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Diversity of macrophytes and differences in the contents of metals between macrophyte species in alpine Lake Bohinj (Slovenia)

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Abstract Alpine Lake Bohinj is the largest natural lake in Slovenia. It is located in the Triglav National Park, which provides protection and limits activities in its catchment area. In the present study, we determined the aquatic macrophyte richness in Lake Bohinj and the trace metal content of Cu, Pb, Cr, Cd, Co, Mn, Fe, Zn, Hg, and Ni in macrophyte and sediment samples. Samples of *Myriophyllum spicatum* and *Chara* spp., and sediment were collected on five sampling plots chosen according to the highest plants diversity and sediment accessibility. The following species were found in the littoral of the lake: *M. spicatum*, *Potamogeton alpinus*, *P. crispus*, *P. filiformis*, *P. lucens*, *P. perfoliatus*, *P. pusillus*, *Ranunculus circinatus*, *Phragmites australis*, *Chara aspera* and *C. delicatula*. The most diverse genus in Lake Bohinj was *Potamogeton* and the most abundant species was *M. spicatum*, which was in accordance with previous studies. The content of most elements (Cd, Co, Cu, Hg, Mn, Ni, and Zn) and bioconcentration factors (BCFs) were higher in *M. spicatum* than in stoneworts. Bioconcentration of elements from sediments to plants was high in *M. spicatum* (BCF >1 for Cd, Co, Mn and Zn), confirming the suitability of this species in the phytoremediation of sediments of polluted water bodies.

Keywords: bioconcentration factor, bioaccumulation trace metals, lake pollution, Charophytes, vascular underwater plants, alpine macrophyte meadows, macrophyte survey

1 Introduction

Chemical pollution is one of the most pressing global environmental problems (Geng et al., 2019). Trace metals are essential and non-essential elements which average concentration in plants is below 100 mg kg-1 d.w. (Marchand et al., 2014). They pose threat for environment and biota because they are non-degradable, persistent, easy to bioaccumulate and toxic (Harguinteguy et al., 2014). Trace metals reach the aquatic ecosystems from both anthropogenic and natural sources. As they occur naturally in the earth's crust (Al-Abbawy et al., 2021), their concentrations in natural waters depend on many factors, most importantly on climatic, morphological, and hydrological conditions in the area, the type of minerals present in drained rocks, the mobility during erosion and transport, the physical parameters of water, and also the residence time of water into the rocks or soils (Féraud et al., 2009). However, uncontrolled anthropogenic activity strongly impacted these metals' natural biogeochemical cycles (Al-Abbawy et al., 2021). The main sources of trace metals are: mining, sewage, smelters, tanneries, textile industry, chemical industry, agriculture and transport (Geng et al., 2019; Eid et al., 2020). Significant amounts of metals are released directly to rivers and lakes with wastewaters as the soluble fraction (Geng et al., 2019). Consequently, the quality of the aquatic ecosystems and the macrophytes are largely affected by human activities (Brucet et al., 2013). In particular, pollution negatively affects self-cleaning capacity, violates the stability of the ecosystem and directly harm living organisms (Eid et al., 2020). In the recent study by Sojka et al. (2022), authors reported lower trace metals concentrations in surface sediments of the lakes, which are located in areas with a higher proportion of national parks and nature reserves. Among these, alpine lakes seem to be particularly vulnerable to the negative effects of pollution due to their remote position and ultra-oligotrophic character (Tornimbeni et al., 2012). Biogeochemical studies of biota in alpine lakes are scarce so the interactions between trace metals and these ecosystems are still poorly understood.

The aquatic macrophytes colonize standing and flowing waters in all climatic zones and their photosynthetic tissues are permanently, or at least during a major part of the year, submerged in freshwater, float on its surface or emerge above it. Macrophytes are the largest primary producers and the initial source of energy and organic matter. They play a role in nutrient retention and regulation of the trophic state of water bodies (e.g., Teubner et al. 2020, 2022) and act as filters for contaminants and suspended solids. In addition, plant tissues serve as food for fish, invertebrates and waterfowl, and their communities provide shelter and a safe nursery habitat for numerous organisms. Thus, a healthy macrophyte community is a vital component of a lake ecosystem (Wetzel, 2001; Pokorný et al., 2007; Teubner et al., 2022). Macrophytes are capable of accumulating elements from the water and sediment. For that reason, they are used as bioindicators to detect the changes or alternations in ecosystem and in phytoremediation processes (Valitutto et al., 2007).

Our research aimed to assess the macrophyte species diversity and to compare the amount of trace metals (Hg, Cu, Pb, Cr, Cd, Co, Mn, Fe, Zn, and Ni) accumulated in the most abundant species in alpine Lake Bohinj.

2 Material and methods

2.1 STUDY AREA

Lake Bohinj (Triglav National Park, Julian Alps) is the Slovenia's deepest and the largest permanent natural lake. The lake's surface comprises 3.18 km^2, and the maximum depth is 45 m in Fužina bay. It has several permanent and temporary surface and sub-surface tributaries and one outflow and its water is changed approximately four times a year. The major inflow is the River Savica. The area is dominated by mixed forest (mostly spruce and beech) (Urbanc-Bercic, 1995; Andrič et al., 2020). Lake Bohinj has been covered by the national environmental monitoring program for years and, based on biological indicators, was mostly classified to very good ecological status (ARSO, 2020). About the concentration of heavy metals, however, is little known. Some data suggest possible pollution of the lake e.g., high content of Hg and brominated diphenyl were detected in fish in 2006-2021 (ARSO, 2021). The soils of the Western Alps region in Slovenia were reported to be naturally enriched with Cd and Hg (Camarero et al., 2009; Gosar et al., 2019). According to Andrič et al. (2020) further increased heavy metal deposits can be also attributed to human activity in the Bronze Age. In contemporary times, the impacts on the lake are mostly due to summer tourism and non-point pollution sources in the catchment (Urbanc-Bercic, 1995). Yet, Lake Bohinj received very little scientific attention up to date (Andrič et al., 2020). Our previous study (Germ et al., 2023), which was one of the first analysing metal loads in different compartments of the Bohinj lake ecosystem, showed that the sediments and macrophytes in the eastern part of the lake were enriched in trace metals (especially Cr, Co, Cd and Hg) due to human activities in past centuries. These observations indicated that macrophytes may play a role in metal transfer from sediments to trophic web.

2.2 SAMPLING

The first aim of our research was to find out the presence and abundance of macrophytes all over the lake's littoral. To this aim, macrophytes were recorded by observation from the boat. The abundance of macrophytes was estimated for five sampling plots (Figure 1) located in the lake's south, east, and west parts. The method of the macrophyte survey follows Kohler et al. (1995), suggesting an abundance rating at the following scale: 1—very rare, 2—rare, 3—commonly present, 4—frequent, and 5—predominant species. Depth distribution of the species was assessed with a depthmeter (Speedtech Instruments; USA).

The same five sampling plots in Lake Bohinj (Figure 1) were chosen for elemental analysis of the two most abundant taxa *Chara* spp. and *Myriophyllum spicatum*. Samples were collected from boat with a rake with hooks. Plants samples were rinsed with lake water in order to remove adhering sediments. The samples were then put in separate clean, polyethylene bags stored in a cooler bag (around 4°C) and immediately transported to the laboratory. About 0.5 kg of an upper layer of sediment sample below the plants was collected, stored in cotton bags, and kept at a temperature of 4°C until analyses (Polechońska et al., 2022).

2.3 PREPARATION OF THE SAMPLES AND CHEMICAL ANALYSIS

In laboratory, plant and sediment material was cleaned, dried, homogenised and digested in open system. A detailed description of samples preparation and digestion procedures can be found in Germ et al. (2023). Concentrations of Cd, Co, Cr, Cu, Ni, and Pb were determined by graphite furnace atomic absorption spectrometry, and concentrations of Ca, Fe, K, Mg, Mn and Zn by flame atomic absorption spectroscopy (Atomic Absorption Spectrometer Avanta PM GBC, Braeside, Australia). Mercury content in plants was measured using atomic absorption spectrometer (AMA 254 mercury analyzer, Spectro-Lab, Warsaw, Poland).

All elements were analysed against Atomic Absorption Standard Solutions (Sigma Chemical Co., St. Louis, MO, USA) and blanks consisting of the same reagents and subjected to the same procedures.

Figure 1: Map of Lake Bohinj with the location of sampling plots at five littoral sites (green dots) where having taken sediment samples and plant tissue material and carried out vegetation surveys of macrophyte stands.

Results for plants and sediments were calculated on a dry weight basis. The accuracy of the methods was checked using Certified Reference Materials: ICINCT-OBTL-5 (Oriental basma tobacco leaves, LGC, Teddington, UK) and ISE sample 847 (soil from the Philippines, WEPAL, Wageningen, The Netherlands). The recovery rates for all elements were found to be in ranges of 94–104%.

2.4 DATA ANALYSIS

Bioconcentration factor (BCF) was calculated as a ratio of the content of each element in macrophytes and its content in bottom sediments to evaluate (1) the mobility of metals between sediment and tissue of various plants and (2) the relative difference in the bioavailability of metals among plant species (Ali et al., 2013).

For statistical data treatment, normal distribution was confirmed by passing the Shapiro-Wilk test. Homogeneity of variance from the means was assessed using Levene's tests.

Differences in content of elements between plant taxa were tested using t-test. The level of significance was accepted at $p < 0.05$. The XLSTAT (Addinsoft, 2022; New York, USA) was used for these calculations.

3 Results and discussion

3.1 SPECIES COMPOSITION AND ABUNDANCE OF MACROPHYTES

The macrophyte vegetation of Lake Bohinj was surveyed since 1986, which allows some comparisons and insights to its changes. Our results showed that *M. spicatum* was the most abundant species in Lake Bohinj in 2021 – it was observed in all sampling plots usually with abundance 2-3 (Table 1). This observation corresponds to the former studies e.g. (Urbanc-Bercic, 1995) when it was classified as the second most abundant species and its rapid spreading was recorded.

Table 1: Presence, abundance and maximal depth of the species, determined in five sampling plots in Lake Bohinj

Transect/species	**Plot 1** abundance (depth in m)	**Plot 2** abundance (depth in m)	**Plot 3** abundance (depth in m)	**Plot 4** abundance (depth in m)	**Plot 5** abundance (depth in m)
Chara aspera	4 (6.2)	1 (5.9)	2 (2.4)		
Chara delicatula	1 (0.6)	3 (5.9)	2 (3.8)		
Myriolphyllum spicatum	3 (6.2)	2 (5.9)	3 (3.8)	2 (5.5)	1 (2.5)
Phragmites australis				4 (0.5)	
Potamogeton alpinus			1 (3.0)		
Potamogeton crispus				1 (0.7)	
Potamogeton filiformis				1 (2.5)	
Potamogeton lucens				1 (5.5)	
Potamogeton perfoliatus	2 (1.9)				
Potamogeton pusillus		1 (1.0)			1 (2.5)
Ranunculus circinatus				1 (3.5)	

The dominance of this species supports the hypothesis that it is the fittest one to lakes conditions. *M. spicatum* is the third most common species along the whole Danube River (Janauer et al., 2021) and became the predominant submerged macrophyte species in mesotrophic Alte Donau having passed lake restoration (oxbow lake of Danube River, Teubner et al., 2020, 2022). *Chara aspera* and *C. delicatula* were also still present in the lake. However, *C. rudis* and *C. contraria* were not recorded in 2021 any more. As in the 1990s (Urbanc-Bercic, 1995), *Potamogeton* was the most species-rich genus in Lake Bohinj. The following species were found in 2021: *P. alpinus*, *P. crispus*, *P. filiformis*, *P. lucens*, *P. perfoliatus*, and *P. pusillus*. *Potamogeton filiformis* was found in the lake for the first time, while formerly observed *P. praelongus* (Urbanc-Bercic, 1995) was not recorded. Still, the abundance of the representatives of genus *Potamogeton* was low in comparison to *Chara* spp. and *M. spicatum* (Table 1). The lowest number of species (only 2) was found in the sampling plot 5, where the human impact is high. The shore is very gentle and swimmers resuspend materials, hindering macrophyte growth in this location. The number of species was the highest in sampling plot 4, which is close to the autocamp. However, the abundance of the majority of the species is low in this location.

The abundance and species diversity of mountain lakes are usually limited and most commonly recorded species belong to the genus *Chara* and *Potamogeton*. Plant communities dominated by Charophytes and other highly sensitive taxa are characteristic for alpine lakes littoral zones (Cantonati, 2006; Dynowski et al., 2019). The diversity of species in Lake Bohinj is comparable with other lakes from alpine region in Austria and Italy. In the Alpine area in Carinthia Austria, in the zones with natural vegetation, *Phragmites australis*, *Scirpus lacustris*, *Nymphaea alba*, *Nuphar lutea*, *Potamogeton* spp., *Myriophyllum* spp., *Ceratophyllum submersum*, *Chara* spp. were recorded (Myrbo et al., 2012). High diversity of macrophytes indicate stability and effective transformation of energy in the ecosystem (Urbanc-Bercic, 1995). Among the recorded species *P. alpinus*, *P. filiformis*, and *C. aspera* are regarded as the reference indicators for alpine lakes. *Potamogeton lucens* and *P. crispus* favor relatively deep, alkaline calcareous water like Lake Bohinj (Preston et al., 1995) while *P. perfoliatus* and *M. spicatum* are species with wide ecological tolerance and the latter one is most abundant species in Slovenian water bodies (Preston, 1995; Zelnik et al., 2021). The most vigorous *P. perfoliatus* usually grows in water over 1 m deep (Preston, 1995) as it is the case also in Lake Bohinj. Preston (1995) reported, that smaller individuals of *P. perfoliatus* might be found in much shallower waters, which fits well with our observation in Lake Bohinj. Also *P. australis* has a wide ecological tolerance and grows in oligotrophic, mesotrophic and eutrophic habitats. It prefers the shores of the reservoirs where water level is up to 1 m, but in the highly transparent water can reach depth up to 4 m (Köbbing et al., 2013). Furthermore, the presence of *P. filiformis* might show that the conditions in Lake Bohinj got worse as it is characteristic for eutrophic waters (Preston, 1995). Also, the presence *R. circinatus* may indicate to higher water trophy as it is a specifc indicator of mesotrophic and eutrophic conditions (Gebler et al., 2022). However, these species were recorded in only one sampling plot each and with usually low abundance indicating to local changes in habitat conditions. Also, the situation in 2021 was exceptional regarding the water level. It was lower compared to other years due to dry conditions in Europe. Therefore, this aspect needs further investigations.

3.2 TRACE METAL BIOCONCENTRATION IN M. SPICATUM AND CHARA SPP.

The content of Cd, Co, Cu, Hg, Mn, Ni, and Zn was higher in the tissue of *M. spicatum* compared to stoneworts (Figure 2). This agrees with Samecka-Cymerman and Kempers (2004) who found that *Myriophyllum* species have a higher accumulation capacity than other submerged macrophytes. Moreover, some studies show that the concentration of Cd in *M. spicatum tissues* were higher than in *Potamogeton pectinatus* (Samecka-Cymerman and Kempers, 2004) and *Ceratohyllum demersum* (Fawzy et al., 2012). Also, Mazej Grudnik and Germ (2010) stated that *M spicatum* from lakes in central Slovenia contained higher concentrations of Cd and Zn in its shoots than *Najas marina*.

Following the same trend as metal contents in vascular plants, *Chara* spp. had a lower BCF than *M. spicatum* for almost all elements (Cd, Co, Cu, Ni, Mn, Fe and Hg). The greatest differences between species were noted for Cd, Co, and Zn (Figure 3). The differences between plants might be due to different uptake mechanisms. Charophytes do not have vascular tissue but can incorporate nutrients and other elements as trace metals through their cell wall as all microalgae living in aquatic environment, including charophyte microalgae, do. Large thalli of Charophytes, which build up underwater meadows together with vascular submerged macrophytes, live attached to the substrate by rhizoids.

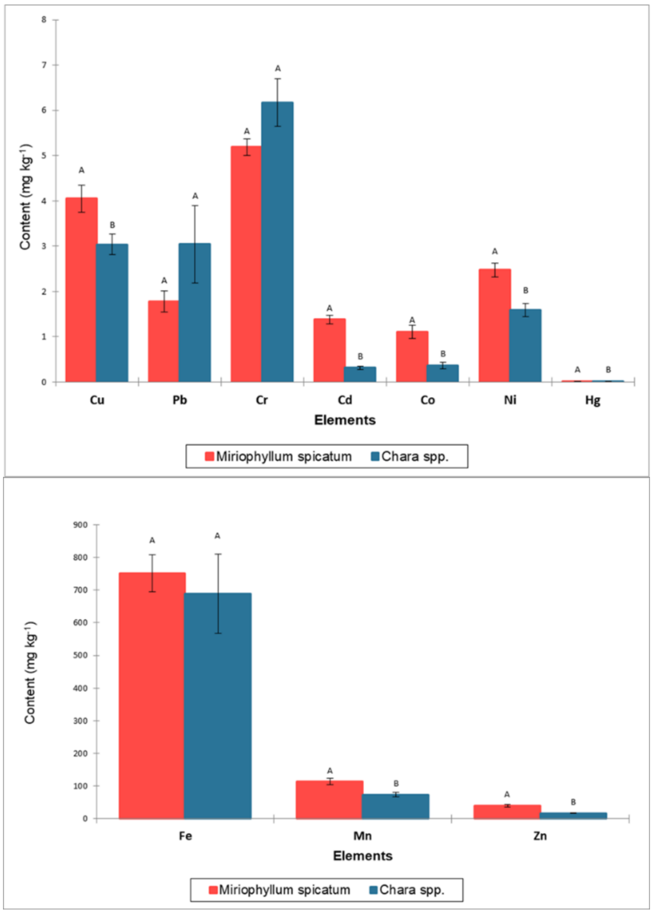

Figure 2: Content of trace metals in *Myriophyllum spicatum* and *Chara* spp. in Lake Bohinj. Data are means ± SE, different letters indicate statistical differences between species (t-test, $p < 0.05$).

The biomass of rhizoids is small in comparison to their above-ground green parts and much smaller than root systems of most aquatic angiosperms (Kufel et al., 2002). Thus, it is assumed that *Chara* spp. absorb elements mainly through submerged organs from water column (Asaeda et al., 2013), where concentrations of metals are usually diluted and much lower than in sediments (Polechońska et al., 2023). *Myriophyllum spicatum* is rooted so it can absorb metals both through roots from sediments and through leaves from water (Cardwell et al., 2002). Myrophyllum spicatum leaves have a thin cuticle that is easy to pass by elements which facilite the uptake (Geng et al., 2019).

The values of BCF > 1 indicate the active transport of elements from the sediment to the leaves (Polechońska et al., 2022). In case of *M. spicatum* an enhanced bioaccumulation of trace metals than compared to the respective concentration in the sediment (BCF > 1) was observed for four of the ten elements examined: Cd, Co, Mn and Zn. Thus, our study confirms other investigations that this vascular macrophyte species has a high capacity to accumulate metals (Fawzy et al., 2012; Galal et al., 2014; Harguinteguy et al., 2016). In Fawzy et al. (2012), the BCF was calculated as heavy metal concentration ratio between plant tissue and water, and thus these results are not directly comparable with our data. Notwithstanding, many studies report an effective bio-accumulation for trace metal elements as Cd, Pb, As, Cr and Hg (Keskinkan et al., 2007; Fawzy et al., 2012; Yabanli et al., 2014; Zamani-Ahmadmahmoodi et al., 2020). The highest BCF for Cd and Zn (BCF > 2) (between sediment and plant tissue) in the case of *M. spicatum* shown in Figure 3 confirms its suitability for the phytoremediation of polluted water bodies (Fawzy et al., 2012). In our study, BCF values for Charophytes have not exceeded the value of one (BCF > 1) for any examined trace element. According to Asaeda et al., (2013), Charophytes are proposed for phytoremediation applications but mostly for nutrients. *Chara* was observed to be a hyperaccumulator for Mn and Cd in unpolluted habitats only (Rai et al., 1995; Asaeda et al., 2013), while other report in general a rather low capacity accumulating trace metals (Laffont-Schwob et al., 2015).

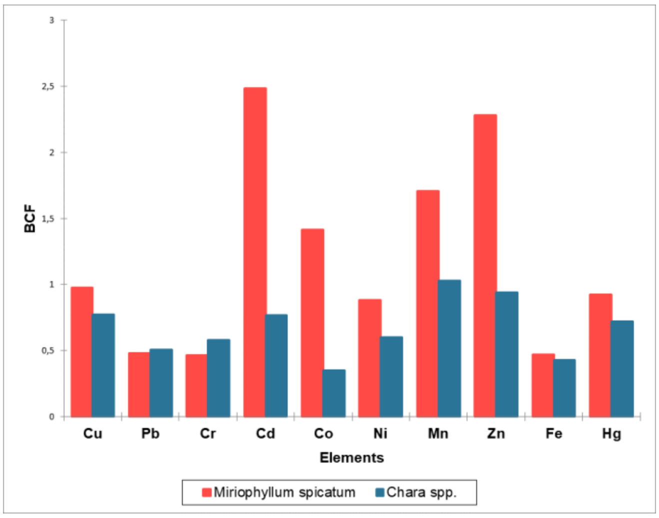

Figure 3: Bioconcentration factor (BCF) of *Myriophyllum spicatum* and *Chara* spp. for different elements.

4 Conclusions

A high number of macrophytes was found in the oligotrophic alpine Lake Bohinj. Species of both wide ecological tolerance such as *P. australis*, *P. perfoliatus* and *M. spicatum* and more sensitive, indicator species such as *P. alpinus*, *P. filiformis*, and *C. aspera* were observed. The diversity of species in Lake Bohinj was comparable to other lakes from the alpine region. *Myriophyllum spicatum* and *Chara* spp. were the most abundant taxa and therefore suited best for trace metal analyses. *Myriophyllum spicatum* showed a higher ability to uptake elements (Cd, Co, Cu, Hg, Mn, Ni, and Zn) compared to stoneworts. According to the high elements content and BCFs values, *M. spicatum* is an appropriate candidate species for the phytoremediation of trace metal in contaminated water bodies, especially for Cd, Co, Mn and Zn.

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References

Al-Abbawy D.A.H., Al-Thahaibawi B.M.H., Al-Mayaly I.K.A., Younis K.H. (2021). Assessment of some heavy metals in various aquatic plants of Al-Hawizeh Marsh, southern of Iraq. *Biodiversitas* 22:338–345. **DOI:10.13057/biodiv/d220141**

Ali H., Khan E., Sajad M.A. (2013). Phytoremediation of heavy metals-Concepts and applications. *Chemosphere* 91:869–881. **DOI:10.1016/j.chemosphere.2013.01.075**

Andrič M., Sabatier P., Rapuc W., Ogrinc N., Dolenec M., Arnaud F., von Grafenstein U., Šmuc A. (2020). 6600 years of human and climate impacts on lake-catchment and vegetation in the Julian Alps (Lake Bohinj Slovenia). *Quaternary Science Reviews* 227:106043. **DOI:10.1016/j.quascirev.2019.106043**

ARSO (2020). Rezultati monitoringa ekološkega stanja jezer v letu 2020.

ARSO (2021). Jezera - Ocena kemijskega stanja jezer od leta 2006 do 2021.

Asaeda T., Zaman T. (2013). Heavy Metal Uptake and Tolerance of Charophytes. In. Gupta D. F. Corpas & J. Palma (eds.) *Heavy Metal Stress in Plants*. Springer Berlin Heidelberg. **DOI:10.1007/978-3-642-38469-1_6**

Brucet S., Poikane S., Lyche-Solheim A., Birk S. (2013). Biological assessment of European lakes. Ecological rationale and human impacts. *Freshwater Biology* 58:1106–1115. **DOI:10.1111/fwb.12111**

Camarero L., Botev I., Muri G., Psenner R., Rose N., Stuchlik E. (2009). Trace elements in alpine and arctic lake sediments as a record of diffuse atmospheric contamination across

Europe. *Freshwater Biology* 54:2518–2532. **DOI:10.1111/j.1365-2427.2009.02303.x**

Cantonati M. (2006). Flora. In. Stoch F. (ed.) High-altitude lakes Pearls in the mountain landscape. Museo Friulano di Storia Naturale Udine.

Cardwell A.J., Hawker D.W., Greenway M. (2002). Metal accumulation in aquatic macrophytes from southeast Queensland Australia. *Chemosphere* 48:653–663. **DOI:10.1016/S0045-6535(02)00164-9**

Dynowski P., Senetra A., Źróbek-Sokolnik A., Kozłowski J. (2019). The Impact of Recreational Activities on Aquatic Vegetation in Alpine Lakes. *Water* 11:173. **DOI:10.3390/w11010173**

Eid E.M., Galal T.M., Shaltout K.H., El-Sheikh M.A., Asaeda T., Alatar A.A., Alfarhan A.H., Alharthi A., Alshehri A.M.A., Picó Y., Barcelo D. (2020). Biomonitoring potential of the native aquatic plant *Typha domingensis* by predicting trace metals accumulation in the Egyptian Lake Burullus. *Science of The Total Environment* 714:136603. **DOI:10.1016/j.scitotenv.2020.136603**

Fawzy M.A., Badr N.E. sayed, El-Khatib A., Abo-El-Kassem A. (2012). Heavy metal biomonitoring and phytoremediation potentialities of aquatic macrophytes in River Nile. *Environmental Monitoring and Assessment* 184:1753–1771. **DOI:10.1007/s10661-011-2076-9**

Féraud G., Potot C., Fabretti J. F., Guglielmi Y., Fiquet M., Barci V., Maria P.C. (2009). Trace elements as geochemical markers for surface waters and groundwaters of the Var River catchment (Alpes Maritimes France). *Comptes Rendus Chimie* 12:922–932. **DOI:10.1016/j.crci.2009.02.002**

Galal T.M., Shehata H. S. (2014). Evaluation of the invasive macrophyte *Myriophyllum spicatum* L. as a bioaccumulator for heavy metals in some watercourses of Egypt. *Ecological Indicators* 41:209–214. **DOI:10.1016/j.ecolind.2014.02.004**

Gebler D., Zalewska-Gałosz J., Jopek M., Szoszkiewicz K. (2022). Molecular identification and habitat requirements of the hybrid *Ranunculus circinatus* × *R. fluitans* and its parental taxa *R. circinatus* and *R. fluitans* in running waters. *Hydrobiologia* 849:2999–3014. **DOI:10.1007/s10750-022-04909-6**

Geng N., Wu Y., Zhang M., Tsang D.C.W., Rinklebe J., Xia Y., Lu D., Zhu L., Palansooriya K.N., Kim K.H., Ok Y.S. (2019). Bioaccumulation of potentially toxic elements by submerged plants and biofilms. A critical review. *Environment International* 131. **DOI:10.1016/j.envint.2019.105015**

Germ M., Golob A., Zelnik I., Klink A., Polechońska L. (2023). Contents of metals in sediments and macrophytes differed between the locations in an alpine lake revealing Human impacts - A case study of Lake Bohinj (Slovenia). *Water* 15(7), 1254. **DOI:10.3390/w15071254**

Gosar M., Šajn R., Bavec Š., Gaberšek M., Pezdir V., Miler M. (2019). Geochemical background and threshold for 47 chemical elements in Slovenian topsoil. *Geologija* 62:7–59. **DOI:10.5474/geologija.2019.001**

Harguinteguy C.A., Cirelli A.F., Pignata M.L. (2014). Heavy metal accumulation in leaves of aquatic plant *Stuckenia filiformis* and its relationship with sediment and water in the Suquía river (Argentina). *Microchemical Journal* 114:111–118. **DOI:10.1016/j.microc.2013.12.010**

Harguinteguy C.A., Cofré M.N., Fernández-Cirelli A., Pignata M.L. (2016). The macrophytes *Potamogeton pusillus* L. and *Myriophyllum aquaticum* (Vell.) Verdc. as potential bioindicators of a river contaminated by heavy metals. *Microchemical Journal* 124:228–234. **DOI:10.1016/j.microc.2015.08.014**

Janauer G.A., Exler N., Anačkov G., Barta V., Berczik Á., Boža P., Dinka M., Georgiev V., Germ M., Holcar M., Hrivnák R., Igić R., Ozimec S., Sârbu A., Schmidt B., Schmidt-Mumm U., Schütz W., Sipos K., Szalma E., Topić J., Tsoneva S., Valachovič M., Valchev V., Vukov D., Zelnik I., Gaberščik A. (2021). Distribution of the macrophyte communities in the Danube reflects river serial discontinuity. *Water* 13 (7), 918. **DOI:10.3390/w13070918**

Keskinkan O., Goksu M. Z. L., Yuceer A., Basibuyuk M. (2007). Comparison of the Adsorption Capabilities of *Myriophyllum spicatum* and *Ceratophyllum demersum* for Zinc Copper and Lead. *Engineering in Life Sciences* 7:192–196. **DOI:10.1002/elsc.200620177**

Köbbing J.F., Thevs N., Zerbe S. (2013). The utilisation of reed (*Phragmites australis*): A review. *Mires and Peat* 13 (1):1–14. **http://www.mires-and-peat.net/, ISSN 1819-754X**

Kohler A., Janauer G.A. (1995). Zur Methodik der Untersuchung von aquatischen Makrophyten in Fließgewässern. In. Steinberg C.H. Bernhardt and H. Klapper (eds.) Handbuch Angewandte Limnologie. *Ecomed*, Landsberg am Lech:3–22. **DOI:10.1002/9783527678488.hbal1995011**

Kufel L., Kufel I. (2002). *Chara* beds acting as nutrient sinks in shallow lakes—a review. *Aquatic Botany* 72:249–260. **DOI:10.1016/S0304-3770(01)00204-2**

Laffont-Schwob I., Triboit F., Prudent P., Soulié-Märsche I., Rabier J., Despréaux M., Thiéry A. (2015). Trace metal extraction and biomass production by spontaneous vegetation in temporary Mediterranean stormwater highway retention ponds. Freshwater macroalgae (*Chara* spp.) vs. cattails (*Typha* spp.). *Ecological Engineering* 81:173–181. **DOI:10.1016/j.ecoleng.2015.04.052**

Marchand L., Nsanganwimana F., Cook B.J., Vystavna Y., Huneau F., Le Coustumer P., Lamy J.B., Oustrière N., Mench M. (2014). Trace element transfer from soil to leaves of macrophytes along the Jalle d'Eysines River France and their potential use as contamination biomonitors. *Ecological Indicators* 46:425–437. **DOI:10.1016/j.ecolind.2014.07.011**

Mazej Grudnik Z., Germ M. (2010). *Myriophyllum spicatum* and *Najas marina* as bioindicators of trace element contamination in lakes. *Journal of Freshwater Ecology* 25(3):421-426. **DOI:10.1080/02705060.2010.9664385**

Myrbo A., Fairbridge R. W., Sampl H., Bolgov M.V., Lemoalle J., Bader J. C., Leblanc M., Sarkar S.K., Bhattacharya A., Bhattacharya A.K., Satpathy K.K., Mohanty A.K., Panigrahi S., Zhang L.T., Yang X., Bengtsson L., Bengtsson L., Bengtsson L., Blenckner T. et al. (2012). Carinthia Lakes and Reservoirs in Austria pp. 128–136.

Pokorný J., Květ J. (2007). Aquatic Plants and Lake Ecosystems. The Lakes Handbook Volume 1. John Wiley & Sons Ltd pp. 309–340.

Polechońska L., Klink A., Golob A., Germ M. (2022). Evaluation of *Nuphar lutea* as bioindicator of metal pollution in freshwater ecosystems. *Ecological Indicators* 136.108633. **DOI:10.1016/j.ecolind.2022.108633**

Polechońska L., Klink A. (2023). Macrophytes as passive bioindicators of trace element pollution in the aquatic environment. *Wiley Interdisciplinary Reviews. Water* 10(2), e1630. **DOI:10.1002/wat2.1630**

Preston C.D. (1995). Pondweeds of Great Britain and Ireland. Botanical Society of Britain & Ireland.

Preston C. D. (Christopher D., Ellis L. T. (1995). Pondweeds of Great Britain and Ireland. Botanical Society of the British Isles London.

Rai U.N., Sinha S., Tripathi R D., Chandra P. (1995). Wastewater treatability potential of some aquatic macrophytes. Removal of heavy metals. *Ecological Engineering* 5:5–12. **DOI:10.1016/0925-8574(95)00011-7**

Samecka-Cymerman A., Kempers A.J. (2004). Toxic metals in aquatic plants surviving in surface water polluted by copper mining industry. *Ecotoxicology and Environmental Safety* 59(1):64–69. **DOI:10.1016/j.ecoenv.2003.12.002**

Sojka M., Jaskuła J., Barabach J., Ptak M., Zhu S. (2022). Heavy metals in lake surface sediments in protected areas in Poland. concentration pollution ecological risk sources and spatial distribution. *Scientific Reports* 12(1), 15006. **DOI:10.1038/s41598-022-19298-y**

Teubner K., Teubner I., Pall K., Kabas W., Tolotti M., Ofenböck T. and Dokulil M.T. (2020) New Emphasis on Water Transparency as Socio-Ecological Indicator for Urban Water: Bridging Ecosystem Service Supply and Sustainable Ecosystem Health. *Frontiers in Environmental Science* 8:573724. **DOI:10.3389/fenvs.2020.573724**

Teubner K., Teubner I.E., Pall K., Tolotti M., Kabas W., Drexler S.-S., Waidbacher H., Dokulil M.T. (2022) Macrophyte habitat architecture and benthic-pelagic coupling. Photic habitat demand to build up large P storage capacity and bio-surface by underwater vegetation. *Frontiers in Environmental Science* 10.901924. **DOI:10.3389/fenvs.2022.901924**

Tornimbeni O., Rogora M. (2012). An Evaluation of Trace Metals in High-Altitude Lakes of the Central Alps. *Water Air & Soil Pollution* 223:1895–1909. **DOI:10.1007/s11270-011-0993-4**

Urbanc-Bercic O. (1995). Aquatic vegetation in two pre-alpine lakes of different trophic levels (Lake Bled and Lake Bohinj). Vegetation development from the aspect of bioindication. *Acta Botanica Gallica* 142:563–570. **DOI:10.1080/12538078.1995.10515280**

Valitutto R.S., Sella S.M., Silva-Filho E.V., Pereira R.G., Miekeley N. (2007). Accumulation of metals in macrophytes from water reservoirs of a power supply plant Rio de Janeiro State Brazil. *Water Air and Soil Pollution* 178:89–102. **DOI:10.1007/s11270-006-9154-6**

Wetzel R.G. (2001). Limology: Lake and River Ecosystems 3rd ed. Low Temperature Physics Vol. Academic P.

Yabanli M., Yozukmaz A., Sel F. (2014). Heavy metal accumulation in the leaves stem and root of the invasive submerged macrophyte *Myriophyllum spicatum* L. (Haloragaceae). an example of Kadin Creek (Mugla Turkey). *Brazilian Archives of Biology and Technology* 57:434–440. **DOI:10.1590/S1516-8913201401962**

Zamani-Ahmadmahmoodi R., Gharahi N., Martin J.A.R., Aazami J., Jafari A., Bahmani M., Jiménez-Ballesta R. (2020). Cd and Pb bioaccumulation in Eurasian watermilfoil (*Myriophyllum spicatum*) in relation to the role of metal contents in wetland sediments. *Environmental Monitoring and Assessment* 192:1-11. **DOI:10.1007/s10661-020-08473-1**

Zelnik I., Kuhar U., Holcar M., Germ M., Gaberščik A. (2021). Distribution of vascular plant communities in Slovenian watercourses. Water (Switzerland) 13(8), 1071. **DOI:10.3390/w13081071**