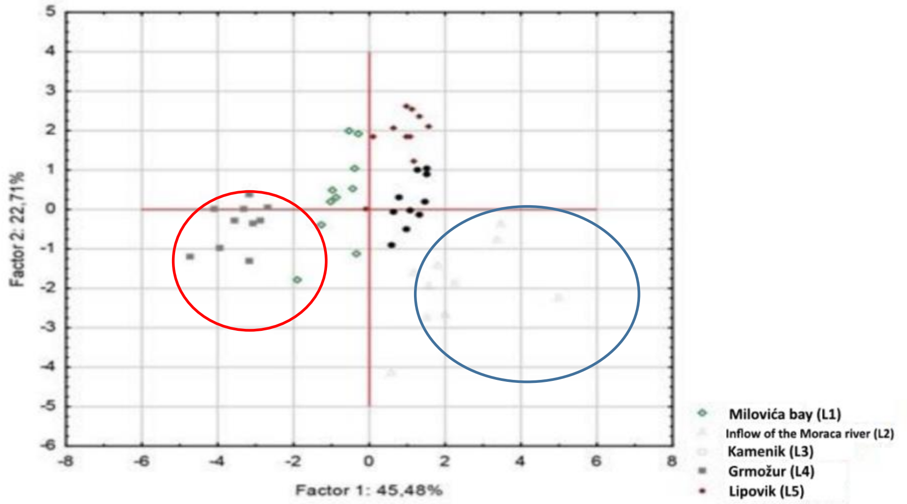

Figure 5. The projection of the cases of the first two components of the Principal Component Analysis based on anatomical characteristic.

The results of the Multivariate Discriminant Analysis (MDA) also showed that investigated populations could be clearly separated into three groups according to the first discrimination axis (Figure 6): the first group consists of individuals from the inflow of the Morača river (L2) another group of an individual from the locality Grmožur (L4) and the third from the samples from the Milović bay (L1), Kamenik (L3) and Lipovik (L5). On the second discriminatory axis, two groups are clearly distinguished: the first group consists of individuals from the inflow of the Morača river (L2) and Grmožur (L4), and the second one from the Milović bay (L1), Kamenik (L3) and Lipovik (L5).

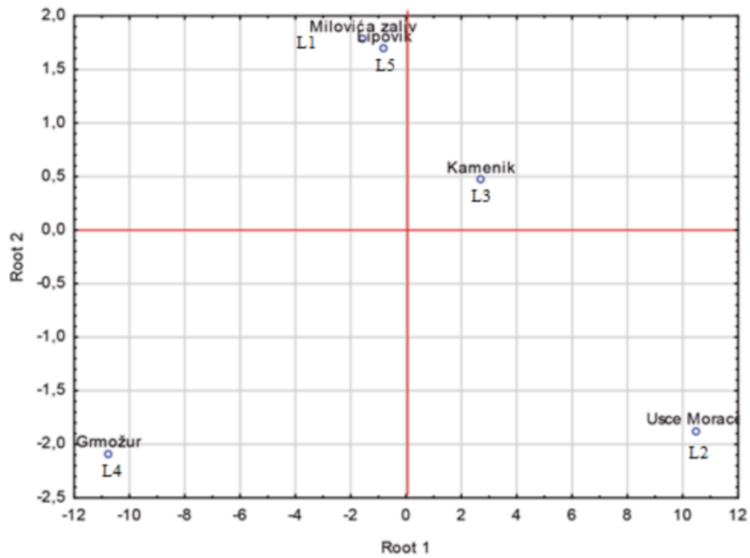

Figure 6. The results of the Multivariate Discriminant Analysis, projection of the first two factors based on anatomical characteristic.

The results of Discriminant Analysis showed that plants from different location could be clearly classified into three groups according to their stem quantitative anatomical characteristics which corresponds with heavy metals content.

The locality L2 (inflow of the Morača river) is under the direct influence of various pollutants of organic and inorganic origin brought by the river Morača to its current through urban and industrial areas. On the left and right banks of the mouth of the Moraca water quality is affected by the numerous impurities in the river collected throughout its course. Probably the most noticeable impact on the changing quality of the Lake Skadar ecosystem relates to the technological processes at the Aluminium Plant in Podgorica (Kastratović at al. 2018). At this location maximum content were recorded for most of the metals analyzed in the tissues of the *T. natans*.

The locality L4 (Grmožur) is spatially farthest from the Morača mouth of the locality. There are significantly different ecological conditions in this area, since it is located in the open part of lake, which is not affected by the tributary waters (potentially the largest polluters of lake water). In its immediate vicinity, there is a sublacustric "eye" Raduš, where the water depth is 60m in dry season. This feature is of particular importance for the intensity of the lake water self-purification process, so in this part of the lake the containment of polluting particles is minimized. Minimum contents for the majority of investigated metals in the tissues of the *T. natans* are registered in this location.

Correlation between anatomical characters and metal contents in stem of *Trapa natans*

Mn is an essential micronutrient for plants (Santandrea et al. 2000), involved in many redox reactions as a cofactor for numerous enzymes and in protection against oxidative stresses (Bowler et al. 1994; Buchel et al. 1999). Unfortunately, while essential for metabolic processes, in recent years, Mn has emerged in some areas as one of the dangerous toxicants, and especially as a consequence of uncontrolled anthropogenic activities, which could cause significant damage to vegetation (Kabata-Pendias 2011).

Table 2. The values of Pearson's correlation coefficient ($p < 0.05$) between heavy metal content in stem and anatomical characters.

Characters	Co	Cu	Mn	Pb	Zn
Stem cross-sectional area	-0.45	-0.57	-0.88	-0.70	-0.78
Thickness of hypodermis	-0.10	-0.45	-0.75	-0.69	-0.84
Number of hypodermal cell layers	-0.58	-0.35	-0.90	-0.83	-0.62
Cortex cavities	0.12	0.31	0.74	0.66	0.68
The surface of the central cylinder	0.11	-0.17	-0.65	-0.72	-0.74
The surface of the cylinder parenchyma	-0.05	-0.34	-0.72	-0.68	-0.77
The cross-sectional area of the cylinder parenchyma cells	-0.27	-0.12	-0.76	-0.74	-0.51
The cross-sectional area of individual vessels	0.03	-0.40	-0.65	-0.61	-0.80
Number of vessels per cross-section	-0.27	-0.59	-0.80	-0.66	-0.86

The results of the correlation analysis (using Pearson's correlation coefficient, $p< 0.05$) between the metal content in stem of *T. natans* and values of anatomical characters, show (Table 2) the existence of a highly significant negative correlation between the Mn cotent in stem and values of two anatomical characters (stem cross-sectional area and Mn cotent, $r = - 0.88$; $p < 0.05$; number of hypodermal cell layers and Mn cotent, $r = - 0.90$; $p < 0.05$) (Figure 7). It should also be noted that no statistically significant correlations were registered between other investigated metals (Co, Cu, Pb and Zn) and the values of anatomical parameters.

The influence of heavy metals on the morpho-anatomical characteristics of many plant species has been investigated in numerous papers (Farzadfar and Zarinkamar 2012; Al-Saadi et. al. 2013; Gupta and Chakrabarti 2013). They suggest that the increased concentrations of heavy metals lead to numerous anatomical changes in different plant organs (root, stem end leaf). Our results, the existence of a highly significant negative correlation between the Mn cotent in stem and values of two anatomical characters agree with the results presented by Baldisserotto et al. (2007). They reported that *T. natans* can accumulate a very high concentration of Mn (>2000 $\mu g\ g^{-1}$) in floating laminae, which certainly leads to numerous morpho-anatomical and physiological changes, but also points to the importance of this species (as a Mn-hyperaccumulator) in phytoremediation process.

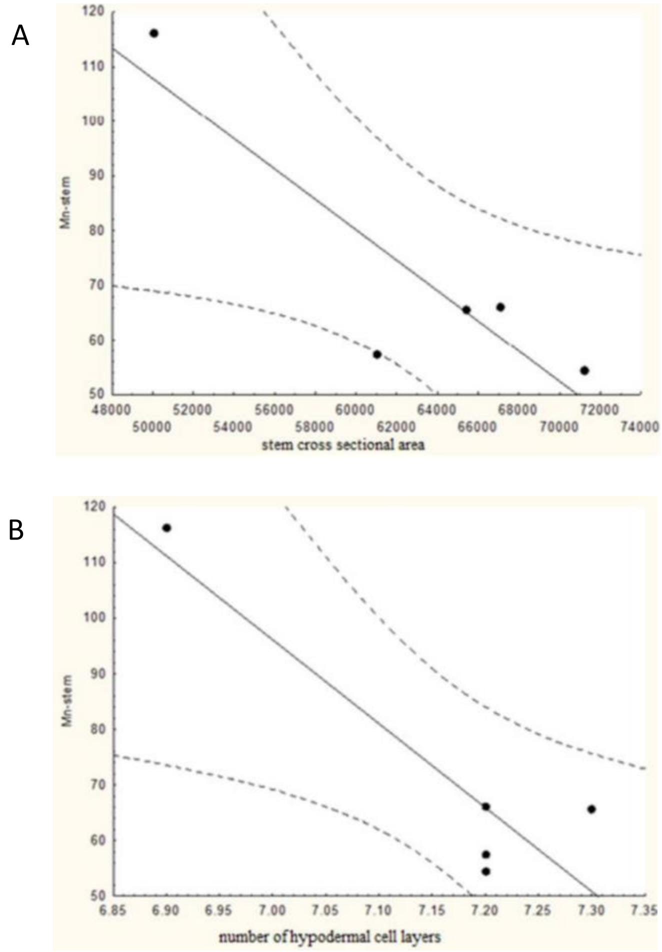

Figure 7. Correlation between Mn content in stem of *Trapa natans* and values of two anatomical characters. A – stem cross-sectional area and Mn cotent (Pearson; r = - 0.88; p < 0.05); B – number of hypodermal cell layers and Mn cotent (Pearson; r = - 0.90; p < 0.05).

4. Conclusions

In general, it can be concluded that the different ecological conditions in certain localities have an impact on the variability of stem anatomical characters of *T. natans*, but also the characteristics of water, sediment, different metal concentration, can potentially influence the variability of anatomical characters in individuals from the different localities. Our research also showed that there is no statistically significant correlation between the content of most investigated metals (Co, Cu, Pb and Zn) and the values of anatomical parameters. However, the existence of a statistically significant negative correlation between the content of Mn (as the most accumulated metal) and values of two anatomical parameters was noted, potentially can be guideline in biomonitoring of aquatic ecosystems which are directly exposed to heavy metals.

Authors' Contributions: PETROVIĆ, D.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, critical review of important intellectual content; KRIVOKAPIĆ, S.: conception and design, critical review of important intellectual content; ANAČKOV, G.: analysis and interpretation of data, critical review of important intellectual content; LUKOVIĆ, J.: analysis and interpretation of data, critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

Acknowledgments: Not applicable.

References

Al-SAADI, S.A.A.M., Al-ASAADI, W.M. and Al-WAHEEB, A.N.H. The effect of some heavy metals accumulation on physiological and anatomical characteristic of some *Potamogeton* L. plant. *Journal of Ecology and Environmental Sciences*. 2013, **4**, 100-108.

BALDANTONI, D., et al. Assessment of macro and microelement accumulation capability of two aquatic plants. *Environmental Pollution*. 2004, **130**, 149–156. https://doi.org/10.1016/j.envpol.2003.12.015

BALDISSEROTTO, C., et al. Responses of *Trapa natans* L. Floating laminae to high concentrations of manganese. *Protoplasma.* 2007, **231**, 65– 82. https://doi.org/10.1007/s00709-007-0242-2

BOES, X., et al. Evaluation of conservative lithogenic elements (Ti, Zr. Al and Rb) to study anthropogenic element enrichments in lake sediments. *Journal of Paleolimnology*. 2011, **46**, 75-87. https://doi.org/10.1007/s10933-011-9515-z

BOWLER, C., VAN CAMP, W., VAN MONTAGU, M. and INZE, D. Superoxide dismutase in plants. *Critical Reviews in Plant Sciences*. 1994, **13**, 199–218. https://doi.org/10.1080/07352689409701914

BUCHEL, C., et al. Photo assembly of the manganese cluster and oxygen evolution frommonomeric and dimeric CP47-reaction center photosystem II complexes. *Proceedings of the National Academy of Sciences USA 96*. 1999, **96**(25), 14288–14293. https://doi.org/10.1073/pnas.96.25.14288

BUTA, B., et al. The Influence of Heavy Metals on Growth and Development of *Eichhornia crassipes* Species, Cultivated in Contaminated Water. *Notulae Botanicae Horti Agrobotanici*. 2011, **39**(2), 135-141. https://doi.org/10.15835/nbha3926095

CARDWELL, A.J., HAWKER, D.W. and GREENWAY, M. Heavy metal accumulation in aquatic macrophytes from southeast Queensland, Australia. *Chemosphere*. 2002, **48**, 653–663. https://10.0.3.248/s0045-6535(02)00164-9

CLEMENS, S., PALMGEREN, M.G. and KRAMER, U. A long way ahead: Understand and engineering plant metal accumulation. *Trends in Plant Science*. 2002, **7**, 309-315. https://10.0.3.248/s1360-1385(02)02295-1

ENT, A.V.D., et al. Hyper accumulators of metal and metalloid trace elements: facts and fiction. *Plant and Soil*. 2013, **362**(1-2), 319-334. https://doi.org/10.1007/s11104-012-1287-3

FARZADFAR, S. and ZARINKAMAR, F. Morphological and anatomical responses of *Matricaria chamomilla* plants to cadmium and calcium. *Advances in Environmental Biology*. 2012, **6**(5), 1603-1609.

GUILIZZONI, P. The role of heavy metals and toxic materials in the physiological ecology of submeresed macrophytes. *Aquatic Botany*. 1991, **41**, 87-109. https://doi.org/10.1016/0304-3770(91)90040-C

GUPTA, S. and CHAKRABARTI, S.K. Effect of heavy metals on different anatomical structures of *Bruguiera sexangula*. *Internacional Journal of Bio-resource and Stress Management*. 2013, **4**(4), 605-609.

KABATA-PENDIAS, A. *Trace elements in soils and plants.* 4th ed. Boca Raton, London, NewYork: CRC Press, Taylor and Francis Group, LLC, 2011.

KASIM, W.A. Changes induced by cooper and cadmium stress in the anatomy and grain yield of Sorghum bicolor (L.)Moench. *International Journal of Agriculture and Biology*. 2006, **8**, 123-128.

KASTRATOVIĆ, V., KRIVOKAPIĆ, S., ĐUROVIĆ, D. and BLAGOJEVIĆ, N. Seasonal changes in metal accumulation and distribution in the organs of *Phragmites australis* (common reed) from Lake Skadar, Montenegro. *Journal of the Serbian Chemical Society*. 2013, **78**, 1241–1258. https://doi.org/10.2298/JSC121026153K

KASTRATOVIĆ, V., et al. Bioaccumulation and translocation of heavy metals by *Ceratophyllum demersum* from the Skadar Lake, Montenegro. *Journal of the Serbian Chemical Society*. 2014, **79**, 1445–1460. https://doi.org/10.2298/jsc140409074k

KASTRATOVIĆ, V., et al. Seasonal patterns of Cu in a system of sediment-water-macrophytes. *Fressenius Environmental Bulletin*. 2017, **26**(2), 1247-1253.

KASTRATOVIĆ, V., et al. Levels and distribution of cobalt and nickel in the aquatic macrophytes found in Skadar Lake, Montenegro. *Environmental Science and Pollution Research*. 2018, **25**(27), 26823-26830.

KUMAR, J.I.N., SONI, H. and KUMAR, R.N. Biomonitoring of selected freshwater macrophytes to assess lake trace element contamination: a case study of Nal Sarvar Bird Sanctuary, Gujarat, India. *Journal of Limnology*. 2006, **65**, 9-16. https://doi.org/10.4081/jlimnol.2006.9

KUMAR, M., CHIKARA, S., CHAND, M.K. and BHATNAGAR, A.K. Accumulation of lead, cadmium, zinc and copper in the edible aquatic plants Roxb. and Nelumbo nucifera Gaertn. *Bulletin of Environmental Contamination and Toxicology*. 2002, **69**, 649–654. https://doi.org/10.1007/s00128-002-0110-x

MARCHAND, L., MENCH M., JACOB, D.L. and OTTE, M.L. Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: A review. *Environmental Pollution*. 2010, **158**, 3447–3461. http://dx.doi.org/10.1016/j.envpol.2010.08.018

PANDEY, N., PATKAK, G.C., PANDEY, D.K. and PANDEY, R. Heavy metals, Co, Ni, Cu, Zn and Cd, produce oxidative damage and evoke differential antioxidant responses in spinach. *Brazilian Journal of Plant Physiology*. 2009, **21**(2), 103–111. https://doi.org/10.1590/S1677-04202009000200003

PETROVIĆ, D., et al. Aquatic plant *Trapa natans* L. as bioindicator of trace metal contamination in a freshwater lake (Skadar Lake, Montenegro). *Acta Botanica Croatica*. 2016, **75**(2), 236-243. https://doi.org/10.1515/botcro-2016-0031

RAI, P.K. Heavy metal phytoremediation ftom aquatic ecosystem with special reference to macrophytes. *Critical Review in Environmental Science and Technology*. 2009, **39**, 697-753. https://doi.org/10.1080/10643380801910058

RAI, U.N., SINHA, S. and CHANDRA, P. Metal biomonitoring in water resources of Eastern Ghats, Karaput (Orissa), India by aquatic plants. *Environmental Monitoring and Assessment*. 1996, **43**, 125–137. https://doi.org/10.1007/BF00398603

RAI, U.N. and SINHA, S. Distribution of metals in aquatic edible plants *Trapa natans* (Roxb.) Makino and Ipomoea aquatica Forsk. *Environmental Monitoring and Assessment*. 2001, **70**, 241–252. https://doi.org/10.1023/a:1010727325662

SANTANDREA, G., PANDOLFI, T. and BENNICI, A. A physiological characterization of Mn-tolerant tobacco plants selected by in vitro culture. *Plant Science*. 2000, **150**, 163–170.

STEŠEVIĆ, D., et al. Application of a new sediment contact test with *Myriophyllum aquaticum* and of the aquatic Lemna test to assess the sediment quality of Lake Skadar. *Journal of Soils and Sediments*. 2007, **7**, 342–349. http://doi.org/10.1065/jss2007.08.249

SWETA, BAUDDH, K., SINGH, R.P. and SINGH, R.P. The suitability of *Trapa natans* for phytoremediation of inorganic contaminants from the aquatic. *Ecological Engineering*. 2015, **9**, 39–42. http://doi.org/10.1016%2Fj.ecoleng.2015.06.003

WANG, Q., CUI, Y. and DONG, Y. Phytoremediation of polluted waters Potentials and prospects of wetland plants. *Acta Biotechnological.* 2002, **22**, 199–208. http://doi.org/10.1002/1521-3846(200205)22:1/2<199

WHITTON, B. and KELLY, M. Use of algae and other plants for monitoring rivers. *Australian Journal of Ecology*. 1995, **20**, 45–56. https://doi.org/10.1111/j.1442-9993.1995.tb00521.x

VEMIĆ, M., ROUSSEAU, D., DU LAING, G. and LENS, P. Distribution and fate of metals in the Montenegrin part of Lake Skadar. *International Journal.* 2016, **5**(1), 39-48. https://doi.org/10.1016/j.fshw.2015.11.003

Received: 23 April 2020 | **Accepted:** 6 October 2020 | **Published:** 29 December 2021