

## *Myriophyllum aquaticum* as a biomonitor of water heavy metal input related to agricultural activities in the Xanaes River (Córdoba, Argentina)

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### ABSTRACT

The aim of the present study was to assess the temporal variation of the heavy metal content (Co, Cu, Fe, Mn, Ni, Pb, and Zn) in surface water and sediments in relation to agricultural practices in the Xanaes River (Córdoba, Argentina). A second objective was to analyze possible relationships between the input of heavy metals on surface water and sediment, heavy metal accumulation and physiological changes in the aquatic plant *Myriophyllum aquaticum*. Samples were taken from the river at two contrasting sites (between April 2010 and August 2010): (1) a pristine area (mountain site), and (2) an area with intensive agricultural activity located at 60 km down river (agricultural site). The total concentration of heavy metals in surface water was higher in samples collected at the agricultural site but in sediments only the Mn concentration was higher than at the mountain site. The Fe and Mn concentrations in surface water at the agricultural site exceeded the recommended values for Argentinean Legislation of 300  $\mu\text{g L}^{-1}$  for Fe and 100  $\mu\text{g L}^{-1}$  for Mn. The accumulations of Zn and Mn in *M. aquaticum* were higher at the agricultural site and more elevated than the Zn and Mn concentrations in sediments at the same sites and sampling times. At the agricultural site, temporal variations of Cu, Fe and Zn were relatively similar for plants and water column, but the levels of the metals in plants were displaced over time. These results suggest that the levels of pollutants in the river came in pulses from the riverbank. These results show the potential use of *M. aquaticum* as a suitable accumulation biomonitor at the early stages of heavy metal pollution in rivers.

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### 1. Introduction

Rivers play an important role in population development by supplying water for humans, agriculture, and industrial consumption. However, aquatic environments suffer the consequences of human activities, due to these environments being a sink for waste storm water, surface runoff, and leaching or effluent discharges. Anthropogenic activities such as mining, agriculture and industrial plants systematically discharge dissolved pollutants and suspended matter into rivers, thereby decreasing the water quality significantly (Wang et al., 2010). Consequently, the polluted inflows of rivers can have a severe impact on human health as well as on the terrestrial and aquatic ecosystems (Shinn et al., 2009). Furthermore, pollution by persistent toxic pollutants, such as heavy metals, is of great concern around the world due to the continuous increase in these emissions. The contribution of these contaminants may

be discontinuous or in pulses, with the duration in the river water usually being ephemeral, as in surface runoff (Jergentz et al., 2005; Udeigwe et al., 2011). Measuring of persistent pollutant accumulation and physiological damage in aquatic plants can provide time integrated information about the presence of toxic compounds in these aquatic ecosystems.

The aquatic macrophytic species of the genus *Myriophyllum*, very common in the Southern Hemisphere, have frequently been used for biomonitoring studies of aquatic pollution, either as bioindicators of water quality through changes in the physiological response of the monitor organism, and/or as bioaccumulators of aquatic pollutants through the concentrations of heavy metals in the plant tissues (Cardwell et al., 2002; Ferrat et al., 2003; Kamal, 2004; Ngayila et al., 2007; Nimptsch et al., 2005).

In the central region of Argentina, high heavy metal levels in air and soil have been demonstrated through several studies (Pignata et al., 2002; Bermudez et al., 2010, 2012; Wannaz et al., 2006, 2012; Rodriguez et al., 2011). Also, research carried out in Córdoba province has identified agrochemicals (fertilizers and pesticides) to be potential sources of heavy metals (Pignata et al., 2002). In addition, in a recent investigation carried out by Bermudez et al.

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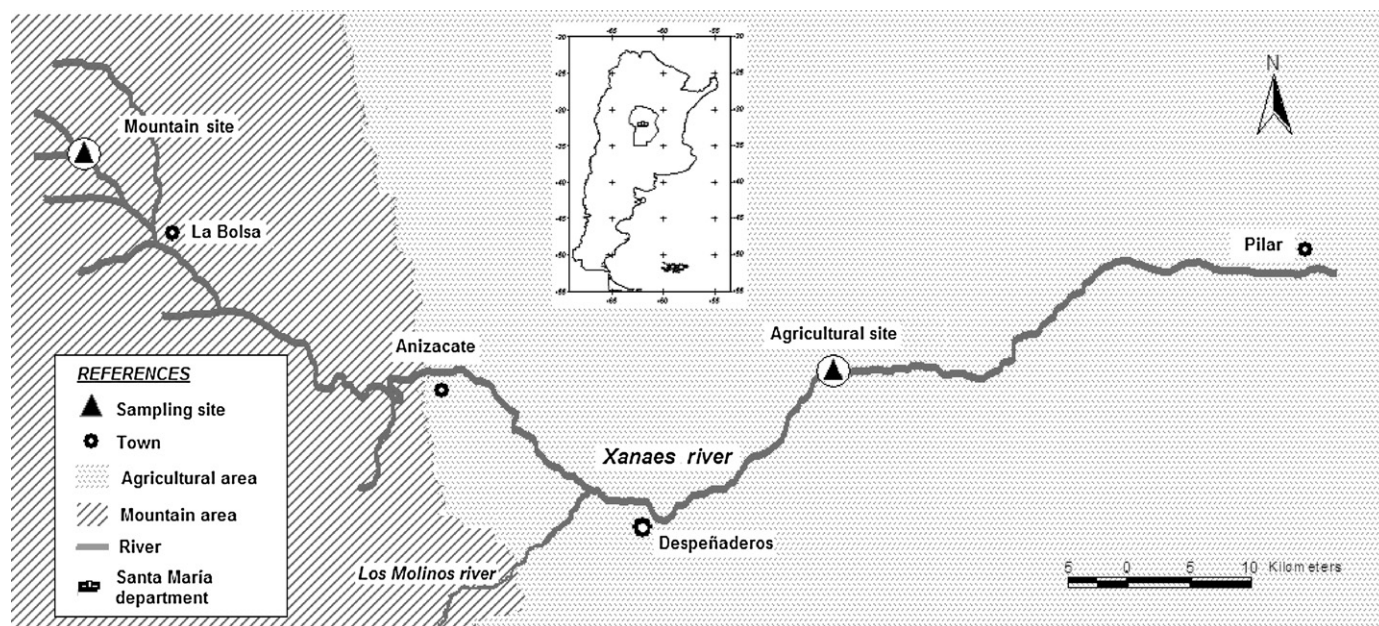


Fig. 1. Surface water, sediment and *M. aquaticum* sampling sites at the Xanaes River, Córdoba (Argentina).

(2010) in the same area that was evaluated in the present study, the authors encountered elevated concentrations of heavy metals in agricultural soils and in wheat grains produced in Córdoba province and particularly in the Xanaes River basin (Bermudez et al., 2011). However, at the present time, heavy metal pollution of the Xanaes River basin is not being monitored.

Plants form the basis of the trophic structure of aquatic ecosystems and can use light energy to synthesize organic biomass that can be utilized through grazing by either planktivorous or omnivorous organisms. Also, plants are part of the aquatic habitat providing cover and spawning for fish and other aquatic organisms. Thus, phytotoxic compounds may not only have an effect on plants but also on the aquatic ecosystem (United States Environmental Protection Agency, US EPA, 2011).

The water quality based limits procedures manual recommends the use of traditional critical design periods or the use of a biologically based design flow to limit acute and chronic toxicity (US EPA, 1996). In addition, impacts might be restricted to a fraction of the river discharge to restrict the spatial extent of a mixing zone (Yoder and Barbour, 2009). The allowance of an averaging period is based on the speed at which effects appear in different aquatic organisms exposed to different concentrations. This biological criterion has a different role in water resource study and management than in chemical water quality criteria or whole effluent toxicity. Biological assessments provide direct measures of the cumulative response of the biological community to all sources of stress, toxic levels of pollutants and unhealthy physical or chemical conditions (US EPA, 2011).

One of the most meaningful ways to answer basic questions about the quality of the surface waters is to observe directly the communities of plants that live in them. Because aquatic plants are constantly exposed to the effects of various stressors, these communities and individuals reflect not only current conditions, but also stresses and changes in conditions over time as well as their cumulative impacts. The physiological and accumulative responses of bioindicator species are invaluable for managing the aquatic resources and ecosystems, because the traditional chemical, physical and toxicity assessments cannot fully answer questions about the ecological integrity of a water body (US EPA, 2011).

Considering that the Xanaes River is an important river (as it is flow and long) in the Córdoba province as a source of drinking water supply for Córdoba city, the study of the background levels of heavy metals and their temporal variations in water, sediments and *Myriophyllum aquaticum*, was the principal aim. An integral assessment was performed based on two main approaches: (a) to determine the heavy metal concentrations in surface water and sediments at two sites of the Xanaes River and (b) to evaluate the temporal variation of the concentrations of heavy metals in both water and sediment, as well as their effects on the aquatic macrophyte *M. aquaticum* by measuring the heavy metal accumulations in leaves and physiological parameters at plants collected in the same two sampling sites.

This information is particularly important with respect to detecting diffuse sources of contaminants and for evaluating to study the physiological response and the role of the aquatic plant *M. aquaticum* associated with the concentration pulses of toxic heavy metals in rivers.

## 2. Materials and methods

### 2.1. Study area and sampling

The present study was carried out in a section of the Xanaes River in the central region of Argentina, Córdoba province (Fig. 1). The source of the Xanaes River is the mountainous formation called the Sierras Grandes. It has an annual flow of  $309 \text{ Hm}^3$ , a mean flow of  $14.5 \text{ m}^3 \text{ s}^{-1}$ , and a length of 340 km, being the second most important river in Córdoba province. The Xanaes River then flows through agricultural and urban areas until reaching the large saline lake Mar Chiquita (Ramsar site No. 1176), which is considered to be one of the most important wetlands in Argentina and the Chaco eco-region in terms of the richness of its biodiversity (Ramsar, 2002).

The geology of the area is characterized by metamorphic proterozoic rocks with sparse calcoalkaline granitoids (Zárate, 2003). There are medium sized towns and villages in the area (e.g. Despeñaderos with 7000 inhabitants), and the weather in the region is temperate, continental, with a semiarid tendency. The annual rainfall is highly seasonal, varying from 900 mm in the Sierras Grandes to 500 mm further westward, with spring and summer being the rainy seasons

**Table 1**  
Mean values and standard deviation ( $\pm$ SD) of the concentrations of Co, Cu, Fe, Mn, Ni, Pb and Zn in water (in  $\text{mg L}^{-1}$ ), sediments (in  $\text{mg kg}^{-1}$  DW) and *M. aquaticum* (in  $\mu\text{g g}^{-1}$  DW) collected at different times at two sampling points at the Xanaes River, Córdoba, Argentina. Results of the variance analysis (Kruskal–Wallis test) between Agricultural and Mountain sites (*p*-value) are shown for each metal along with the % OM in sediments.

	Water ( $\mu\text{g L}^{-1}$ )			Sediment ( $\text{mg kg}^{-1}$ DW)			<i>M. aquaticum</i> ( $\text{mg kg}^{-1}$ DW)		
	Mountain site	Agricultural site	<i>p</i> -Value	Mountain site	Agricultural site	<i>p</i> -Value	Mountain site	Agricultural site	<i>p</i> -Value
Co	2.1 $\pm$ 0.7	7.6 $\pm$ 1.5	<0.0001	5.7 $\pm$ 0.9	5.3 $\pm$ 0.5	0.0559	2.0 $\pm$ 0.3	2.9 $\pm$ 0.3	<0.0001
Cu	4.5 $\pm$ 0.7	19.7 $\pm$ 12.3	<0.0001	5.3 $\pm$ 0.6	5.4 $\pm$ 0.6	0.9823	5.3 $\pm$ 2.6	8.2 $\pm$ 3.8	0.0008
Fe	25 $\pm$ 8	290 $\pm$ 190	<0.0001	6431 $\pm$ 2410	5171 $\pm$ 1182	0.0428	8.7 $\pm$ 4.8	16 $\pm$ 7.8	0.0002
Mn	3 $\pm$ 1	58 $\pm$ 36	<0.0001	310 $\pm$ 87	462 $\pm$ 154	0.0001	4.9 $\pm$ 1.1	8.3 $\pm$ 1.9	<0.0001
Ni	12.1 $\pm$ 3.1	31.4 $\pm$ 5.1	<0.0001	11.7 $\pm$ 2	11.4 $\pm$ 0.9	0.7060	14 $\pm$ 6.8	14 $\pm$ 8.7	0.5444
Pb	3.2 $\pm$ 5.1	17.7 $\pm$ 7.1	<0.0001	10 $\pm$ 6.8	8.3 $\pm$ 1.2	0.4778	1.6 $\pm$ 0.7	2.0 $\pm$ 0.8	0.0760
Zn	5.1 $\pm$ 2.3	15.4 $\pm$ 15.1	<0.0001	47.6 $\pm$ 9.7	46.5 $\pm$ 6.1	0.7172	51 $\pm$ 30	87 $\pm$ 84	0.5742
OM%				9.13 $\pm$ 1.30	5.25 $\pm$ 0.58	<0.0001			

DW, dry weight.

(Bertolino et al., 2007; García et al., 2007). The maximum water temperatures occur during the rainy season (mean temperature of 20 °C), with the minimum temperatures occurring in winter (mean temperature of 8 °C).

In the Xanaes River, two sampling sites were selected: a mountain site (31°42'19.64"S and 64°29'36.44"W) and an agricultural site (31°48'12.81"S and 64°18'21.94"W) (Fig. 1). The mountain site was located 10 km upstream of La Bolsa town and was considered to be the reference site because it still maintains predominantly pristine forest features (Luti et al., 1979). The agricultural site was located at 15 km downstream of Despeñaderos city (at 60 km approximately from mountain site), and was characterized by the presence of soybean, wheat, and maize crops the surroundings. The area has 221,300 ha, with agricultural activity occupying 60% of the land (Ministerio de Agricultura, Ganadería y Alimentos de la provincia de Córdoba, República Argentina, 2012). Sampling of surface water, sediment and *M. aquaticum* plants was performed every 14 days between April and August 2010 (dry season). Thus, a total of 10 sampling campaigns were carried out at each site.

For each campaign, three replicates of surface water, sediment and *M. aquaticum* plants were collected (Table 1). The precipitation in this area for this time period was between 6.0 and 25.0 mm (Servicio Meteorológico Nacional, Argentina, 2012). Environmental data for conductivity, pH, temperature and dissolved oxygen in water were analyzed in situ by using a multiparameter probe.

## 2.2. Sample preparation for surface water and sediment analysis

All glassware used in the field and laboratory were left in 10% (v/v) HNO<sub>3</sub> (Merck, Química Argentina) solution for 24 h, and then washed with ultra-pure water. Ultrapure water was obtained using a water purification system consisting of a tandem of filtration through activated carbon, reverse osmosis and ionic exchange, with membrane (0.45 μm) filtration (Arium 611 UV system, Sartorius, Germany).

Surface water samples (1 L) for metal analysis were collected in the littoral zone, at approximately 10 cm below the surface of the river water. Three replicates of water samples were collected at each sampling site in clean polyethylene bottles, immediately acidified using 63% HNO<sub>3</sub> until pH  $\leq$  2 and then filtered with a 2.0 μm filter paper (Scheicher & Schüll, Blauband 589<sup>3</sup>, No. 300210) (Monferrán et al., 2011). The Co, Cu, Fe, Mn, Ni, Pb and Zn analyses were carried out by GFAAS (graphite furnace atomic absorption spectrometer, Analyst 600, equipped with AS 800 autosampler, Perkin Elmer, USA). A spiked sample was also prepared in order to verify recovery percentages of different elements by spiking a 5 mL water sample with variable amounts of individual atomic absorption spectrometry standard solutions (AccuStandard®, Fluka, 1000 mg L<sup>-1</sup> in 1% nitric acid) to yield twice the starting concentration for each element. The rest of the

procedure was the same as that used for non-spiked samples. Spiked samples were further analyzed to evaluate the recovery percentage, with 90–110% recovery percentages being obtained (data not shown). Appropriate dilutions and addition of matrix modifiers (CsCl, La<sub>2</sub>O<sub>3</sub>, CaCl<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>) were performed before measuring individual elements. Repeatability of atomic absorption measurements was usually  $\geq$ 97% comparing values obtained from triplicates (three measurements on each triplicate sample or spiked sample). The stability of the equipment was verified by measuring the calibration curve before and after sample measurements. Blank solutions, containing only reagents without river water, were used to deduct the content of metals arising from reagents and laboratory tools.

Submerged fine bed sediments for metal analysis were collected from the littoral zone, from approximately the first 10 cm of the surface of the river sediment. Three replicates of sediment samples were collected at each sampling point. Sediment samples were collected with a clean plastic core tube and sealed in polyethylene bags, before being returned in coolers to the laboratory where they were oven dried for 48 h at 60  $\pm$  2 °C. Prior to analysis, the samples were dry-sieved using a 63 μm acrylic mesh. Then, 5 g of the <63 μm dried material was carbonized in an oven at 450 °C for 4 h, and ashes were digested using a mixture of HCl (20%) and concentrated HNO<sub>3</sub> (3:1) (v/v) (Franco-Uría et al., 2009). The solid residue was separated by centrifugation and the sample was diluted with ultrapure water to a final volume of 25 mL. Finally, the Co, Cu, Fe, Mn, Ni, Pb and Zn contents were analyzed using FAAS (air-acetylene flame atomic absorption spectrometer, Perkin Elmer 3110, USA). Blanks were prepared using the same protocol without sediment (only reagents). Recovery studies were also carried out by adding individual atomic absorption spectrometry standard solutions (AccuStandard®, 1000 mg L<sup>-1</sup> in 1% nitric acid) to three slurry sediments corresponding to each studied area (1 mg Fe or 10 mg of other metals kg<sup>-1</sup> sediment), which were homogenized before drying. Spiked samples were further treated and analyzed as normal sediments to evaluate the recovery percentage, and 80–95% recovery percentages were obtained after correcting to dry weight (data not shown). Ultra clean conditions were maintained during all stages of sample collection, transport, handling, processing and analysis. The percentage of organic matter (% OM) was determined according to Peltola and Åström (2003) by the combustion of the samples at 500 °C for 4 h.

## 2.3. Sample preparation for aquatic plant analysis

Three replicates of *M. aquaticum* plant samples were collected at each sampling site, at a constant depth of about 50 cm. Each sample consisted of a pool of 5–7 plants, which were thoroughly washed in river water before being placed in polyethylene bags and returned to the laboratory in coolers. Once there, the plants were again rinsed

with distilled water to remove any material adhering to their surfaces, dissected using a lyophilizer chamber of acrylic and trays (Rifcor® Model L-A-B4) and preserved in plastic bags until analysis. For the physiological parameter determinations, the procedures followed for the quantification of chlorophyll a (Chl-*a*), chlorophyll b (Chl-*b*), phaeophytin a (Phaeoph-*a*), and malondialdehyde (MDA) have been previously described by Pignata et al. (2002). In addition, 5 g of the dried aquatic plant were carbonized in an oven at 450 °C for 4 h, and ashes were digested using a mixture of HCl (20%) and concentrated HNO<sub>3</sub> (3:1) (v/v) (Nekrasova et al., 2011). For metal determinations, the solid residue was separated by centrifugation and the sample was diluted with ultrapure water to a final volume of 25 mL. Finally, the Co, Cu, Fe, Mn, Ni, Pb and Zn contents were analyzed using FAAS (air-acetylene flame atomic absorption spectrometer, Perkin Elmer 3110, USA). Digestion blanks were prepared and analyzed in the same manner, with results being expressed as mg kg<sup>-1</sup> DW (dry weight). The quality control was evaluated with certified material ( $\pm$ 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. All the extractions of heavy metals in the analyzed samples were performed according to the modified technique of Bermudez et al. (2009).

Samples of the certified material (Oriental Tobacco Leaves (ICHTJ-CTA-OTL-1)) were analyzed ( $\pm$ uncertainty for certified value, with 95% confidence) and revealed that the Cu, Mn, Ni, Pb and Zn concentrations were  $13.9 \pm 0.485$ ,  $394 \pm 10.6$ ,  $5.94 \pm 0.75$ ,  $5.08 \pm 1.03$  and  $51.2 \pm 4.09$  mg kg<sup>-1</sup> DW, respectively. The method, therefore, provided concentrations that were within the certified ranges for Cu, Mn and Zn, but 12.6% greater for Ni and 20.3% higher for Pb.

#### 2.4. Data analysis

Statistical analyses were based on the mean values of the determinations performed on the three replicates obtained at each sampling site during the months studied. Assumptions of normality were tested using the Shapiro–Wilk test. Since not all the variables satisfied these assumptions, non-parametric tests were used (Kruskall–Wallis). This procedure is in agreement with Reimann and Filzmoser (2000), who showed that geochemical data are strongly bimodal and non-normal. When the Kruskal–Wallis null hypothesis was rejected (significance level < 0.05), post hoc pairwise comparisons were performed to investigate differences between pairs of means.

Spearman's correlation coefficients were calculated in order to study the relationships among the chemical elements of the three systems and between each of them and the physiological variables in plants. Tukey's multiple range test was used to differentiate the means where appropriate. A significance level of  $p < 0.05$  was used in all comparisons.

### 3. Results and discussion

#### 3.1. Heavy metal in water and sediment

Temperature, pH and the dissolved oxygen measured in water of the Xanaes River did not show significant changes between the mountain site ( $9.8 \pm 4.3$  °C;  $8.3 \pm 0.2$ ;  $9.4 \pm 1.5$  mg L<sup>-1</sup>) and the agricultural one ( $13 \pm 4.2$  °C;  $8.4 \pm 4.2$ ;  $9.7 \pm 1.9$  mg L<sup>-1</sup>) ( $p = 0.1017$ ;  $p = 0.7299$  and  $p = 0.8721$ , respectively) over the study time. However, a higher conductivity was observed at the agricultural site (agricultural site:  $360.4 \pm 76.3$   $\mu$ S cm<sup>-1</sup>, mountain site:  $119.1 \pm 28.3$   $\mu$ S cm<sup>-1</sup>).

The total concentrations of all the heavy metals measured in surface water were significantly higher in samples collected at the agricultural site (Table 1). In sediments collected at the agricultural site, only Mn showed levels significantly higher than in sediments collected at the mountain site, whereas the % OM was greater at the mountain site sediments (Table 1).

The mean concentrations of heavy metals in water (Table 1) were within the limits of the recommended values in Argentine legislation except Fe and Mn (Fe: 300  $\mu$ g L<sup>-1</sup>, Cu: 2  $\mu$ g L<sup>-1</sup>, Mn: 100  $\mu$ g L<sup>-1</sup>, Ni: 25  $\mu$ g L<sup>-1</sup>, Pb: 1  $\mu$ g L<sup>-1</sup> and Zn: 30  $\mu$ g L<sup>-1</sup>) (Secretaría de Ambiente y Desarrollo Sustentable, 2012; Argentinean Law 24051). Indeed, adverse effects of manganese on freshwater aquatic organisms have been previously reported in a number of studies, although the cause-effect evidence is not extensive (Thurston et al., 1979; US EPA, 1976, 1986). The US EPA's 1976 and 1986 Quality Criteria for Water established 50  $\mu$ g L<sup>-1</sup> as the recommended water quality criterion of manganese in domestic water supplies. This criterion for manganese that US EPA recommends under the Clean Water Act is the same as the secondary maximum contaminant level established by US EPA in their National Secondary Drinking Water Regulations (US EPA, 1992a).

Iron is a “non-priority” pollutant (US EPA, 1994). The US EPA Quality Criteria for Water established 300  $\mu$ g L<sup>-1</sup> as the recommended water quality criterion for iron in domestic water supplies (US EPA, 1976, 1986). According to these regulations, this criterion of 300  $\mu$ g L<sup>-1</sup> in water is to prevent objectionable tastes or laundry staining and is of an esthetic rather than toxicological significance (US EPA, 1973). The US EPA's human health iron criterion under the Clean Water Act is the same as the secondary maximum contaminant level given in US EPA's National Secondary Drinking Water Regulations (US EPA, 1992b).

The heavy metal concentrations in surface water were higher at the agricultural site for all sampling times and significant differences among them at each sampling site were found (Table 2). The higher concentrations of metals at the agricultural site were probably related to the use of fertilizers in agricultural practices and run-off from top soils. At the agricultural site, at several sampling times the values of the concentrations of Cu, Ni and Pb were higher than those fixed by Argentinean Legislation (Secretaría de Ambiente y Desarrollo Sustentable, 2012) and sometimes Zn, Fe and Mn were also higher. These results demonstrated that the concentrations of heavy metals in surface water of Xanaes River were higher than those concentrations cited by Gaiero et al. (1997) for other rivers of Córdoba province.

Spearman's correlation analysis did not reveal any association between heavy metal concentrations in water with heavy metal concentrations in sediment, except for Mn ( $r = 0.49$ ;  $p = 0.00019$ ) being higher at the agricultural site (data not shown). Therefore, the presence of the heavy metals measured in the water matrix, was not related to another matrix, such as the sediment. Thus, the sediments did not seem to be the source of the heavy metals measured in the water phase.

The temporal heavy metal concentration variations in the sediments (Table 3) over the sampling period showed significant differences for Co, Fe, Mn, Ni, Pb and Zn at the agricultural site among sampling times, and for Fe, Mn, Ni, Pb and Zn at the mountain site. However, only Mn showed concentrations significantly higher at the agricultural site (Table 1).

#### 3.2. Heavy metal accumulation in *M. aquaticum* and physiological changes

In aquatic plants collected at the agricultural site, Co, Cu, Fe and Mn revealed higher concentrations than plants collected at the mountain site (Table 1), with these accumulations revealing

**Table 2**  
Mean values and standard deviation ( $\pm$ SD) of the concentrations (in  $\mu\text{g L}^{-1}$ ) of Co, Cu, Fe, Mn, Ni, Pb and Zn in surface water at two sites (Agricultural and Mountain sites) in the Xanaes River, Córdoba, Argentina over the sampling period April–August 2010. Results of the variance analysis (Kruskal–Wallis) between sampling times for each site are shown. Values in each vertical column followed by the same letter do not differ significantly at  $p < 0.05$  (pairwise comparisons of means).

Sampling times	Surface water ( $\mu\text{g L}^{-1}$ )							
	Co <sub>[Agricultural site]</sub>	Co <sub>[Mountain site]</sub>	Cu <sub>[Agricultural site]</sub>	Cu <sub>[Mountain site]</sub>	Fe <sub>[Agricultural site]</sub>	Fe <sub>[Mountain site]</sub>	Mn <sub>[Agricultural site]</sub>	Mn <sub>[Mountain site]</sub>
04.21.2010 (0)	6.8 ± 0.4 bcd	2.2 ± 0.2	17.4 ± 2.2 abc	5.7 ± 0.8 a	349 ± 24 abc	42 ± 3 a	85.9 ± 0.3 abc	3.9 ± 0.2 ab
05.05.2010 (14)	9.2 ± 0.1 ab	2.4 ± 0.7	43.5 ± 3.4 a	4.0 ± 0.3 b	661 ± 29 a	27 ± 1 abc	103.5 ± 2.2 ab	2.6 ± 0.1 c
05.19.2010 (28)	5.2 ± 0.4 d	2.6 ± 0.5	13.2 ± 4.7 c	5.1 ± 0.2 a	206 ± 11 cde	31 ± 7 ab	37.5 ± 0.7 bcd	2.8 ± 0.7 c
02.06.2010 (42)	6.4 ± 0.5 cd	2.2 ± 0.3	15.6 ± 0.2 bc	4.1 ± 0.1 b	297 ± 10 bcde	19 ± 0 d	66.8 ± 1.7 abcd	2.6 ± 0.8 c
06.16.2010 (56)	9.5 ± 0.2 a	0.8 ± 0.4	39.5 ± 6.2 ab	4.5 ± 0.1 ab	506 ± 42 ab	17 ± 10 cd	120.5 ± 3.8 a	3.2 ± 0.4 abc
06.30.2010 (70)	8.2 ± 1.2 abc	2.3 ± 0.6	18.7 ± 9.9 abc	4.4 ± 0.3 ab	241 ± 163 bcde	22 ± 2 bcd	48.8 ± 32.7 bcd	2.8 ± 0.4 c
07.14.2010 (84)	6.8 ± 0.6 bcd	2.7 ± 0.3	15.9 ± 0.6 abc	4.2 ± 0.2 b	338 ± 36 abcd	20 ± 1 bcd	45.9 ± 9.0 bcd	2.9 ± 0.5 bc
07.28.2010 (98)	7.5 ± 0.5 abc	2.1 ± 1.2	8.2 ± 0.3 c	3.5 ± 1.2 b	74 ± 19 de	21 ± 3 bcd	23.7 ± 7.3 d	3.0 ± 0.8 bc
08.11.2010 (112)	7.9 ± 1.1 abc	1.8 ± 0.5	8.1 ± 0.4 c	4.4 ± 0.4 ab	55 ± 9 e	26 ± 5 abcd	15.4 ± 1.1 d	3.7 ± 1.1 abc
08.23.2010 (126)	8.3 ± 2.0 abc	2.5 ± 0.6	16.9 ± 5.9 abc	4.9 ± 0.2 a	166 ± 137 cde	22 ± 2 bcd	37.0 ± 28.8 cd	4.6 ± 0.6 a
Kruskal Wallis	$p = 0.0146$	n.s.	$p = 0.0112$	$p = 0.0116$	$p = 0.0041$	$p = 0.0223$	$p = 0.0047$	$p = 0.0487$

Sampling times	Surface water ( $\mu\text{g L}^{-1}$ )					
	Ni <sub>[Agricultural site]</sub>	Ni <sub>[Mountain site]</sub>	Pb <sub>[Agricultural site]</sub>	Pb <sub>[Mountain site]</sub>	Zn <sub>[Agricultural site]</sub>	Zn <sub>[Mountain site]</sub>
04.21.2010 (0)	27.8 ± 2.0 bc	12.8 ± 0.5 abc	11.2 ± 1.5 de	0.5 ± 0.2 d	17.2 ± 2.7 ab	3.2 ± 0.1 cd
05.05.2010 (14)	35.8 ± 0.3 a	10.3 ± 3.0 bcd	23.0 ± 3.9 ab	1.4 ± 0.7 abcd	48.4 ± 11.1 a	3.3 ± 0.4 bcd
05.19.2010 (28)	22.5 ± 1.2 c	12.7 ± 2.7 abcd	15.6 ± 0.7 bcde	6.5 ± 7.3 ab	6.0 ± 0.3 bc	7.4 ± 2.9 ab
02.06.2010 (42)	28.1 ± 1.8 bc	9.6 ± 2.0 cd	7.8 ± 2.1 e	5.2 ± 6.8 abc	8.3 ± 0.1 bc	2.7 ± 0.6 d
06.16.2010 (56)	36.4 ± 0.2 a	11.3 ± 4.2 abcd	15.1 ± 2.0 bcde	1.1 ± 1.2 cd	20.5 ± 6.9 ab	8.3 ± 1.1 a
06.30.2010 (70)	38.4 ± 4.2 a	7.3 ± 0.4 d	13.4 ± 1.3 cde	1.0 ± 0.3 bcd	10.5 ± 4.1 abc	4.6 ± 2.1 abcd
07.14.2010 (84)	26.8 ± 1.3 bc	15.6 ± 0.2 a	17.0 ± 0.3 abcd	12.6 ± 7.9 a	25.4 ± 25.0 ab	5.5 ± 0.7 abcd
07.28.2010 (98)	33.4 ± 1.0 ab	14.9 ± 0.1 ab	23.2 ± 6.1 abc	0.6 ± 0.2 cd	5.3 ± 0.2 c	6.0 ± 1.2 abc
08.11.2010 (112)	32.0 ± 1.1 abc	11.4 ± 0.5 abcd	18.0 ± 0.4 abc	1.6 ± 0.7 abcd	5.1 ± 0.3 c	7.4 ± 1.5 ab
08.23.2010 (126)	32.9 ± 2.4 ab	15.6 ± 1.5 ab	32.3 ± 1.2 a	1.3 ± 1.0 abcd	7.3 ± 3.4 bc	3.0 ± 0.1 d
Kruskal Wallis	$p = 0.0018$	$p = 0.0240$	$p = 0.0015$	$p = 0.0320$	$p = 0.0037$	$p = 0.0048$

<sup>a</sup> In parenthesis are shown the days lapsed from the start of the monitoring.

the relationship with the higher concentrations of these metals in superficial water at the agricultural site. Because the plant has rhizomatous stems (Orchard, 1981), these elements might have been taken from the water column by the plant. Related to this,

Kamal (2004) showed that *M. aquaticum* may be effective in the bio-absorption of Cu and Fe from polluted water.

It should be pointed out that the Mn and Zn maximum values of accumulation in plants collected at the agricultural site, duplicated

**Table 3**  
Mean values and standard deviation ( $\pm$ SD) of the concentrations (in  $\text{mg kg}^{-1}$  DW) of Co, Cu, Fe, Mn, Ni, Pb and Zn in sediments at two sites (Agricultural and Mountain sites) in the Xanaes River, Córdoba, Argentina over the sampling period April–August 2010. Results of the variance analysis (Kruskal–Wallis) between sampling times for each site are shown. Values in each vertical column followed by the same letter do not differ significantly at  $p < 0.05$  (pairwise comparisons of means).

Sampling times	Sediment ( $\text{mg kg}^{-1}$ DW)							
	Co <sub>[Agricultural site]</sub>	Co <sub>[Mountain site]</sub>	Cu <sub>[Agricultural site]</sub>	Cu <sub>[Mountain site]</sub>	Fe <sub>[Agricultural site]</sub>	Fe <sub>[Mountain site]</sub>	Mn <sub>[Agricultural site]</sub>	Mn <sub>[Mountain site]</sub>
04.21.2010 (0)	5.6 ± 0.4 a	6.5 ± 0.8	5.8 ± 0.5	5.8 ± 0.3	4312 ± 69 cde	7900 ± 1513 abc	162 ± 3 d	381 ± 28 ab
05.05.2010 (14)	4.8 ± 0.2 bc	6.2 ± 0.7	4.9 ± 0.1	5.4 ± 0.2	3581 ± 42 e	9813 ± 291 a	377 ± 17 bcd	307 ± 11 abcc
05.19.2010 (28)	5.1 ± 0.5 abc	5.4 ± 0.1	5.3 ± 0.2	5.7 ± 0.4	4710 ± 166 bcde	9540 ± 229 ab	341 ± 42 cd	204 ± 43 c
02.06.2010 (42)	5.7 ± 0.4 a	6.2 ± 0.9	6.1 ± 0.3	5.2 ± 0.6	5117 ± 158 abcde	4751 ± 61 cde	630 ± 42 a	284 ± 5 bcc
06.16.2010 (56)	5.9 ± 0.4 a	5.8 ± 0.3	5.6 ± 0.7	5.9 ± 0.4	5326 ± 388 abcd	6182 ± 115 abcd	655 ± 109 a	358 ± 2 abc
06.30.2010 (70)	5.4 ± 0.1 ab	5.2 ± 1.4	5.7 ± 0.7	4.0 ± 0.4	7488 ± 190 a	2398 ± 273 e	505 ± 14 abc	182 ± 1 c
07.14.2010 (84)	5.4 ± 0.1 abc	5.7 ± 1.4	5.3 ± 0.4	4.8 ± 0.5	6237 ± 390 ab	4473 ± 87 de	468 ± 11 abcd	230 ± 10 cc
07.28.2010 (98)	4.5 ± 0.6 c	5.7 ± 1.3	5.2 ± 0.4	5.5 ± 0.4	5841 ± 841 abc	5087 ± 316 cde	424 ± 89 bcd	431 ± 10 a
08.11.2010 (112)	4.9 ± 0.2 bc	5.2 ± 0.3	4.9 ± 0.5	5.6 ± 0.4	3683 ± 126 de	5438 ± 208 bcde	462 ± 137 abc	427 ± 8 a
08.23.2010 (126)	5.4 ± 0.2 ab	4.7 ± 1.0	5.3 ± 0.6	5.4 ± 0.4	5414 ± 201 abc	8727 ± 217 abc	595 ± 65 ab	297 ± 10 abcc
Kruskal Wallis	$p = 0.0136$	n.s.	n.s.	n.s.	$p = 0.0013$	$p = 0.0009$	$p = 0.0034$	$p = 0.001$

Sampling times	Sediment ( $\text{mg kg}^{-1}$ DW)							
	Ni <sub>[Agricultural site]</sub>	Ni <sub>[Mountain site]</sub>	Pb <sub>[Agricultural site]</sub>	Pb <sub>[Mountain site]</sub>	Zn <sub>[Agricultural site]</sub>	Zn <sub>[Mountain site]</sub>	OM% <sub>[Agricultural site]</sub>	OM% <sub>[Mountain site]</sub>
04.21.2010 (0)	11.9 ± 0.5 ab	12.8 ± 1.0 ab	9.7 ± 1.7 a	8.9 ± 3.0 abcd	51.7 ± 4.1 ab	52.1 ± 0.8 ab	5.2 ± 0.2 abc	8.1 ± 0.3 cde
05.05.2010 (14)	11.1 ± 0.7 bc	11.0 ± 0.8 abc	7.3 ± 0.3 bc	10.8 ± 2.3 abc	44.4 ± 1.7 bcd	49.9 ± 1.3 abc	4.7 ± 0.2 c	7.9 ± 0.1 de
05.19.2010 (28)	11.1 ± 0.7 bc	12.8 ± 0.3 ab	8.6 ± 0.4 ab	8.1 ± 1.6 bcd	54.3 ± 1.8 a	49.0 ± 4.2 abc	5.0 ± 0.5 abc	7.3 ± 0.2 e
02.06.2010 (42)	11.8 ± 0.5 abc	13.3 ± 2.8 ab	9.1 ± 0.4 a	22.5 ± 18.0 a	44.3 ± 1.2 bcd	56.5 ± 8.0 ab	5.4 ± 0.6 abc	8.3 ± 0.4 bcde
06.16.2010 (56)	12.7 ± 0.5 a	12.9 ± 1.3 ab	8.0 ± 0.9 abc	9.6 ± 2.6 abcd	40.4 ± 1.1 cd	64.8 ± 2.9 a	6.0 ± 0.6 a	9.0 ± 0.7 abcde
06.30.2010 (70)	12.1 ± 0.5 ab	8.8 ± 0.4 c	8.4 ± 0.1 abc	6.0 ± 0.8 d	46.0 ± 4.1 abcd	39.0 ± 0.6 cd	5.8 ± 0.1 a	10.0 ± 0.9 ab
07.14.2010 (84)	11.5 ± 0.8 abc	9.3 ± 0.6 c	9.7 ± 1.0 a	6.9 ± 0.7 cd	45.1 ± 4.1 abcd	31.5 ± 1.6 d	5.6 ± 0.4 ab	9.7 ± 0.2 abc
07.28.2010 (98)	10.3 ± 0.5 c	11.0 ± 0.8 abc	6.8 ± 0.6 c	9.5 ± 1.8 abcd	46.4 ± 2.0 abc	43.2 ± 1.6 bcd	4.7 ± 0.7 bc	9.8 ± 0.4 abc
08.11.2010 (112)	9.9 ± 0.8 c	10.6 ± 0.5 bc	6.8 ± 0.2 c	6.1 ± 0.9 d	55.3 ± 2.8 a	38.9 ± 0.6 cd	4.7 ± 0.3 c	11.6 ± 0.3 a
08.23.2010 (126)	11.9 ± 0.5 ab	14.3 ± 1.4 a	8.8 ± 0.7 ab	11.7 ± 0.6 ab	36.5 ± 0.9 d	50.8 ± 1.1 abc	5.4 ± 0.1 abc	9.6 ± 0.9 abcd
Kruskal Wallis	$p = 0.0205$	$p = 0.0088$	$p = 0.0096$	$p = 0.015$	$p = 0.0044$	$p = 0.0022$	$p = 0.0337$	$p = 0.0026$

DW, dry weight.

<sup>a</sup> In parenthesis are shown the days lapsed from the start of the monitoring.

**Table 4**

Mean values and standard deviation ( $\pm$ SD) of the concentrations (in  $\text{mg kg}^{-1}$  DW) of Co, Cu, Fe, Mn, Ni, Pb and Zn in *M. aquaticum* leaves at two sites (Agricultural and Mountain sites) in the Xanaes River, Córdoba, Argentina over the sampling period April–August 2010. Results of the variance analysis (Kruskall–Wallis) between sampling times for each site are shown. Values in each vertical column followed by the same letter do not differ significantly at  $p < 0.05$  (pairwise comparisons of means).

Sampling times	<i>M. aquaticum</i> ( $\text{mg kg}^{-1}$ DW)							
	Co <sub>[Agricultural site]</sub>	Co <sub>[Mountain site]</sub>	Cu <sub>[Agricultural site]</sub>	Cu <sub>[Mountain site]</sub>	Fe <sub>[Agricultural site]</sub>	Fe <sub>[Mountain site]</sub>	Mn <sub>[Agricultural site]</sub>	Mn <sub>[Mountain site]</sub>
04.21.2010 (0)	2.9 $\pm$ 0.3 abcd	2.7 $\pm$ 0.2 a	6.7 $\pm$ 0.2 bcdef	11.5 $\pm$ 0.4 a	4.8 $\pm$ 0.3 d	16.7 $\pm$ 0.3 a	4.2 $\pm$ 0.1 e	5.7 $\pm$ 0.0 abc
05.05.2010 (14)	3.0 $\pm$ 0.1 abc	1.8 $\pm$ 0.3 b	7.2 $\pm$ 0.3 abcde	7.7 $\pm$ 0.1 ab	8.5 $\pm$ 0.2 cd	16.7 $\pm$ 0.3 a	6.1 $\pm$ 0.1 de	5.7 $\pm$ 0.0 abc
05.19.2010 (28)	3.2 $\pm$ 0.1 ab	2.3 $\pm$ 0.0 a	15.1 $\pm$ 0.4 a	7.2 $\pm$ 0.4 ab	11.9 $\pm$ 0.2 bcd	10.6 $\pm$ 0.0 ab	9.6 $\pm$ 0.1 ab	3.5 $\pm$ 0.0 e
02.06.2010 (42)	3.0 $\pm$ 0.0 abc	1.9 $\pm$ 0.2 b	11.4 $\pm$ 0.3 abc	5.5 $\pm$ 0.1 abc	27.6 $\pm$ 0.2 a	10.0 $\pm$ 0.1 abc	8.5 $\pm$ 0.3 bcde	6.8 $\pm$ 0.1 a
06.16.2010 (56)	3.4 $\pm$ 0.3 a	2.0 $\pm$ 0.0 ab	12.6 $\pm$ 0.2 ab	3.3 $\pm$ 0.1 cd	25.1 $\pm$ 0.9 ab	8.5 $\pm$ 0.5 abcd	9.1 $\pm$ 0.1 abcd	5.4 $\pm$ 0.1 abcd
06.30.2010 (70)	2.5 $\pm$ 0.1 d	2.1 $\pm$ 0.1 ab	10.6 $\pm$ 0.2 abcd	3.8 $\pm$ 0.0 bcd	25.2 $\pm$ 1.3 ab	5.9 $\pm$ 0.1 bcde	10.7 $\pm$ 0.2 a	4.5 $\pm$ 0.1 bcde
07.14.2010 (84)	2.8 $\pm$ 0.1 bcd	2.1 $\pm$ 0.2 ab	5.1 $\pm$ 0.0 def	3.2 $\pm$ 0.1 cd	19.4 $\pm$ 0.7 abc	7.8 $\pm$ 0.1 abcde	6.8 $\pm$ 0.1 cde	6.3 $\pm$ 0.1 ab
07.28.2010 (98)	2.7 $\pm$ 0.2 cd	1.9 $\pm$ 0.1 b	4.6 $\pm$ 0.1 ef	2.8 $\pm$ 0.0 d	15.0 $\pm$ 0.1 abc	3.9 $\pm$ 0.1 cde	9.4 $\pm$ 0.1 abc	4.1 $\pm$ 0.0 cde
08.11.2010 (112)	2.5 $\pm$ 0.2 cd	1.9 $\pm$ 0.2 b	3.9 $\pm$ 0.0 f	4.4 $\pm$ 0.1 abc	11.5 $\pm$ 0.3 bcd	3.7 $\pm$ 0.1 de	9.7 $\pm$ 0.1 ab	3.9 $\pm$ 0.0 cde
08.23.2010 (126)	2.6 $\pm$ 0.2 cd	1.8 $\pm$ 0.1 b	5.3 $\pm$ 0.0 cdef	3.9 $\pm$ 0.2 bcd	8.4 $\pm$ 0.1 cd	3.0 $\pm$ 0.1 e	8.8 $\pm$ 0.0 bcde	3.7 $\pm$ 0.1 de
Kruskal Wallis	$p = 0.0041$	$p = 0.0205$	$p = 0.0007$	$p = 0.0008$	$p = 0.0008$	$p = 0.0008$	$p = 0.0008$	$p = 0.0008$

Sampling times	<i>M. aquaticum</i> ( $\text{mg kg}^{-1}$ DW)					
	Ni <sub>[Agricultural site]</sub>	Ni <sub>[Mountain site]</sub>	Pb <sub>[Agricultural site]</sub>	Pb <sub>[Mountain site]</sub>	Zn <sub>[Agricultural site]</sub>	Zn <sub>[Mountain site]</sub>
04.21.2010 (0)	5.5 $\pm$ 0.2 f	15.6 $\pm$ 1.2 abcd	2.2 $\pm$ 0.5	1.9 $\pm$ 0.9	179.5 $\pm$ 19.4 ab	69.0 $\pm$ 6.5 ab
05.05.2010 (14)	8.9 $\pm$ 0.2 cdef	25.7 $\pm$ 0.9 a	1.5 $\pm$ 0.9	1.8 $\pm$ 0.9	66.1 $\pm$ 11.1 abcd	14.5 $\pm$ 3.8 d
05.19.2010 (28)	33.0 $\pm$ 3.2 a	20.3 $\pm$ 1.3 abc	2.3 $\pm$ 0.1	1.7 $\pm$ 0.3	246.8 $\pm$ 21.5 a	44.6 $\pm$ 1.6 bcd
02.06.2010 (42)	12.9 $\pm$ 1.3 abcd	14.7 $\pm$ 0.7 abcde	2.5 $\pm$ 0.2	2.0 $\pm$ 0.6	86.2 $\pm$ 14.2 abc	40.8 $\pm$ 5.9 bcd
06.16.2010 (56)	10.1 $\pm$ 0.2 abcde	7.8 $\pm$ 0.2 def	3.0 $\pm$ 0.6	1.0 $\pm$ 1.1	183.0 $\pm$ 50.2 ab	46.1 $\pm$ 2.1 abcd
06.30.2010 (70)	9.8 $\pm$ 0.1 bcdef	22.2 $\pm$ 1.1 ab	1.8 $\pm$ 1.0	1.7 $\pm$ 1.1	29.6 $\pm$ 3.9 bcde	24.6 $\pm$ 5.7 cd
07.14.2010 (84)	6.1 $\pm$ 0.2 ef	6.6 $\pm$ 0.2 ef	1.2 $\pm$ 0.6	1.6 $\pm$ 0.4	32.4 $\pm$ 5.3 bcde	105.4 $\pm$ 9.7 a
07.28.2010 (98)	17.4 $\pm$ 1.9 abc	9.8 $\pm$ 0.0 cdef	2.0 $\pm$ 1.1	2.0 $\pm$ 0.4	11.8 $\pm$ 2.2 de	92.0 $\pm$ 4.6 ab
08.11.2010 (112)	7.1 $\pm$ 0.1 def	5.1 $\pm$ 0.2 f	1.7 $\pm$ 0.3	1.3 $\pm$ 0.5	11.4 $\pm$ 0.3 e	51.0 $\pm$ 5.1 abc
08.23.2010 (126)	24.5 $\pm$ 0.4 ab	11.8 $\pm$ 0.8 bcdef	1.7 $\pm$ 0.7	1.4 $\pm$ 0.7	22.6 $\pm$ 5.6 cde	17.1 $\pm$ 1.4 d
Kruskal Wallis	$p = 0.0007$	$p = 0.0008$	n.s.	n.s.	$p = 0.001$	$p = 0.0012$

DW, dry weight.

<sup>a</sup> In parenthesis are shown the days lapsed from the start of the monitoring.

the maximums observed at the mountain site (Tables 1 and 4). On the other hand, significant differences in the concentration of heavy metals in leaves of *M. aquaticum* were observed over the studied period (Table 4), except for Pb.

Table 5 shows the mean values of the physiological parameters measured in *M. aquaticum* and the Kruskal–Wallis results between sampling sites. The levels of chlorophyll ( $a + b$ ) were significantly higher at the mountain site, whereas MDA and the Phaeoph- $a$ /Chl- $a$  ratio were significantly higher at the agricultural site. The loss of photosynthetic pigments is a common in response of plants to stress (i.e. pollution), which has been observed after metal treatment in various species of *Myriophyllum* genus such as *M. quitense* (Nimptsch et al., 2005), *M. heterophyllum* (Sivaci et al., 2008), *M. spicatum* (Grudnik and Germ, 2010; Jiao et al., 2009), *M. alterniflorum* (Delmail et al., 2011; Ngayila et al., 2009) and *M. mattogrossense* (Nimptsch and Pflugmacher, 2007).

The temporal variation in the physiological parameters showed the same pattern in both sampling sites as well as differences between sites at each sampling time (Fig. 2). The concentrations of Chl- $a + b$  were lower at the agricultural site in all the sampling periods (Fig. 2A). The concentration of MDA (Fig. 2B) as well

**Table 5**

Mean values and standard deviation ( $\pm$ SD) of the concentrations of the physiological parameters measured in *M. aquaticum* leaves at two sites (Agricultural and Mountain sites) in the Xanaes River, Córdoba, Argentina. Results of the variance analysis (Kruskall–Wallis) between Agricultural and Mountain sites ( $p$ -value) are shown for each parameter.

	<i>M. aquaticum</i>		
	Mountain site	Agricultural site	$p$ -Value
Chl- $a + b$ ( $\text{mg g}^{-1}$ DW)	2.75 $\pm$ 0.40	2.26 $\pm$ 0.47	0.0001
MDA ( $\mu\text{mol g}^{-1}$ DW)	0.03 $\pm$ 0.01	0.06 $\pm$ 0.02	<0.0001
Phaeoph- $a$ /Chl- $a$	1.09 $\pm$ 0.03	1.11 $\pm$ 0.04	0.0112

Chl, chlorophyll; MDA, malondialdehyde; Phaeoph, phaeophytin.

**Table 6**

Spearman correlation coefficients among physiological parameters and heavy metal contents in the aquatic plant *M. aquaticum*.

<i>M. aquaticum</i>	Physiological parameters		
	Chl ( $a + b$ )	MDA	Phaeoph- $a$ /Chl- $a$
<i>Heavy metals</i>			
Co	-0.51***	0.68***	0.52***
Cu	-0.32**	0.65***	0.66***
Fe	-0.66***	0.49***	0.32**
Mn	-0.67***	0.42***	0.15
Ni	0.11	-0.01	0.18
Pb	-0.15	0.30*	0.27*
Zn	-0.12	0.36*	0.48***

Chl, chlorophyll; MDA, malondialdehyde; Phaeoph, phaeophytin.

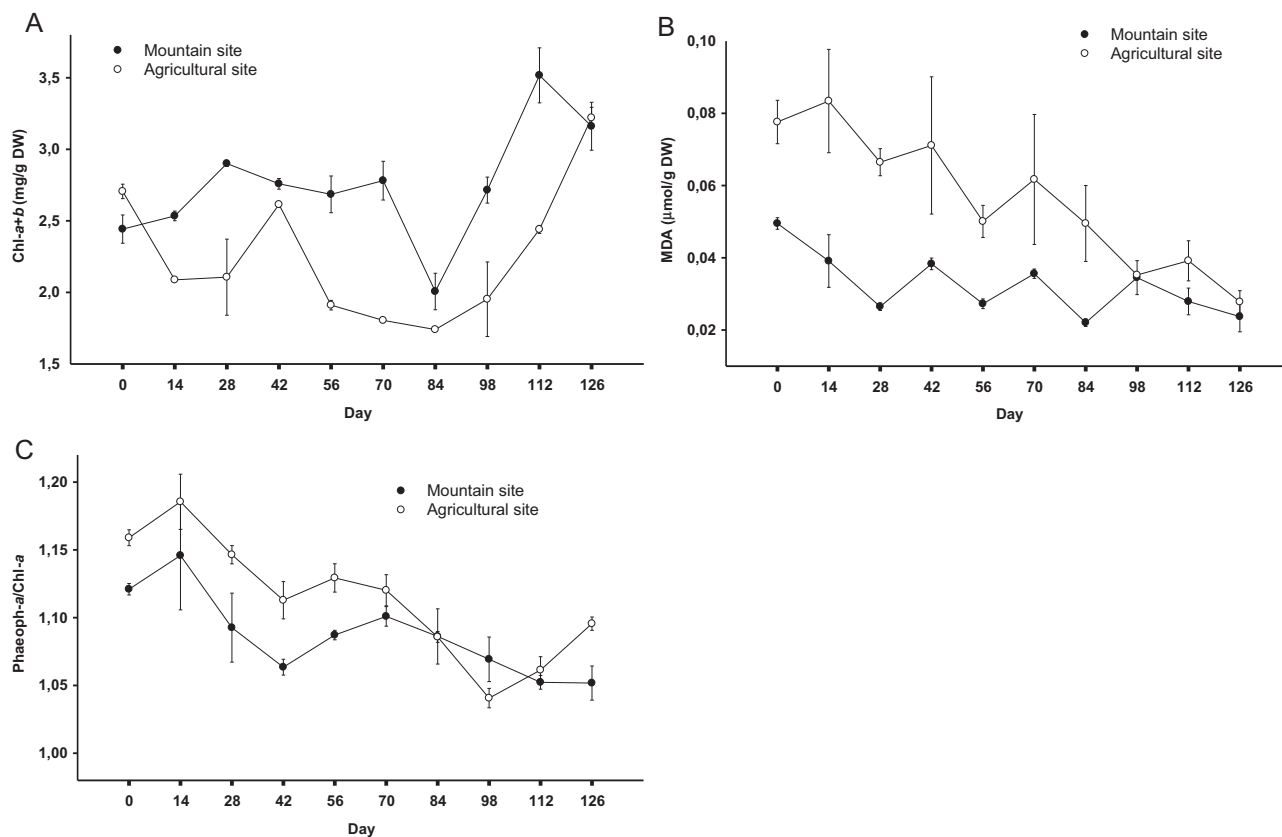
\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

as the Phaeoph- $a$ /Chl- $a$  ratio (indicative of the degradation of chlorophyll- $a$ ) (Fig. 2C), although showing a temporal variations at both sampling sites, presented more elevated values at the agricultural site, which may explain the physiological damage observed in plants collected at that site during the period studied.

The Spearman correlation analysis among physiological parameters and heavy metal content in plants (Table 6) showed a significant negative association of Chl- $a + b$  with Co, Cu, Fe and Mn accumulation. Prasad (2001) attributed the drop in chlorophyll to the metal-induced modification of chlorophyll degradation and to its structural and functional damage. Trace metals may substitute for the magnesium ion in the chlorophyll molecule, leading to an inability to catch photons and thus to a decrease in the photosynthetic activity. On the other hand, heavy metals at low concentrations may induce oxidative stress, which subsequently inhibits the chlorophyll biosynthesis (Ferrat et al., 2003). Therefore, our results showing that heavy metals in *M. aquaticum* tissues



**Fig. 2.** Variation pattern of average concentrations (with standard deviation) of the physiological parameters measured in *M. aquaticum* leaves at the two sampling sites (agricultural and mountain) over the sampling period.

may induce oxidative stress, might explain the differences observed in chlorophyll pigments and lipid peroxidation between the two sampling sites.

The Phaeoph-a/Chl-a ratio showed a positive correlation with Co, Cu, Fe, Pb and Zn. Thus, we can infer that Pb and Zn may have produced degradation of chlorophyll-a in this plant species but did not seem to affect the synthesis of chlorophylls expressed as Chl-a + Chl-b.

At the same time, Pb and Zn as well as Co, Cu, Fe and Mn correlated positively with MDA concentration in *M. aquaticum*, which could probably explain the effects of these metals on lipid peroxidation and membrane damage (Table 6). Peroxidation of the membranes via metal-induced free radicals causes destabilization and permeability to arise (Gill and Tuteja, 2010). Other authors have demonstrated that a metal excess was able to generate oxidative stress from increased lipid peroxidation in plants (measured as MDA) (Nikookar et al., 2005; Rama Devi and Prasad, 1998). The physiological damage observed in *M. aquaticum* plants collected at the agricultural site may be attributed not only to the heavy metals related to them, but also to the presence of other pollutants associated with these practices.

### 3.3. Correlation analysis among heavy metal content in *M. aquaticum* vs. surface water and sediment

As *M. aquaticum* is a rooted water plant, it is able to take up heavy metals from the water and the sediment phase. To investigate in the present study if either the sediment or the surface water was the main source for heavy metals in leaves of *M. aquaticum*, the Spearman correlation between heavy metal levels in sediment, surface water and plant was applied (Table 7), and a significant high

positive correlation for Cu, Fe and Mn was found between heavy metal content with *M. aquaticum* and sediment.

Therefore, these elements may be incorporated by *M. aquaticum* through their root system from the sediment. This is in agreement with Jackson (1998), who reported that the root uptake of elements with subsequent translocation to above-ground tissues has been shown for a variety of elements, and thus may be an important vector exiting from the sediments for elements that would otherwise remain buried. This author also pointed out the importance of macrophytes in the element cycling in aquatic ecosystems.

In addition, the significantly high positive correlations found for Co, Cu, Fe, Mn and Zn in aquatic plant and in surface water (Table 7), are in agreement with Demirezen and Aksoy (2006), who postulated that the aquatic flora reflects the metal content of their environment.

In the previous section, we reported that the metal concentrations in the sediments were not significantly different between sites except for Mn. However, significant differences between sites were found for both plant and water metal content (Table 1). The exposure of the aquatic plant to heavy metals in water and the integrated response time to the storage and assimilation processes in their tissues, may reveal whether the contaminants came in pulses or at a steady stream to the surface waters (Bonanno and Lo Giudice, 2010; Cardwell et al., 2002; Collins et al., 2005).

The mean values of Cu, Fe and Zn showed relatively similar variations in surface water and in the aquatic plant, but the levels of metals in the plant were displaced over time (Tables 2 and 4). These results suggest that the input of pollutants into water at the agricultural site was in the form of pulses and that *M. aquaticum* is a biomonitor able to detect this phenomenon. Although there are no previous studies on the Xanaes River, where the aquatic environment is presented as a sink by surface runoff, leaching and/or

**Table 7**

Spearman correlation coefficients between heavy metal concentrations in water and sediment and heavy metal concentrations in the aquatic plant *M. aquaticum* collected at the same sites.

	<i>M. aquaticum</i>						
	Co	Cu	Fe	Mn	Ni	Pb	Zn
<i>Water</i>							
Co	0.69***	0.38**	0.42***	0.63***	−0.05	0.27*	−0.01
Cu	0.83***	0.56***	0.43***	0.49***	−0.06	0.24	0.19
Fe	0.82***	0.61***	0.50***	0.48***	−0.10	0.27*	0.21
Mn	0.81***	0.48***	0.36*	0.49***	−0.20	0.30*	0.26*
Ni	0.68***	0.32*	0.41**	0.64***	−0.17	0.24	0.06
Pb	0.59***	0.19	0.27*	0.62***	−0.04	0.09	−0.10
Zn	0.55***	0.19	0.16	0.12	−0.45***	0.14	0.40**
<i>Sediment</i>							
Co	−0.09	0.22	0.21	−0.09	0.09	0.18	0.21
Cu	0.15	0.31*	0.24	−0.09	−0.07	0.27*	0.25*
Fe	−0.25*	0.2	0.30*	−0.19	0.24	−0.11	−0.29*
Mn	0.39**	0.24	0.50***	0.55***	−0.07	0.15	−0.04
Ni	0.05	0.32*	0.15	−0.16	0.08	0.07	0.12
Pb	−0.15	0.20	0.10	−0.11	0.15	0.10	0.04
Zn	−0.02	0.17	0.15	0.02	0.10	−0.01	−0.08

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

effluent discharge of anthropic activities, it is known that agricultural activities close to rivers can have an important impact on the water quality (Hesterberg et al., 2006) and also affect the biota living there (Beasley and Kneale, 2003). Finally, Cu, Fe and Zn incorporated as pollutants in surface waters were able to be removed by *M. aquaticum* rapidly by self-purification processes. Therefore, this species could be proposed as a suitable heavy metals accumulation biomonitor at the early stages of river pollution.

The information that allows to obtain *M. aquaticum* will be employed in future works in order to studying the impact of human activities on aquatic ecosystems by means of the analysis of multiple criterions for selecting ecologically significant input variables (Zhao et al., 2006; Muttill and Chau, 2007) and the implementation de water quality modeling methodologies (Chau et al., 2002).

#### 4. Conclusions

The agricultural activities were a source of heavy metals in surface water at the Xanaes River, with Fe and Mn levels in water exceeding the limits permitted by both international and national laws. The input of Co, Cu, Ni, Pb and Zn in the river followed a pulse pattern, possibly due to runoff, fertilizer application or effluents discharged close to the river.

The concentrations of Co, Cu, Fe and Mn in leaves of *M. aquaticum* at the agricultural site were higher than those in plants collected at the mountain site, and they did not respond to temporal changes. The heavy metal concentration variations in *M. aquaticum* were similar to those in the water column, although the accumulation by plants was delayed over time. The suitability of *M. aquaticum* as a good bioindicator for the early stages of heavy metal input in rivers is proposed in this study.

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