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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)

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Abstract: Metals stored in sediments of lakes can bioaccumulate through the food chain, posing a risk to the environment and human health. Alpine lakes are supposed to be less affected by pollution than lowland lakes and are vulnerable to any changes and impacts in their catchment areas because of their remote position and ultra-oligotrophic character. Therefore, we used a model Alpine lake, Bohinj (in the Triglav National Park, Julian Alps, Slovenia), to evaluate the load of metals in the abiotic and biotic compartments of the ecosystem, in order to assess the spatial distribution of metals, and finally, to determine whether past and present human activities in the lake's catchment area may be causing pollution. To this aim, the contents of Cu, Pb, Cr, Cd, Co, Mn, Fe, Zn, Hg and Ni in the sediment, water, and macrophyte samples were determined. The results showed that the average content of some toxic elements, especially in the sediments (Cd 0.52 mg/kg; Hg 0.03 mg/kg) and plants (Co 0.71 mg/kg; Cr 5.88 mg/kg) was elevated compared to natural background values. High Hg contents could be connected with natural geological sources, while other elements were probably of anthropogenic origin. High levels of all elements in the eastern part of the lake indicated long-term pollution, which could be a consequence of past iron extraction and military activities in the vicinity. On the other hand, high contents of elements in the water suggests that intensive touristic activities in the area may cause temporal pollution in the summer. The study sheds light on complicated processes governing the distribution of trace metals in Alpine lakes.

Keywords: oligotrophic lake; macrophytes; pollution; potentially hazardous elements

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1. Introduction

The quality of water, especially in lake ecosystems, is largely influenced by human activities within the reservoir as well as in its catchment area [1]. Anthropopressure is strongly connected with emissions of Cd, Cr, Cu, Hg, Ni, Pb, and Zn, usually termed as trace metals, which characterize increasing environmental concentrations, persistence and toxicity [2]. Another potential danger of trace metals is their ease of accumulation in organisms [3]. Due to these features, trace metal pollution may pose a risk to the natural environment and human health. The World Health Organization has estimated that as many as two-thirds of diseases are the consequence of polluted water [4]. Therefore, water pollution control is one of the most pressing and challenging issues of the modern world.

Many factors influence the concentrations and bioavailability of trace metals in aquatic ecosystems: climatic (temperature, precipitation, evaporation, etc.), morphological and hydrological conditions, geological features of the catchment area, the physical parameters of the water and the retention time of water in the substrates r [5,6]. Metals in pristine aquatic ecosystems occur in trace concentrations and their contents largely depend on the geology of the catchment area [7], but various human activities can substantially increase their concentrations [7–9]. It was indicated that, beside current impacts such as cattle livestock farming, the massive pressure of bathers in the summer, vessels and traffic, past activities leave their mark on the environment as well. In particular, industrial activity such as mining, melting and the extraction of metals could be detected decades and even centuries after their abandonment [10]. For most of the toxic trace metals, anthropogenic sources are more important than natural ones [11]. Anthropogenic metals are, in comparison with lithogenic metals, reported as highly bioavailable and transportable in the aquatic environment [12]. Lake sediments are known to be a crucial storage sink for metals and other substances deposited from the water [13]. On the other hand, these elements can also be easily circulated back into the water [14], which process significantly increases their concentration—even to a toxic level [15]. Liu et al. [16] observed that hypoxia or a high content of inorganic nitrogen also increased the release of certain metals from sediments, as well as possibly leading to higher metal bioavailability [4]. Beside the sediments, macrophytes also have an important function regarding intake and deposition of trace elements in aquatic environments [17]. Plants are good indicators of trace elements since they accumulate pollutants during their whole growing period and are not affected by temporal fluctuations. Shah et al. [18] confirmed that macrophytes have the ability to intake high amounts of metals from surrounding water, which suggests that they can be used as in situ biomonitors of lake pollution.

Alpine lakes are especially vulnerable to any changes and impacts in their catchment areas because of their remote position and ultra-oligotrophic character, and for the same reasons, they are particularly valuable ecosystems [3]. Compared to lowland lakes, the cycling of the metals in Alpine lakes has been rarely studied; therefore, the accumulation of trace metals in their compartments has still only been partly described. An example of a model Alpine lake is Lake Bohinj, which is the deepest and the largest permanent natural lake in Slovenia. The lake and most of its catchment area are within the Triglav National Park and one of Slovenia's favourite recreational areas and is supposed to be relatively free from pollution. Monitoring of Lake Bohinj has been taking place for more than 40 years, but up-to-date monitoring programs have mostly included measurements of the contaminants in water and sediments only, which do not allow us to adequately predict levels in aquatic biota and ecotoxic effects [19]. There has been no study examining the content of the metals in both the abiotic and biotic compartments of Lake Bohinj. European Commission Water Framework Directive (WFD) [20] guidelines obliged the monitoring of the ecological state of the waterbodies as well as the presence of priority substances. Thus, according to phytoplankton indices, in 2020 Lake Bohinj was classified as having a very good ecological status [21]. On the other hand, high contents of Hg and brominated diphenyl were detected in fish in 2006–2021 [22], indicating possible pollution from trace metals. The water quality guidelines of the WFD list Hg, Pb, Cd and Ni as dangerous substances, while Cu, Cr and Zn are on the list of specific hazardous chemical substances. Elements such as Hg, Pb and Cd are not essential trace elements and can only have negative effects on biota [23]. The results of these monitoring surveys drew attention to possible pollution of Lake Bohinj and indicated the need for more complex research to evaluate the metal contents in both abiotic and biotic compartments of the lake ecosystem, and to identify possible sources and risks.

Therefore, we decided to measure the content of several metals (Cu, Pb, Cr, Cd, Co, Mn, Fe, Zn, Hg and Ni), which are considered as potentially hazardous elements, in the water, sediment, and macrophyte samples from Lake Bohinj to find out the relation between elements in the abiotic and biotic compartments of the ecosystem, to assess the spatial distribution of metals, and, finally, to determine if past and present human activities in the lake's catchment area have caused the ecosystem pollution.

2. Materials and Methods

2.1. Study Area

Lake Bohinj is located in the Julian Alps in the Alpine region (north-west Slovenia), and is the deepest and largest natural lake in Slovenia. It has many permanent as well as temporary surface and subsurface tributaries and one outflow (the River Jezernica, which confluences with the River Mostnica and forms the River Sava Bohinjka). The major inflow is the River Savica with an average discharge of 5.5 m^3/s, representing approximately 60% of the outflow from the River Jezernica, with an average discharge of 9 m^3/s. The lake's surface is 3.18 km^2 and its maximum depth in Fužina bay is 45 m. The lake is subjected to winds and considerably high throughflow at high discharges of the inflowing River Savica, which accelerate the mixing of water masses and shorten the retention time of the water in the lake. The latter is extremely important for the slow progress of succession processes. The retention time of the water is 3–4 months; therefore, the water is changed approximately four times a year. The watershed area of Lake Bohinj is larger than 100 km^2 [24] and has the characteristics of Alpine karst as Triassic limestones and dolomites which form the vast majority of the bedrock. The water draining this catchment area is rich in carbonate and bicarbonate and has a pH between 7.2 and 8.4.

The most diverse plant genus in Lake Bohinj is *Potamogeton*. In 2022, six species of this genus were found in the lake: *P. perfoliatus*, *P. alpinus*, *P. lucens*, *P. crispus*, *P. pusillus*, and *P. filiformis*. In addition to pondweeds, we also found *Myriophyllum spicatum*, *Ranunculus circinatus*, *Phragmites australis*, *Chara aspera* and *Chara delicatula*. Despite the many species of pondweeds in the lake, their abundance is low in comparison to *Chara* spp. and *M. spicatum*.

2.2. Sampling

Five sampling plots in Lake Bohinj (Figure 1) were chosen, where the macrophytes had been frequently sampled within the national monitoring program. Plots were located perpendicularly to the shore, 6 m wide and 6 m long. Plots were located in the south, east and west part of the lake, where the coverage of the littoral with macrophyte stands is considerable, and species diversity is at its highest. Sampling plot 5 is adjacent to a front-moraine of the glacier reaching towards the village of Stara Fužina, where iron-ore smelting activities occurred. Macrophytes were identified with the help of a steel tube with a transparent glass bottom and collected from a boat using a telescopic rod with hooks. All species present in the plots were collected (Table 1). At least three samples of each species were collected at each plot. Samples were rinsed with lake water to remove adhering sediments, put in separate clean, polyethylene bags and transported to the laboratory in a cooler bag (around 4 °C) on the same day. The depth distribution of species was assessed with a depth meter (Speedtech Instruments; Unionville, VA, USA).

At the same plot, we also collected samples of the water and bottom sediment (three composite samples of the water and three composite samples of the sediments at each site). Water samples (0.5 L) were collected from the lake at a depth of 0.5 m and stored in clean polyethylene bottles. We collected about 0.5 kg of a bottom sediment sample below the plant stand at a depth of 0–3 cm with a handheld stainless-steel collector and then stored the samples in cotton bags. Samples were transported to a laboratory in a cooler bag and kept at a temperature of 4 °C until analyses [25].

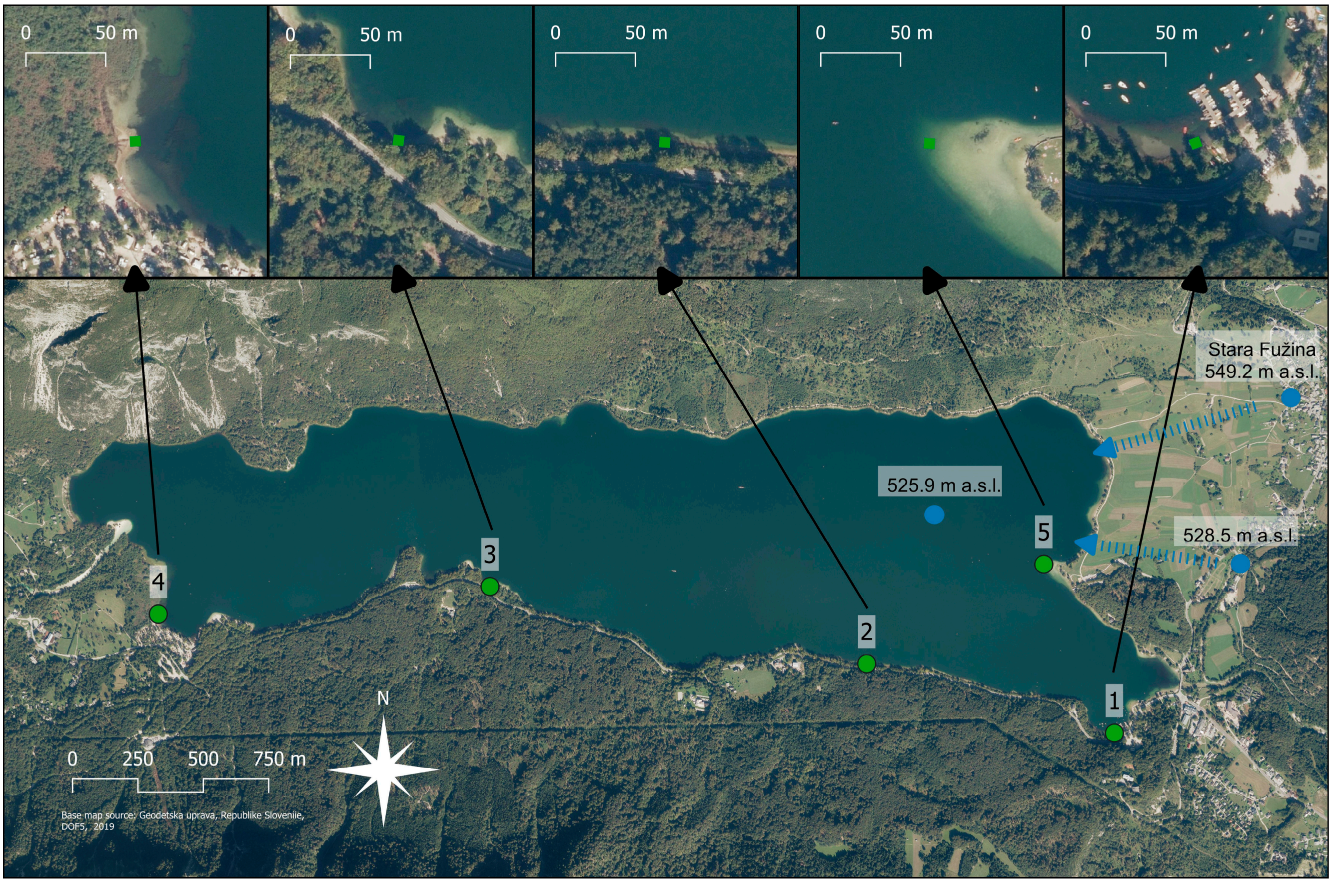

Figure 1. A map showing the location of the sampling plots in Lake Bohinj. Pictures on the upper site represent the size of each sampling plots with a dimension 6 × 6 m. The area east of the lake (and sampling plot 5) is a front-moraine of the former glacier reaching towards the village of Stara Fužina, where iron-ore smelting activities occurred. Blue circles show the altitude of the specific area, while blue arrows display the directions of the subsurface water currents.

Table 1. List of species collected in each plot.

Plot	Species Collected
1	*C. delicatula*, *C. aspera*, *M. spicatum*, *P. perfoliatus*
2	*C. delicatula*, *C. aspera*, *M. spicatum*
3	*C. delicatula*, *M. spicatum*
4	*P. crispus*, *P. filiformis*, *P. lucens*, *M. spicatum*
5	*M. spicatum*

2.3. *Preparation of the Samples and Chemical Analyses*

Waters were filtered through a Whatman glass microfiber filter (GF/C) and acidified to pH ≤ 2 with HNO_3 (65% ultra-pure pro-analysis, Merck KGaA, Darmstadt, Germany). Plant samples were manually cleaned of parts of other species, dead plant parts and other adhering materials, and subsequently washed in distilled water to clean them of periphyton, before being dried at 70 °C in a laboratory dryer. Dry plant material was ground in a mortar to a fine powder. Sediments were air-dried (at a temperature of 20 °C) for seven days. Next, all external materials (such as plants residues) were removed manually using stainless tweezers. Samples were passed through a 2 mm diameter sieve to remove stones and ground to fine powder in an automatic mortar grinder (Pulverisette 2, Fritsch, Idar-Oberstein, Germany) [26]. The mercury content in plants and sediments was measured using an atomic absorption spectrometer (AMA 254 mercury analyzer, Spectro-Lab, Warsaw, Poland). Next, sediment and plant samples (0.5 g; all in two replicates) were digested with HNO_3 (65% ultra-pure pro-analysis, Merck KGaA, Germany) and H_2O_2 (30% ultra-pure, Chempur, Piekary Śląskie, Poland) in an open system. The digests were cooled down and diluted with deionised water to a volume of 50 mL [27]. Metal contents were determined in the digests and acidified water samples: concentrations of Cd, Co, Cr, Cu, Ni, and Pb were determined by ETAAS, and concentrations of Ca, Fe, K, Mg, Mn and Zn by FAAS (Atomic Absorption Spectrometer Avanta PM GBC, Braeside, Australia).

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. Results for plants and sediments were calculated on a dry weight basis. The accuracy of the methods was checked using Certified Reference Materials: IC-INCT-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 Analyses

Data are presented as box plots, where the sides of the box represent the lower and upper quartile. The line inside the box is median, the red plus sign represents the average value for the parameter, the lower whisker represents the lower extreme, and the upper whisker represents the upper extreme. The squares outside the box plot are outliers.

Normal distributions of the data and homogeneity of variance from the means were assessed with Shapiro–Wilk tests and Levene's tests, respectively. Differences in the content of the elements in sediments and plants between the plots were tested using one-way ANOVA and differences between the groups were tested with a Tukey post hoc test. The level of significance was accepted at $p < 0.05$. The calculations were made with XLSTAT (Addinsoft, 2022; New York, NY, USA).

The anthropogenic impact on the studied sediment chemistry was evaluated by the contamination factor (CF), calculated as the following ratio: $CF = C_i/C_n$, where: C_i—the content of the specific heavy metal investigated and C_n—the local uncontaminated background level for the same metal [28,29]. The mean pre-industrial concentration of metals in the European Alpine lakes was employed as a background value and the CFs were calculated only for Cd, Cu, Hg, Pb and Zn for which biogeochemical background values are available [30]. Contamination factor values below 1 indicate low contamination from metal, those in the range of 1–3—moderate contamination, 3–6—considerable contamination, and >6 very high contamination [28,29].

3. Results

The average concentrations of Cu, Pb, Cd and Zn in the lake water were higher than values in waterbodies on limestone bedrock, such as the Plitvice lakes (Croatia), published by Vukosav et al. [19]. However, only Cu and Cd concentrations were higher than the Environmental Quality Standards of the European Commission WFD (Table 2). Except for Cd and Hg, the average contents of all measured elements in the lake sediment were lower than values in sediments of Lake Zürich 200 years ago, published by von Gunten et al. [31] and in natural water bodies, according to Kabata-Pendias and Pendias [32]. The average contents of Cd and Ni exceeded literature values in some study sites (Table 2 and Figure 2). The average contents of Cr (5.88 ± 1.03 mg/kg DW) and Cd (0.73 ± 0.47 mg/kg DW) in macrophytes from Lake Bohinj were higher than natural contents in uncontaminated macrophytes (4.0 mg/kg DW for Cr and 1.0 for Cd) [33], while the contents of Cu, Pb, Mn, Fe, Zn, Ni and Hg were lower (Table 2).

Table 2. Average concentrations ± SD of elements in water, bottom sediments and macrophytes in five plots in Lake Bohinj and literature values representing quality standards and natural contents.

Elements	Water (μg L^{-1})			Sediments (mg kg^{-1}DW)		Macrophytes (mg kg^{-1}DW)	
	Lake Bohinj	Vukosav et al. [£]	EQS * (WFD)	Lake Bohinj	Literature Values	Lake Bohinj	Natural Content of Macrophytes
Cu	3.96 ± 1.09	0.012 [£]	1.1	4.88 ± 2.04	20–23 [•]	3.91 ± 1.30	7.9 [$]
Pb	0.68 ± 0.29	0.012 [£]	7.2	5.46 ± 2.67	10–19 [•]	2.17 ± 1.88	6.1 [$]
Cr	0.40 ± 0.14			14.73 ± 8.06	50 [#]	5.88 ± 1.03	4.0 [$]
Cd	0.09 ± 0.03	0.011 [£]	0.08	0.52 ± 0.20	0.2 [•]	0.73 ± 0.47	1.0 [$]
Co	0.10 ± 0.20			0.96 ± 0.66	13 [#]	0.71 ± 0.60	0.32 [$]
Mn	2.42 ± 0.92			91.32 ± 44.05	770 [#]	96.24 ± 37.23	370.0 [$]
Fe	76.52 ± 36.10			2207± 1100	18,000 [#]	771.3 ± 360.2	1000 [$]
Zn	2.92 ± 1.32	0.26 [£]	≤7.8	21.55 ± 9.82	50–84 [•]	33.99 ± 19.51	52.0 [$]
Ni	3.44 ± 3.03			4.96 ± 4.55	6 [#]	2.15 ± 0.62	4.2 [$]
Hg				0.03 ± 0.02	0.025 [®]–0.4 [•]	0.02 ± 0.02	0.029 [®]

Notes: [£] Vukosav et al., 2014 [19]; * Environmental Quality Standards WFD [20]; [•] von Gunten et al., 1997 [31]; [#] Kabata-Pendias and Pendias, 1999 [32]; [$] Brooks and Robinson, 1998 [33]; [®] Fontanella et al., 2009 [34].

The spatial distribution of the metals in lake sediments was heterogeneous (Figure 2). The contents of Cu, Pb, Co, Cr, Zn, and Fe were statistically the lowest in plot 3 (2.36 ± 0.19 mg/kg DW, 2.49 ± 0.35 mg/kg DW, 0.22 ± 0.01 mg/kg DW, 6.01 ± 0.35 mg/kg DW, 8.98 ± 0.94 mg/kg DW and 797 ± 151 mg/kg DW, respectively), while the contents of Pb, Cd, Co, Mn, Ni, Zn and Hg were statistically the highest in plot 5 (8.75 ± 0.22 mg/kg DW, 0.83 ± 0.09 mg/kg DW, 1.75 ± 0.05 mg/kg DW, 151 ± 2 mg/kg DW, 11.91 ± 0.41 mg/kg DW, 34.69 ± 1.19 mg/kg DW and 0.05 ± 0.001 mg/kg DW, respectively). For all elements, a similar pattern showing lower content of sediments in plots 3 and 4 and higher content in plots 2 and 5 could be seen (Figure 2). According to the CF values (Table 3), sediments of all plots indicated low contamination of Cu, Hg, Pb and Zn and moderate to considerable of Cd.

Table 3. The contamination factor (CF) for heavy metals in surface sediments from five plots in Lake Bohinj.

Plot	Cd	Cu	Hg	Pb	Zn
1	1.95	0.23	0.11	0.16	0.25
2	3.09	0.34	0.15	0.14	0.33
3	1.75	0.12	0.09	0.06	0.11
4	1.97	0.18	0.16	0.07	0.21
5	4.15	0.35	0.47	0.20	0.43

The concentrations of the elements in macrophytes were less diverse than for sediments. Nevertheless, some patterns can be seen—the highest content of Cd, Co, Ni, and Hg was recorded in macrophytes growing at plot 5 (1.73 ± 0.16 mg/kg DW, 1.98 ± 0.19 mg/kg DW, 3.65 ± 0.11 mg/kg DW and 0.05 ± 0.038 mg/kg DW, respectively), while the content of Fe was the highest in macrophytes, growing at plot 2 (1073 ± 126 mg/kg DW) (Figure 3).

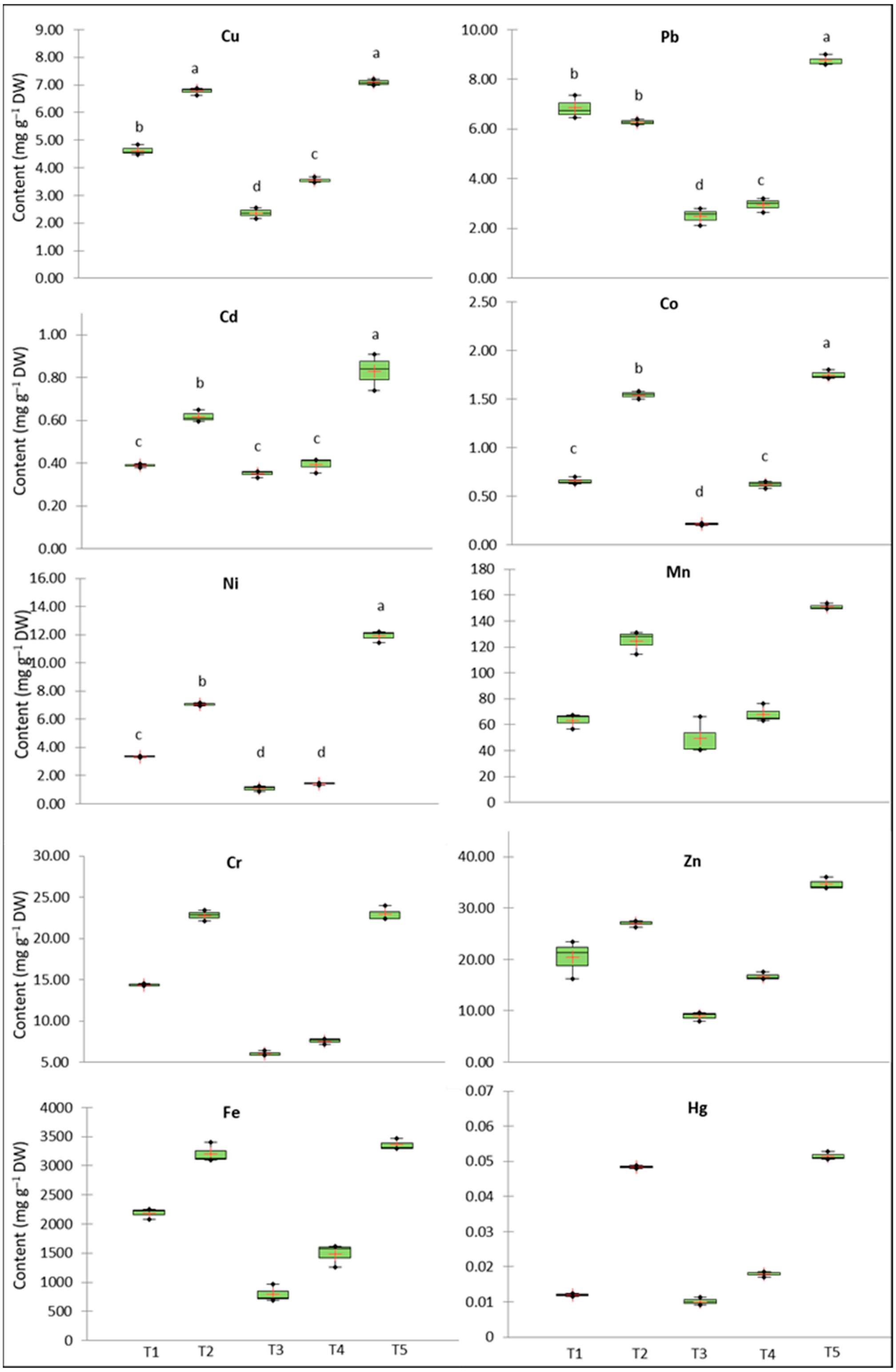

Figure 2. Content of elements in the sediment of Lake Bohinj. Different letters indicate significant differences between plots (ANOVA with the Tukey post hoc test, $p < 0.05$).

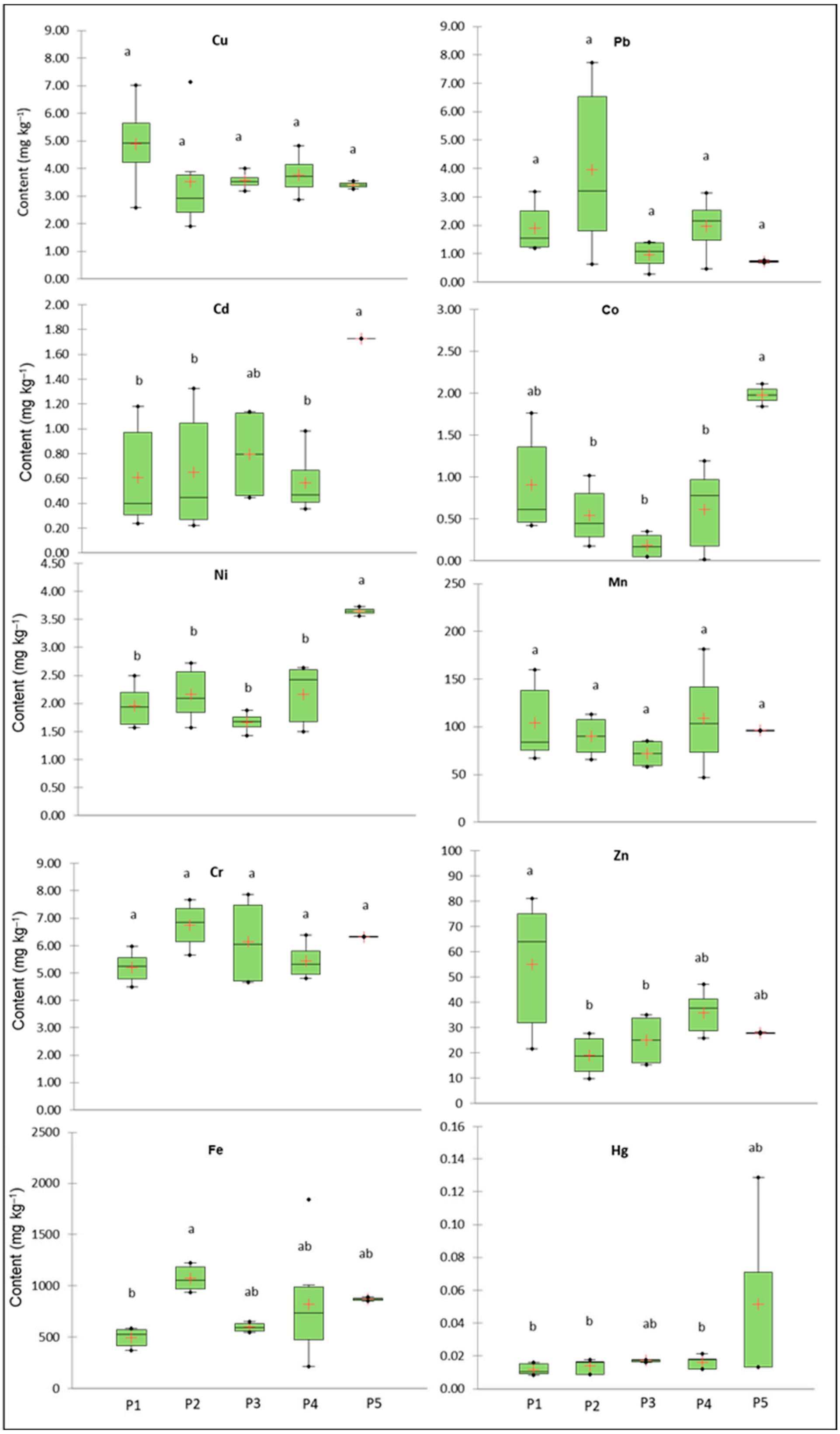

Figure 3. Content of elements in macrophytes growing in different plots of Lake Bohinj. Different letters indicate statistical differences between plots (ANOVA with the Tukey post hoc test, $p < 0.05$).

4. Discussion

Generally, Alpine lakes are less polluted in comparison to lowland lakes. This is mainly due to Alpine tributaries, which are naturally low in nutrients and trace elements, and due to a much lower proportion of intensively cultivated agricultural land and urban areas in their catchment areas [35]. Metal concentrations in lake water are expected to be determined mostly by bedrock and surficial geology, as well as the physical and chemical characteristics of water, such as pH [3]. Contrary to these assumptions, the concentrations of some of the measured elements in water from Lake Bohinj were higher compared to concentrations in similar surface water bodies, e.g., Croatian lakes on limestone bedrock (Cu, Pb, Zn) [19], Alpine lakes in Italy (Cr, Cu, Fe) [3], as well as mean values of the uncontaminated freshwaters found in the literature (Ni) [33] (Table 2). Moreover, the contents of Zn, Ni, and Cu in the water were higher in Lake Bohinj than in the polluted Lake Velenje located in the mining region in the central part of Slovenia, while values for Pb were comparable in these two lakes [36]. These observations suggest some level of pollution of the lake water. However, it must be highlighted that the content of the elements in the water are usually subjected to strong fluctuations and may reflect short-term acute conditions [37]; therefore, the elevated concentrations in Lake Bohinj may have resulted from more intensive usage of the lake in summer and may not represent the overall load of pollutants.

It was assessed that more than 90% of heavy metals present in aquatic systems are related to suspended particles and sediments [38–40]. A small proportion of the free metal ions are in a dissolved form in the water [41]. Thus, the accumulation of trace metals in the sediment enables us to assess the anthropogenic influence on the aquatic system [42]. Therefore, we assumed that comparison of the recorded concentrations of the elements in sediments and plants with the values noted in Lake Velenje would, contrary to aquatic concentrations, show the long-term pollution. The comparison showed that the contents of all measured elements were higher in sediments from Lake Velenje. Further on, the comparison between the contents of the elements in macrophytes from these lakes shows that average contents of Zn and Pb were higher in plants from Lake Bohinj, while the values for Cr, Cu and Ni were comparable for both lakes (Table 2). Moreover, the results from national monitoring of the chemical status of the lakes in 2021 even showed that all measured values for elements in sediments were higher for Lake Bohinj than for Lake Velenje [43]. Lake Velenje was expected to be more polluted with trace elements as it appeared due to land subsidence as a consequence of intensive coal-mining and the large amount of ash that the Šoštanj Thermal Power Plant has been disposing for decades to the shores and to the bottom of the lake becoming a source of trace elements [36]. Lake Bohinj is located in a pristine area of the Triglav National Park, without industry in its surroundings, where human activity is limited mostly to recreation and only small settlements occur. For the reason mentioned, lower contents of the elements both in the sediment and plants from Lake Bohinj were expected. When compared with mean values for Alpine and Arctic lakes, sediments in Lake Bohinj were enriched in Cd, but contents of Cu, Pb and Zn were lower than in uncontaminated reservoirs [30]. Similarly, only Cd content in Lake Bohinj was higher than in sediments of the Dravinja river, a moderately polluted river in Slovenia [44]. In addition, the CF values for Cd indicated a moderate or considerable contamination of the sediments with this metal (Table 3) [29]. The average content of Cr in macrophytes in Lake Bohinj was 5.88 ± 1.03 mg/kg DW, which is slightly higher compared to the average content of Cr in the leaves of *Ceratophyllum demersum* in Lake Skadar [45]. Macrophytes in Lake Bohinj contained more Cr and Cd than *M. spicatum* from the polluted Kadın creek [46], as well as more Cd, Fe and Zn than *P. pectinatus* from the polluted Sarno river [37]. On the other hand, the contents of Cd, Cu, Fe, Mn and Pb were lower than in *Potamogeton* species and *C. demersum* from the rivers heavily affected by industry (e.g., the Danube in Europe and Donghe in China) [47,48].

Consequently, the most worrying results of our study are the high concentrations of Cd and Hg in sediments and high Co and Cr contents in macrophytes (Table 2). All these elements are a major environmental problem because of their long-term persistence and high toxicity to plants, animals and humans [49,50]. The main causes of Cd pollution are anthropogenic activities that contribute 80–90% [51]. Activities connected with Cd releases are animal husbandry and sewage overflow, as well as phosphate fertilizers and the burning of fossil fuels [52,53]. In all mentioned cases, Cd is transferred to groundwater below the adjacent catchment area, which ends up in the lake. Higher concentration of Cd in the soil in the vicinity of the mining areas is frequently a consequence of ore smelting and refining, as well as smelting slag from where Cd is leached into groundwater by rainfall and is then concentrated in sediments [52,53]. Camarero et al. [30] claimed that the Julian Alps are naturally enriched in Hg, which explained a slightly elevated concentration of sediments. The origins of Cr in natural ecosystems are industrial activities such as iron and steel manufacturing, tannery, leather manufacturing and municipal sewage sludges, while Co pollution is usually related to nonferrous metal smelters, fossil fuels combustion, and road dust [50]. In the sediments of the Sava river north of our study area, which drains the same mountain group—the Julian Alps —Milačič et al. [54] measured a lower concentration of Cd (0.28 mg/kg) than in our samples (0.52 ± 0.20 mg/kg) (Table 2). Gosar et al. [55] reported the highest average values for the Cd content in the upper soil layer within Slovenia for the region of the Western Alps, where our study area is also located, which means that naturally elevated values could not be excluded. On the other hand, the weathering and release of Cd from rocks rarely increases its concentration in the environment [56]. In this context, the relatively high content of the studied elements in sediments and macrophytes from Lake Bohinj was probably due to iron production and other past industrial activities [57].

In particular, a high content of all elements in the sediment from plot 5 (Figure 2), exceeding the Interim Sediment Quality Guidelines and the upper limit of normal concentrations according to Kabata-Pendias [50], can be a consequence of the vicinity of the settlement Stara Fužina, known from the pre-Roman era for iron industry. Iron smelters were built along the Mostnica river, on the east side of the lake, near plot 5. The production of iron has taken place there for 2500 years from the Iron Age to the end of the 19th century [57], reaching its highest extent in Roman times and the Middle Ages. The consequence of the former iron-smelting activity for the current high content of the elements in the environment was documented by Gallini et al. [58]. The settlement of Stara Fužina lies on coarse fluvio-glacial deposits, which are easily leached by precipitation, also towards Lake Bohinj (Figure 1). The spreading of the trace metals in the vicinity of smelters by air was documented by Galinha et al. [59]. Frančišković-Bilinski et al. [60] also registered elevated concentrations of metals in river sediments, although industrial pollution has decreased during the past decades. On the other hand, the area around plot 5 is one of the locations on the lake with the greatest recreational activity, especially bathing during the summer, which can substantially contribute to sediment mixing and redistribution of these elements into the water column and upmost layers of sediments. Recent human activities such as agriculture on the east shore of the lake and sewage leaching from the settlements could also be the possible reason for the high content of elements in the lake sediment. The pollution of this area is confirmed by elevated contents of Cd, Co, Hg, Ni in macrophytes compared to other parts of the lake (Figure 3).

Plot 2 also indicates the area of the lake with elevated element concentrations in sediments and Fe content in plants (Figures 2 and 3). This plot is relatively far from the former iron smelter and located upstream; therefore, other possible sources of metals were expected. Exploration carried out by scuba divers revealed remnants of mines and grenades buried in lake sediments near this location. Locals claim that during the First World War, an entire trainset loaded with mines and grenades being transported by the Austro-Hungarian army to the front in the mountains west of lake Bohinj fell into the lake in this area. According to another story, unused grenades were deposited into the lake after the end of the war [61]. It is likely that metals came into the water and sediments from these sources.

The lowest concentrations of metals in sediments were measured in the two most western plots, 3 and 4 (Figure 2), where the main tributary, the River Savica, inflows the lake. Its average discharge represents around 60% of the water entering the lake and it also generates the gentle current of the water masses towards the eastern part of the lake; it is therefore possible that the inflowing water has an influence on concentrations of the measured elements in sediments of Lake Bohinj. The Savica is a non-polluted, fast-flowing river, with temperatures below 7 °C even in summer, that increases the flow rate of Lake Bohinj and supplies it with oxygen. The influence may be similar to the process observed by Vukosav et al. [19], who recorded a decrease in concentration of Cd, Zn and Cu downstream in the water as well as in sediments of the Plitvice Lakes, due to a self-purification process enabled by the presence of authigenic calcite.

The results showed that increased concentration of the elements in sediments is not necessarily followed by an increased content of macrophytes. The content of the pollutants in the sediment of aquatic habitats reflects a long-term loading, while the content of plants depends on the length of the vegetative period and reflects their bioavailability [62]. It is likely that, to some extent, protective mechanisms of macrophytes decrease the transfer of these elements from sediments to plant tissues. Trace elements cause toxicity in higher plants, and therefore, plant cells tightly control their uptake and use [63]. In addition, rooted submerged plants can absorb the trace elements directly from the water if they are not bioavailable in sediments [64], so a part of the metal load in plants may be absorbed from the water. High contents of trace metals in plants confirm high bioavailability of metals in the aquatic environment and their anthropogenic origin [65].

Nevertheless, based on certain biological indicators (e.g., phytoplankton, macrophytes, benthic diatoms), Lake Bohinj was classified with a very good ecological status in recent years [21]. The results of our study showed that the content of certain elements in the sediment and macrophytes was elevated, most probably due to past human activities. Few studies have examined pre-industrial anthropogenic heavy-metal pollution [66]. Alpine lakes were considered to be relatively unaffected, while their possible pollution was usually attributed to deposition of the atmospheric pollutants [3]. Our results confirm that local ancient metallurgy may have left permanent traces in the lake sediments [67] and indicate places which require special attention and may pose a risk.

5. Conclusions

The results brought new insights into the fate and bioavailability of trace elements in the unique habitats of Alpine lakes, which as ultra-oligotrophic and pristine ecosystems are particularly vulnerable to human pressures. In the case of Lake Bohinj, we found that the load of the metals in sediments manifests in the biota to some extent. Metal levels in the water indicated seasonal pressures on the lake, while the content of the elements in the sediment and plants showed long-lasting pollution of some parts of the lake. The spatial analyses of metal concentrations and use of different environmental matrices enabled us to distinguish between past and present pollutions and indicated the location of potential pollution sources. Moreover, the heterogenous spatial distribution of metals allowed us to distinguish between the natural (geological) and anthropogenic sources of trace metals. In particular, human activities in past centuries (iron smelting and military waste) were probably the reasons for the high content of elements in the sediments in the eastern part of the lake. Further investigations are needed to determine factors causing differences in the spatial patterns of metal concentrations shown in sediments and plants. Additionally, our results indicate the need for complex research within the evaluation of the state of lakes before we make assumptions about the pollution levels based on present human pressures only.

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