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The macrophytes *Potamogeton pusillus* L. and *Myriophyllum aquaticum* (Vell.) Verdc. as potential bioindicators of a river contaminated by heavy metals

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ABSTRACT

To evaluate the *Myriophyllum aquaticum* and *Potamogeton pusillus* macrophytes as indicator organisms of heavy metal pollution in biomonitoring studies of the aquatic ecosystem, the aim of this study was to determine the Co, Cu, Fe, Mn, Ni, Pb and Zn accumulation in leaves of the those species and the possible relationship to water pollution by these metals. Surface water, sediment and plants were collected at 10 sampling sites of the Ctalamochita river (Argentina). Cooper and Pb concentrations exceeded the limits established for the protection of aquatic life defined by Argentina Legislation (Cu: 2.0 μgL^{-1}, Pb: 2.0 μgL^{-1}) and international norms (Cu: 1.6 μgL^{-1}, Pb: 2.5 μgL^{-1}) in surface water, while Cu and Zn exceeded the limit for ecological screening levels (Cu: 31.6 mgkg^{-1}, Zn: 121.0 mgkg^{-1}) in sediment. Heavy metal concentrations were found to be higher downstream of Río Tercero city in water and sediments samples, probably related to the contribution of pollutants from the effluent discharge of the city. Both species revealed a high capacity to accumulate heavy metals in its tissues, in areas of the river with higher heavy metals values in the abiotic compartments. Particularly, high accumulation of Co, Cu, Ni and Zn in *P. pusillus* correlated with their concentrations in sediments and Co, Cu, Mn and Zn accumulation in *M. aquaticum* correlated with the concentrations of these metals in water. These macrophytes reflect spatial variations of metals in water and sediments of the Ctalamochita river; therefore they are of potential use as heavy metal bioindicators of river pollution.

1. Introduction

The availability and quality of fresh water are two of the major challenges for humanity in the twenty-first century, with contamination of surface waters with pollutants such as heavy metals, pesticides and persistent organic compounds being of worldwide concern [1]. The presence of high concentrations of metals in aquatic ecosystems, such as rivers, ponds and lakes, is a potential risk to human health and the ecosystem, due to their toxicity, bioaccumulation and high residence times in the atmosphere. Although some metals such as Cu^{2+}, Cr^{3+}, Fe^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} are required as micronutrients for autotrophic organisms, these may have toxic effects at high concentrations [2]. Other non-essential nutrients are toxic to organisms even at low concentrations, such as Hg^{2+}, Cd^{2+} and Pb^{2+} [3].

The entry of heavy metals into surface waters can be continuous or pulsed, with both types resulting from anthropogenic sources such as soil washing by surface runoff [4,5]. In general, these contaminants on entering aquatic systems become attached to particulate material which is transported or decanted, and are thus incorporated into the sediments. In this way, the surface sediments are an important reservoir of heavy metals and other contaminants in the aquatic environment.

The macrophytes contribute significantly to the primary production of water bodies, in the littoral zone being a fundamental part of the trophic structure of aquatic ecosystems and an important link in the recycling of nutrients. Consequently, they can incorporate large amounts of metals from the environment [6]. The absorption capacity of metals through roots and leaves [7], combined with their sedentary nature, makes macrophytes appropriate agencies to detect changes or alterations in the aquatic environment [8]. High concentrations of metals in aquatic plants can be accumulated from the water column and/or from sediments [9] demonstrating the usefulness of macrophytes as biomonitors for aquatic systems.

Native species of the submerged macrophytes, *Myriophyllum aquaticum* and *Potamogeton pusillus*, are widely represented in rivers of the central region of Argentina. These plants are found in the sandy substrate of river beds with moderate currents, with floating rhizomes and roots sometimes present and they can even colonize regions with

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high human impact, where many other species are less common. In this way, these species have the optimum characteristics to be used as bioindicator organisms in biomonitoring studies of water pollution [10]. However, despite its wide distribution, there are scarce studies *in situ* and *in-vitro* of the genus Myriophyllum and Potamogeton [11–13], in relation to its use in biomonitoring of surface water quality or in obtaining experimental evidence of exposure to contaminants. In order to evaluate the *M. aquaticum* and *P. pusillus* macrophytes as indicator organisms of heavy metal pollution in biomonitoring studies of the aquatic ecosystem, the aim of this study was to determine the Co, Cu, Fe, Mn, Ni, Pb and Zn accumulation in leaves of the submerged macrophytes *M. aquaticum* and *P. pusillus* and the possible relationship of the concentrations of these metals with those found in surface water and sediment samples of the Ctalamochita river (Argentina).

2. Materials and methods

2.1. Study area and sampling

The present study was carried out in a section of the Ctalamochita river middle basin in Cordoba province, located in the central region of Argentina (Fig. 1). The confluence of the Santa Rosa (N), Grande (W), Quillinzo (S) and de la Cruz (S) rivers and the Amboy brook (W), is the origin of Río Tercero lake, formed by the dam of the same name. This is the source of the Ctalamochita river, which flows in the direction W–E and runs a length of more than 400 km before emptying into the Paraná river, and is considered to be one of the most important rivers of Argentina. At 10 km downstream of Río Tercero lake, is the Piedras Moras lake formed by the dam of the same name. The climate of the region is temperate, continental and sub-tropical, tending to semi-arid. There is a marked seasonality of rainfall in the summer months with a great ability to retain water until the months of April and May. Maximum precipitation occurs between October and March (725 mm) with the minimum taking place between April and September (143 mm) [14]. The maximum temperature reaches 34 °C in summer and drops to −5 °C in winter.

The Ctalamochita river has a predominant flow direction of W–E, an average flow rate that exceeds 27 m^3s^{-1} (drainage area of approximately 3300 km^2). This river is a main water resource for drinking water, supplying many localities. It is used for irrigation, as well as having both on the river and in its reservoirs plants for electric power generation (hydroelectric and nuclear power), and also areas for recreational use. In the basin, the main activities are agriculture, livestock, forestry and tourism. Demographically, in the region the most populous cities are Río Tercero (46,200 inhabitants), Almafuerte (11,200 inhabitants) and Embalse (8500 inhabitants) [15]. Río Tercero city is characterized by significant industrial activity, such as chemical and petrochemical industries and tanneries. The industrial center of this city is large, and recent studies have identified these industries as being major sources of emissions of heavy metals into the atmosphere [16–18]. In addition, Bermudez et al. [19] found high enrichment factors for Mn, Pb and Zn in the surface soils of this basin.

In previous years, the water quality in the river Ctalamochita has been assessed through physical, chemical and biological analyses, related to the different types of activities that discharge industrial and sewage effluents into the river. Different levels of contamination have been recorded [20], with the need for policies to control contamination, due to the high degree of genotoxicity, having been identified [21]. However, no data exists on the concentrations of heavy metals in water or sediment of the Ctalamochita river basin.

In this study, Sites 1 (Amboy brook) and 2 (de la Cruz river) were located prior to Río Tercero lake in mountainous areas of pristine conditions. All of the Sites 3 to 10 were on the Ctalamochita river, with Sites 3 and 4 being located between the Río Tercero and Piedras Moras lakes, an area influenced by the presence of electric power plants, agricultural and aggregate extraction. Sites 5 to 10 were located downstream of Piedras Moras lake and crossed Río Tercero city. Of these Sites 5 and 6 were located in areas of recreation, watering places and aggregate extraction, whereas Sites 7 to 9 were located in the industrial areas of Río Tercero city. Finally, Site 10 was situated after effluent discharge from a sewage treatment plant and tannery activities. The sampling campaign was conducted in the summer of 2009, and samples from surface water, sediment and aquatic plants for each sampling site were collected for three replicates. Surface water samples (1 L) were collected at about 10 cm deep in plastic bottles. For sediments, fine material was collected from the river bottom of the littoral zone. These samples were taken in the first 10 cm of the surface with a plastic manifold and stored in polyethylene bags and kept refrigerated.

The submerged macrophytes, *M. aquaticum* and *P. pusillus*, were collected at each site, at a constant depth of about 50 cm, with each sample consisting of a group of 5–7 plants which were thoroughly washed *in situ* with river water before being placed in polyethylene bags. In the laboratory, there were again washed with distilled water to remove any material adhering to the surface and kept at −20 °C until processing [22].

2.2. Preparation and analysis of samples

Water samples, immediately after being collected, were acidified with 63% HNO_3 to pH ≤ 2, before being filtered through a filter paper 2.0 μm and preserved until analysis [23].

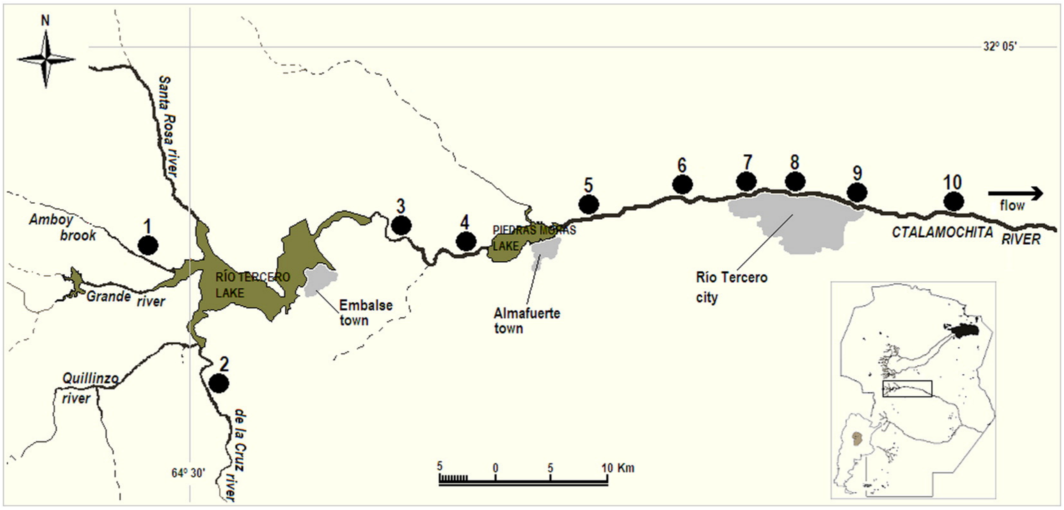

Fig. 1. Sampling sites (●) in the Ctalamochita river middle basin.

The sediment samples were dried in an oven for 48 h at 60 ± 2 °C, and prior to being analyzed they were passed through an acrylic mesh of 63 μm. Then, 5 g of dry matter (<63 μm) was carbonized in an oven at 450 °C for 4 h, with the ashes being digested using a mixture of HCl (20%) and concentrated HNO_3 (3:1) *V/V* [24]. Aquatic plants leaves were dehydrated using a lyophilizer tray (Rificor®-Model-L-A-B4). Then, 5 g of dry material was placed at 450 °C for 4 h, and the ashes were digested with a mixture of HCl (20%) HNO_3 (3:1) *V/V* [25]. The solid residue of sediment and macrophyte samples was separated by centrifugation before being filtered through a filter paper 2.0 μm, and supernatants were diluted with ultrapure water to a final volume of 25 mL.

The Co, Cu, Fe, Mn, Ni, Pb and Zn contents were analyzed using graphite furnace atomic absorption spectrometry (AAnalyst600-AS800, Perkin-Elmer, USA) for water samples and by flame atomic absorption spectrometer (Perkin-Elmer 3110, USA) for sediment and macrophyte samples. Blanks were prepared using the same protocol (only reagents). Recovery studies were also carried out by adding individual atomic absorption spectrometry standard solutions (AccuStandard®, 1000 mgL^{-1} 1% HNO_3) to three slurry sediments corresponding to each studied area (1 mg Fe or 10 mg of other metals kg^{-1} sediment), which were homogenized before drying. Spiked samples were further treated and analyzed as normal sediments to evaluate the recovery percentage, with 80–95% recovery percentages being obtained after correcting to dry weight. The quality control was evaluated with certified material (± uncertainty for certified value, with 95% confidence) of Oriental Tobacco Leaves (ICHTJ-CTA-OTL-1), using the same protocol to check the validity of the analytical method. Certificate material samples revealed that the concentrations of Cu, Mn, Ni, Pb and Zn were 13.9 ± 0.485, 394 ± 10.6, 5.94 ± 0.75, 5.08 ± 1.03 and 51.2 ± 4.09 $mgkg^{-1}$ dry weight, respectively. This method, therefore, gave concentrations that were within the range for certification for Cu, Mn and Zn, but 12.6% higher for Ni and 20.3% greater for Pb. Ultra clean conditions were maintained during all stages of sample collection, transport, handling, processing and analysis.

2.3. Statistical analysis

Statistical analysis of the data was performed using the statistical software Version-1.1 InfoStat. Assumptions of normality and homoscedasticity were tested using the Shapiro–Wilk and Levene tests, respectively, and the variables that were not normally distributed were $\log_{10}$ trans-formed. The data obtained were analyzed using descriptive statistics and analysis of variance (ANOVA) at three levels (*p*-values: 0.001, 0.01, 0.05, respectively) in order to check for significant differences between the sampling sites and between species, followed by a Tukey post-test ($p < 0.05$). The Pearson correlation coefficients were calculated to assess the relationship between metal concentrations in the leaves of plants with their concentrations in water and sediments.

3. Results and discussion

3.1. Concentration of heavy metals in water and sediment

The concentrations of Cu, Ni, Pb, Co, Mn, Fe and Zn in water showed wide variations (Cu: min. 1.96–max. 5.80 μgL^{-1}, Ni: min. 1.20–max. 6.72 μgL^{-1}, Pb: min. 1.20–max. 6.72 μgL^{-1}, Co: min. 0.61–max. 2.27 μgL^{-1}, Mn: min. 10.76–max. 53.92 μgL^{-1}, Fe: min. 33.52–max. 169.4 μgL^{-1}, Zn: min. 1.80–max. 20.8 μgL^{-1}). All the heavy metals measured in surface waters of the Ctalamochita river were significantly ($p <$ 0.001) among sampling sites (Table 1). Particularly, high concentrations of Cu and Pb, along with Mn and Zn (Table 1), were recorded at sampling sites downstream of Río Tercero city on the Ctalamochita river (Sites 7 to 10), possibly due to the negative impact of effluent discharges from human activities and a sewage treatment plant taking place in the city with pollution by direct download either through channels or soil infiltration and runoff. Lerda and Prosperi [20] reported that different types of activities that discharge industrial and sewage effluents into the river Ctalamochita affected the water quality.

The mean concentrations of all heavy metals in surface water, except for Cu and Pb (Table 1), were recorded within the limits for the protection of aquatic life defined by Argentina Legislation [26] (Cu: 2.0 μgL^{-1}, Ni: 25 μgL^{-1}, Pb: 2.0 μgL^{-1}, Mn: 100 μgL^{-1}, Zn: 30 μgL^{-1}) and international norms (Cu: 1.6 μgL^{-1}, Co: 24.0 μgL^{-1} [27]; Mn: 80.0 μgL^{-1} [28]; Ni: 52.0 μgL^{-1}, Pb 2.5 μgL^{-1}, Zn 120.0 μgL^{-1} [29]). Although copper is an essential micronutrient for all organisms, it is known to be toxic at high concentrations in surface waters [2]. In contrast, Pb is not essential, whose toxicity is manifested even at low concentrations [3]. Both these metals, therefore, may be toxic to the aquatic organisms found in the Ctalamochita river.

In sediment, heavy metal concentrations showed a wide variation (Cu: min. 2.93–max. 34.59 $mgkg^{-1}$, Ni: min. 4.63–max. 13.93 $mgkg^{-1}$, Pb: min. 4.65–max. 20.63 $mgkg^{-1}$, Co: min. 3.33–max. 3.75 $mgkg^{-1}$, Mn: min. 80.1–max. 6352.3 $mgkg^{-1}$, Fe: min. 3522.2–max. 9827.8 $mgkg^{-1}$; Zn: min. 12.7–max. 199.9 $mgkg^{-1}$). All the heavy metals measured in sediments of the Ctalamochita river were significantly ($p < 0.001$) among sampling sites (Table 1). In particular, high concentrations of Cu, Fe, Mn and Ni were recorded at Sites 3–6 on Ctalamochita river (Table 1), which could have been due to agrochemicals used in crop areas close to the banks of this river at these sites. For example, in recent years has been shown that agricultural activities in Argentina represent a source of heavy metals such as Cu, Mn and Ni [17,18,30]. Related to this, it is possible found high concentrations of Cu in water and sediment due to use of insecticides and fungicides with this metal as part of the chemical components [31–33]. Also, high concentrations of Zn in the Ctalamochita river sediments were downstream of Río Tercero city (Sites 9–10), with these sampling sites involving deposits of industrial waste (red-mud), as well as sewage treatment plant discharges from the city. In previous studies on rivers of the central region of Argentina, have demonstrated that the contributions of this metal are related to industrial and municipal discharges, as well as to discharge from stormwater runoff [13,22,23].

The average concentrations of all the heavy metals in the sediment analyzed, except for Cu and Zn (Table 1), were within the limits established for ecological screening levels [27] (Cu: 31.6 $mgkg^{-1}$, Ni: 22.7 $mgkg^{-1}$, Pb: 35.8 $mgkg^{-1}$, Co: 50 $mgkg^{-1}$, Zn: 121.0 $mgkg^{-1}$). High concentrations of Cu and Zn in sediments may have a negative impact on aquatic plant communities, as demonstrated by Peng, et al. [8] in surface water of the Donghe river (China).

3.2. Heavy metals in *M. aquaticum* and *P. pusillus* collected in the Ctalamochita river

The *M. aquaticum* and *P. pusillus* aquatic plants collected in the Ctalamochita river showed significant differences in the heavy metal concentrations in their tissues among sampling sites (Table 2). In general, in the Ctalamochita river basin, heavy metal accumulation in both species was greater in areas downstream than at sites located upstream. *P. pusillus* showed higher concentrations of Co ($p < 0.001$), Cu ($p < 0.01$), Ni ($p < 0.05$) and Pb ($p < 0.001$), than *M. aquaticum*, while the latter presented higher concentrations of Fe ($p < 0.05$). No significant differences in the concentrations of Mn and Zn between the two macrophyte species were detected (Table 2).

Although comparing concentrations in different species of submerged macrophytes generates inaccuracies due to different exposure and uptake processes, at least this type of comparison gives an indication of contaminant levels in other areas (Table 3). In general, mean concentrations of Co, Cu, Pb and Zn in *M. aquaticum* and concentrations of Ni and Zn in *P. pusillus* were similar to those reported by Baldantoni et al. [34] in *Potamogeton pectinatus* at Averno lake (Italy), by Duman et al. [35] in *Potamogeton lucens* at Sapanca lake (Turkey), by Grudnik and Germ [36] in *Myriophyllum spicatum* at Velenjsko and Družmirsko

Table 1
Heavy metal concentrations (means ± SD; n = 3) in surface water ($\mu g L^{-1}$) and sediments ($mg kg^{-1}$ DW) from the Ctalamochita river. ANOVA results between sampling sites. Vertical column with the same letter not differing significantly at $p < 0.05$ (TukeyHSD test).

	Co		Cu		Fe		Mn	
Site	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
1	1.93 ± 0.30a	4.45 ± 0.30cd	3.00 ± 0.42bc	4.18 ± 0.30de	167.6 ± 1.6a	3845 ± 477c	11.20 ± 0.44e	93.0 ± 20.5e
2	0.72 ± 0.12d	3.80 ± 0.28d	2.21 ± 0.31c	4.47 ± 0.32de	71.1 ± 22.9c	3874 ± 150bc	22.19 ± 2.48d	117.6 ± 14.1de
3	0.95 ± 0.12cd	5.50 ± 0.12bc	3.76 ± 0.21b	31.76 ± 2.48a	145.7 ± 2.4a	7658 ± 282a	34.83 ± 0.13c	165.9 ± 13.0de
4	1.41 ± 0.16abc	6.92 ± 0.13a	3.21 ± 0.34bc	28.47 ± 1.02b	39.7 ± 3.1de	8430 ± 410a	35.81 ± 3.34c	6290.7 ± 83.6a
5	1.30 ± 0.22bc	3.81 ± 0.09d	3.17 ± 0.31bc	10.78 ± 1.43c	35.0 ± 1.5e	8695 ± 1,134a	42.85 ± 0.34bc	1007.2 ± 91.7c
6	1.79 ± 0.10ab	6.41 ± 1.41ab	3.79 ± 0.47b	6.26 ± 0.07d	45.4 ± 4.0de	8637 ± 220a	47.83 ± 0.69ab	3340.4 ± 134.1b
7	1.21 ± 0.14cd	4.54 ± 0.02cd	3.35 ± 0.45bc	3.91 ± 0.20de	51.8 ± 2.8cde	4173 ± 333bc	51.16 ± 2.06a	213.6 ± 12.0de
8	1.04 ± 0.20cd	3.47 ± 0.11d	3.85 ± 0.40ab	3.04 ± 0.16e	51.9 ± 3.4cde	4516 ± 221bc	48.17 ± 4.99ab	294.5 ± 49.4d
9	1.34 ± 0.19bc	3.47 ± 0.17d	3.96 ± 0.25ab	4.70 ± 0.28de	60.1 ± 3.6cd	4465 ± 463bc	41.80 ± 3.88bc	138.5 ± 22.8de
10	1.09 ± 0.20cd	4.53 ± 0.19cd	5.00 ± 0.75a	5.52 ± 0.20de	105.9 ± 7.1b	5228 ± 196b	44.93 ± 4.03ab	267.9 ± 18.4de
ANOVA	***	***	***	***	***	***	***	***

	Ni		Pb		Zn	
Site	Water	Sediment	Water	Sediment	Water	Sediment
1	5.72 ± 0.16a	6.94 ± 0.22de	6.01 ± 0.26bcd	8.28 ± 0.19d	2.67 ± 0.02c	21.0 ± 2.0def
2	1.63 ± 0.49d	5.04 ± 0.12e	2.47 ± 0.39cd	5.11 ± 0.43d	3.61 ± 0.76c	15.2 ± 2.3f
3	3.56 ± 0.52bc	8.96 ± 0.10bc	3.11 ± 0.51cd	8.89 ± 0.45cd	10.00 ± 0.43b	36.2 ± 0.2cd
4	4.56 ± 0.99abc	10.16 ± 0.87b	5.97 ± 1.54bcd	20.43 ± 0.20a	3.45 ± 0.54c	44.6 ± 1.9c
5	5.71 ± 0.90a	12.51 ± 1.32a	2.22 ± 0.35cd	14.16 ± 1.29bc	4.04 ± 0.77c	35.1 ± 3.5cde
6	4.57 ± 0.59abc	7.58 ± 0.05cd	7.68 ± 1.31abc	14.30 ± 5.78bc	2.28 ± 0.30c	21.2 ± 0.4def
7	3.43 ± 0.47cd	6.21 ± 0.76de	12.13 ± 2.14a	6.14 ± 0.75d	2.20 ± 0.42c	16.7 ± 1.7ef
8	5.16 ± 0.73abc	5.47 ± 0.38e	10.46 ± 3.66ab	6.62 ± 0.33d	2.99 ± 0.47c	20.5 ± 1.0def
9	5.09 ± 0.59abc	5.22 ± 0.98e	10.80 ± 3.63ab	9.14 ± 0.54cd	12.00 ± 0.38b	105.4 ± 11.1b
10	5.40 ± 0.79ab	6.63 ± 0.32de	1.59 ± 0.14d	17.74 ± 1.29ab	18.93 ± 1.64a	183.3 ± 16.3a
ANOVA	***	***	***	***	***	***

DW,dry-weight.
*** $p < 0.001$.

Table 2
Comparisons of Co, Cu, Fe, Mn, Ni, Pb and Zn mean concentrations ($mg kg^{-1}$ DW) among the species of this study, *M. aquaticum* and *P. pusillus*, and species of submerged macrophytes of the other areas.

	Co		Cu		Fe		Mn	
Site	*M. aquaticum*	*P. pusillus*	*M. aquaticum*	*P. pusillus*	*M. aquaticum*	*P. pusillus*	*M. aquaticum*	*P. pusillus*
1	2.71 ± 0.06	8.21 ± 0.47	1.13 ± 0.10	4.58 ± 0.11	962.8 ± 14.5	834.5 ± 25.6	658.9 ± 35.2	693.7 ± 21.6
2	1.23 ± 0.09	4.02 ± 0.51	2.02 ± 0.20	5.63 ± 0.19	1228.4 ± 19.8	1182.0 ± 75.1	544.1 ± 30.0	449.7 ± 24.6
3	2.14 ± 0.55	4.51 ± 0.32	2.79 ± 0.39	28.85 ± 1.64	969.8 ± 148.7	674.8 ± 25.8	657.8 ± 46.6	137.9 ± 12.5
4	0.58 ± 0.10	6.62 ± 0.66	2.85 ± 0.16	3.68 ± 0.20	452.1 ± 34.5	209.0 ± 33.5	889.7 ± 96.4	873.4 ± 58.1
5	1.56 ± 0.07	4.52 ± 0.15	3.17 ± 0.10	4.31 ± 0.17	2224.8 ± 125.5	687.1 ± 23.5	1684.3 ± 54.4	1914.5 ± 82.7
6	3.45 ± 0.10	5.08 ± 0.28	2.91 ± 0.13	11.54 ± 0.54	1154.9 ± 34.6	916.0 ± 94.9	1062.3 ± 36.4	1122.6 ± 56.1
7	1.11 ± 0.21	3.40 ± 0.88	6.16 ± 0.54	18.57 ± 0.63	377.7 ± 11.6 d	201.8 ± 19.8	1388.6 ± 21.9	354.0 ± 22.7
8	0.81 ± 0.06	4.07 ± 1.08	4.25 ± 0.44	9.61 ± 0.35	1221.2 ± 29.1	546.8 ± 37.3	1184.1 ± 130.5	792.2 ± 30.8
9	1.21 ± 0.13	3.12 ± 0.66	4.04 ± 0.76	12.75 ± 0.70	2072.0 ± 66.0	1570.2 ± 181.5	632.1 ± 17.2	1426.3 ± 77.0
10	2.24 ± 0.10	4.85 ± 0.75	4.56 ± 0.15	6.17 ± 0.31	2591.6 ± 199.6	2479.9 ± 114.3	651.0 ± 57.7	730.6 ± 44.4
ANOVA	Co		Cu		Fe		Mn	
Site	*		***		***		***	
Macrophyte	***		**		*		ns	
SitexMacrophyte	ns		ns		ns		***	

	Ni		Pb		Zn	
Site	*M. aquaticum*	*P. pusillus*	*M. aquaticum*	*P. pusillus*	*M. aquaticum*	*P. pusillus*
1	5.10 ± 0.78	7.20 ± 0.21	1.46 ± 0.49	9.15 ± 1.16	9.17 ± 0.54	29.15 ± 0.27
2	6.54 ± 0.61	8.54 ± 0.44	1.78 ± 0.77	5.09 ± 0.44	19.67 ± 0.75	28.80 ± 1.81
3	4.79 ± 1.14	10.89 ± 0.47	1.68 ± 1.07	5.21 ± 1.75	21.25 ± 4.60	47.16 ± 2.46
4	3.86 ± 0.82	4.45 ± 0.34	0.55 ± 0.36	7.96 ± 0.61	15.99 ± 3.60	16.16 ± 0.55
5	9.65 ± 0.38	4.55 ± 0.08	1.13 ± 0.10	5.93 ± 0.57	18.75 ± 1.16	21.18 ± 2.35
6	4.93 ± 0.72	8.50 ± 0.69	5.45 ± 0.83	5.40 ± 0.36	8.73 ± 0.58	11.56 ± 2.38
7	4.66 ± 0.50	6.45 ± 0.41	1.15 ± 0.50	5.66 ± 0.27	7.81 ± 0.85	23.47 ± 1.43
8	6.10 ± 0.31	7.19 ± 0.31	1.05 ± 0.08	5.93 ± 1.28	21.39 ± 3.39	42.81 ± 2.22
9	5.68 ± 0.33	11.42 ± 0.32	0.52 ± 0.18	4.56 ± 0.70	266.61 ± 2.61	279.03 ± 8.34
10	6.26 ± 0.18	8.60 ± 0.94	0.35 ± 0.16	4.82 ± 0.63	101.63 ± 3.99	106.05 ± 5.67
ANOVA	Ni		Pb		Zn	
Site	**		**		***	
Macrophyte	*		***		ns	
SitexMacrophyte	**		ns		ns	

ns, not-significant. DW, dry-weight.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

lakes (Slovenia), by Harguinteguy et al. [13] in *M. aquaticum* in the Xanaes river (Argentina) and by Harguinteguy et al. [22] in *Stuckenia filliformis* in the Suquía river (Argentina). Whereas, the concentrations of Co, Cu and Pb in *P. pusillus* were higher and, in *M. aquaticum*, the concentrations of Ni were lower than those reported by these authors (Table 3).

Iron and Mn revealed higher concentrations in two plant species of this study, indicating accumulation from the aquatic environment. The Fe concentrations in leaves of *M. aquaticum* and *P. pusillus* were similar to those reported by Baldantoni et al. [34] in *P. pectinatus* at Averno lake (Italy), by Demirezen and Aksoy [37] in *Groenlandia densa* and *P. pectinatus* in the Sultan Marsh wetland (Turkey) and by Harguinteguy et al. [13] in *M. aquaticum* in the Xanaes river (Argentina), but were lower than those described by Harguinteguy et al. [22] in *S. filliformis* in the Suquía river (Argentina) (Table 3). In general, Mn concentrations in leaves of *M. aquaticum* and *P. pusillus* were higher than those informed by Baldantoni et al. [34] in *P. pectinatus* at Averno lake (Italy), by Demirezen and Aksoy [37] in *G. densa* and *P. pectinatus* in the Sultan Marsh wetland (Turkey) and by Harguinteguy et al. [13] in *M. aquaticum* in the Xanaes river (Argentina), but were similar to those reported by Harguinteguy et al. [22] in *S. filliformis* in the Suquía river (Argentina) (Table 3).

3.3. Correlation analysis of the concentrations of heavy metals in plants, water and sediment

As *M. aquaticum* and *P. pusillus* are submerged macrophytes with roots in the sediment and floating roots in the water column, it might be able to capture heavy metals from the water and sediment phase. In this study, in order to evaluate whether surface water or sediment was the main source of heavy metals in *M. aquaticum* and *P. pusillus* leaves, a Pearson correlation analysis was carried out between the heavy metal concentrations in surface water and sediments and those in the macrophytes (Table 4). A significant positive association was found between the Co, Cu, Mn and Zn concentrations in leaves of *M. aquaticum*, and those in water (Table 4). Therefore, these elements may have been incorporated by *M. aquaticum* through their leaves from the column water. These results are in agreement with those reported by Harguinteguy et al. [13], who demonstrated an association between the accumulation of these metals in *M. aquaticum* and those in water for the Xanaes river (Argentina). The absorption and accumulation of heavy metals in *M. aquaticum* may have been due to the plants being rhizomatous stems that are capable of capturing nutrients from the water column [38]. For Zn, it was found a significant association between the accumulation of this species and the metal concentrations in sediments. Related to this, Kamal [39] showed that *M. aquaticum* may be effective in the bioabsorption of Cu and Zn in waters contaminated with these metals. *P. pusillus* showed a significant positive correlation for Co, Cu, Ni and Zn (Table 3) with their concentrations in sediments. This species forms clumps in places where fine sediments accumulate in small established banks, and have a developed root system and are completely submerged in river beds. Demirezen and Aksoy [37] postulated that the aquatic flora reflects the metal content of their environment, with submerged macrophytes being able to incorporate metals in their leaves directly from the water and not just through the roots with subsequent translocation to the upper plant tissues [7]. This feature indicates the importance of these plants in the cycling of chemicals in aquatic ecosystems [6]. In addition, in this study was also observed that there was a positive association between the accumulation of Co and Zn in this species with the concentrations of these metals in water (Table 4). Our results are in agreement with those of Peng et al. [8], who observed a linear correlation between the concentrations of Zn in leaves of two species of Potamogeton (*P. pectinatus* and *P. malaianus*) with metal concentrations in water.

In previous studies on rivers of central region of Argentina, the aquatic environment has been presented as a sink surface runoff, leaching and/or effluent discharges from human activity [13,22,23], as it is known that industrial activities and wastewater discharges that reach rivers can have a significant impact on the water quality and biota living there [11,34]. These findings indicate that these macrophytes reflect spatial variations of metals in both water and sediments of the Ctalamochita river and were able to be removed those pollutants for self-purification processes. Therefore, these species could be proposed as suitable heavy metal bioindicators for the early stages of river pollution.

4. Conclusions

The average concentrations of the heavy metals were within the limits established by Argentina Legislation and international norms for the protection of aquatic life, except for Cu and Pb in surface waters and for Cu and Zn in sediments of the Ctalamochita river, which occurred primarily downstream of Río Tercero city.

Table 3
Heavy metal concentrations (means ± SD; n = 3) in *M. aquaticum* and *P. pusillus* leaves (mgkg^{-1} DW) from the Ctalamochita river. ANOVA results between sampling sites and macrophyte species.

Submerged macrophytes	Co	Cu	Fe	Mn	Reference
Myriophyllum aquaticum	1.71 (0.47–3.53)	3.39 (1.02–6.64)	1325.5 (366.1–2742.5)	935.3 (1325.5–1735.3)	In this study
Potamogeton pusillus	4.84 (2.54–8.75)	10.57 (3.48–29.93)	930.2 (172.9–2608.0)	849.5 (123.5–2004.8)	In this study
Potamogeton pectinatus	1.4	10.5	990	–	Baldantoni et al. [34]
Groenlandia densa	–	–	450	320	Demirezen and Aksoy [37]
Potamogeton pectinatus	–	–	1200	480	Demirezen and Aksoy [37]
Potamogeton lucens	–	4.0	–	500	Duman et al. [35]
Myriophyllum spicatum	–	5.0	–	–	Grudnik and Germ [36]
Myriophyllum aquaticum	2.5	6.8	1219	661	Harguinteguy et al. [13]
Stuckenia filliformis	2.6	10.1	4598	1219	Harguinteguy et al. [22]
Species	Ni	Pb	Zn		Reference
Myriophyllum aquaticum	5.76 (3.04–10.04)	1.51 (0.21–6.01)	49.10 (7.07–269.55)		In this study
Potamogeton pusillus	7.78 (4.24–11.77)	5.97 (3.72–9.89)	60.54 (9.43–285.23)		In this study
Potamogeton pectinatus	1.5	2.8	53.5		Baldantoni et al. [34]
Groenlandia densa	–	–	–		Demirezen and Aksoy [37]
Potamogeton pectinatus	–	–	–		Demirezen and Aksoy [37]
Potamogeton lucens	12.0	17.0	62.0		Duman et al. [35]
Myriophyllum spicatum	–	3.0	25.0		Grudnik and Germ [36]
Myriophyllum aquaticum	13.7	1.8	68.7		Harguinteguy et al. [13]
Stuckenia filliformis	7.5	8.1	56.3		Harguinteguy et al. [22]

The data are express in mgkg^{-1} and between parentheses express minimums and maximums values.

Table 4
Pearson correlation analysis of heavy metal concentrations among surface water, sediments and macrophytes in samples collected in the Ctalamochita river (n = 10).

	Co		Cu		Fe		Mn	
Macrophyte	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
M. aquaticum	0.46^{**}	0.27 ns	0.45^{**}	−0.23 ns	−0.02 ns	−0.01 ns	0.55^{**}	0.12 ns
P. pusillus	0.56^{***}	0.45^{**}	0.20 ns	0.36^{*}	0.23 ns	−0.24 ns	0.25 ns	0.21 ns

	Ni		Pb		Zn	
Macrophyte	Water	Sediment	Water	Sediment	Water	Sediment
M. aquaticum	0.21 ns	0.34 ns	0.02 ns	0.02 ns	0.63^{***}	0.65^{***}
Pn pusillus	−0.23 ns	-0.54^{**}	−0.01 ns	0.28 ns	0.64^{***}	0.63^{***}

ns, not-significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

The native submerged macrophytes *M. aquaticum* and *P. pusillus* revealed a high capacity to accumulate heavy metals in their tissues in this study, especially in areas of the river where higher values of heavy metals were observed in the abiotic compartments, surface water and sediments. These aquatic plants might have an influence on nutrient recirculation in the Ctalamochita river and, in turn, even be able to accumulate higher concentrations of metals. Therefore, these species might be used in biomonitoring studies of aquatic pollution produced by these metals, as they reflect their different concentrations in an aquatic ecosystem.

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