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RESEARCH ARTICLE

Effect of the combined addition of Zn and Pb on partitioning in sediments and their accumulation by the emergent macrophyte *Schoenoplectus californicus*

Silvana Arreghini[1] · Laura de Cabo[2] · Roberto Serafini[1] · Alicia Fabrizio de Iorio[1]

Abstract Wetlands usually provide a natural mechanism that diminishes the transport of toxic compounds to other compartments of the ecosystem by immobilization and accumulation in belowground tissues and/or soil. This study was conducted to assess the ability of *Schoenoplectus californicus* growing in natural marsh sediments, with zinc and lead addition, to tolerate and accumulate these metals, taking account of the metal distribution in the sediment fractions. The Zn and Pb were mainly found in available (exchangeable) and potentially available (bound to organic matter) forms, respectively. The absorption of Zn and Pb by plants increased in sediments with added metals. Both metals were largely retained in roots (translocation factor < 1). Lead rhizome concentrations only increased significantly in treatments with high doses of metal independently of added Zn. The addition of Zn increased its concentration in roots and shoots significantly, while its concentration in rhizomes only increased when both metals were added together. Zinc concentration in shoots did not reach the toxic level for plants. Zinc and Pb concentrations in roots were high, but they were not sufficient to reduce biomass growth.

Responsible editor: Elena Maestri

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Introduction

Wetlands usually provide a natural mechanism that diminishes the transport of toxic compounds to other compartments of the ecosystem. This approach usually relies on "phytostabilization," the use of plants to immobilize heavy metals and store them in belowground tissues and/or soil which is conducted especially by emergent macrophytes (de Cabo et al. 2015). The extent of uptake and the distribution of metals within the plant may have a significant effect on their residence time and potential release. This information will contribute to a better understanding of these systems, ensuring that wetlands do not themselves eventually become sources of metal contamination to surrounding areas (Weis and Weis 2004).

A significant removal of dissolved metal ions from the water column to the sediment phase is achieved in wetlands. Metals are removed from solution primarily through the precipitation of insoluble oxyhydroxide compounds or sorption onto particulate materials. The fate of trace metals in this sink depends on the biogeochemical transformations that occur in the sediment layer (Peltier et al. 2003). The partition in various forms of trace metals in sediments and soils is commonly conducted by sequential extraction, which furnishes detailed information about their origin, mode of occurrence, biological and physicochemical availability, mobilization, and transport (Tessier et al. 1979). A portion of the total content of metals in sediments may not be easily available to plants (e.g., the fractions occluded in iron and manganese oxides, or in primary and secondary minerals). However, changes in the physical or chemical environment of sediment can modify metal availability.

Although some tolerant plants are now available for land restoration, there has been a continuing interest in searching for native plants that are tolerant, adapted to local climatic conditions, and are able to colonize metal-enriched soils

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(Shu et al. 2002). *Schoenoplectus californicus* is an indigenous and emergent macrophyte (Lange et al. 1998) which attains dense stands on muddy river banks and coastal areas of lakes and estuaries. Numerous marshes in Argentina are covered by *S. californicus*. Several studies have demonstrated its ability to maintain ecological functions in marshes, to incorporate important quantities of carbon from the atmosphere into biomass, to protect shorelines, and to tolerate high levels of heavy metals (Arreghini et al. 2001; Villar et al. 2001; Kandus et al. 2010; de Cabo et al. 2015). Tolerance is conferred by specific physiological mechanisms which effectively enable it to function normally even in the presence of high concentrations of potentially toxic elements (Baker 1987). However, there is evidence that different populations develop different capabilities to face metal stress. Biomass growth is a useful variable for assessing heavy metal toxicity. Lewis et al. (2001) found that growth assessment was a more sensitive indicator of heavy metal exposition than the stress protein level or photosynthetic efficiency.

The aim of this study was to determine the ability of *Schoenoplectus californicus* to tolerate and accumulate zinc and lead, taking account of the metal distribution in marsh sediment fractions. The interdependence of zinc and lead on the uptake by this macrophyte has been also considered.

Materials and methods

Experimental design and sample collection

An experiment was carried out in summer (January to March). Alluvial sediments and rhizomes of *S. californicus* were collected from a background area, a natural wetland of the Paraná River floodplain. Sediments were air-dried and their main characteristics were determined. One kilogram of air-dry sediment was placed in plastic containers (21-cm diameter and 21-cm deep) to a depth of 10 cm to provide an adequate rooting zone for *S. californicus.* Zinc, as $Zn(NO_3)_2$, and lead, as $Pb(NO_3)_2$, were added separately to the sediments, attaining concentrations of 2000 μg Zn/g air-dry sediment (TZn), 350 μg Pb/g dry sediment (LoPb), and 1400 μg Pb/g dry sediment (HiPb). Zinc and Pb were also added simultaneously (Zn-LoPb and Zn-HiPb). These treatments were compared with control treatments without adding any metals (CT). Before planting the rhizomes, the sediments were kept at a temperature of 28 ± 5°C for 41 days. The added metal concentrations simulated the metal contamination from a variety of anthropogenic sources, including untreated sewage discharge, mining and metal-related industry runoff, and urban and agriculture stormwater runoff (Davis et al. 2001; Councell et al. 2004; O'Geen et al. 2010; Fujiwara et al. 2011; Wen et al. 2015), and metal concentration in water and sediments of an

urban river in Buenos Aires was also considered (Rendina et al. 2001; Magdaleno et al. 2014).

Several rhizomes containing ten buds and some shoots were transplanted into plastic containers. These initial shoots were not considered for either the metal determinations or biomass measurement. Waterlogged soil was maintained throughout the experiment by periodic addition of rainwater. The plants were grown for 63 days. All treatments were undertaken outdoors in triplicate under a natural illumination regime, amounting to roughly 13 h of light per day.

Plant measurements

Shoot heights were measured to the nearest 0.5 cm at 8, 14, 22, 36, 47, 56, and 63 days after rhizome planting. Shoot heights were converted to shoot biomass using a height-weight relationship obtained at the end of the experiment by harvesting and drying all the shoots. Net primary production (NPP) was calculated as the sum of the shoot weights in each pot and referred to square meter. Weights of rhizome and root biomass per pot were measured at the end of the experiment. Aboveground and belowground tissues were oven dried at 70°C to a constant weight and then weighed to the nearest 0.0001 g.

Relative growth rate was calculated from equation proposed by Harper (1977):

$$RGR = (lnw_2 - lnw_1)\big/(t_2 - t_1)$$

w_1 and w_2 being the dry weights of the aboveground biomass (grams) at time t_1 and t_2 (days), respectively.

Analytical procedures

The initial characteristics of the sediments were analyzed. Cation exchange capacity (CEC) was determined by Allison et al. (1985). Sediment pH was measured using 1:2.5 (sediment/deionized water). Kjeldahl nitrogen, extractable phosphorus (Kurtz-Bray), and organic matter content were determined according to Page et al. (1982). Total Zn and Pb concentrations in the sediments were determined by acid digestion with HNO_3, $HClO_4$, and HF (2:1:1).

At the end of the experiment, sediment samples were collected and kept at −20°C until analysis to determine Zn and Pb bound to the following five sediment fractions: exchangeable, carbonates, iron and manganese oxides, organic matter and sulfides, and residual, according to the sequential extraction technique proposed by Tessier et al. (1979). This method consists of sequential extractions with $MgCl_2$ at pH 7, NaOAc at pH 5, HOAc, $NH_2OH·HCl$ 25% (v/v) of HOAc, HNO_3, H_2O_2, and NH_4OAc, and $HF-HClO_4$. This extraction scheme is based on operationally defined fractions. Extraction procedures provide qualitative evidence regarding the forms of

association of trace metals and, indirectly, their bioavailability (Harrison 1981).

Rhizome, root, and shoot tissues of *S. californicus* were analyzed for total Zn and Pb concentrations. Plant samples were carefully washed with tap water and rinsed with deionized water, and scrubbed thoroughly to remove any particulate matter adhering to the plant material. Plants were separated into rhizomes, roots, and shoots and were dried at 70°C to a constant weight and then ground into a powder using a blender. One gram of powder was digested with HNO_3 and $HClO_4$ (2:1). Zinc and Pb concentrations were determined by atomic absorption spectrophotometry (PerkinElmer AAnalyst 200).

The concentration of Zn and Pb in whole plants was calculated from biomass values and metal concentration in each part of the plant. The translocation factor (TF) for metals within a plant was estimated by the ratio of the metal concentration in shoots to the metal concentration in roots. The bioconcentration factor (BCF) was calculated from the ratio of the metal concentration in roots to the total metal concentration in the sediments obtained as the sum of fractions by Tessier sequential extraction method.

Statistical analysis

The values of shoot biomass and Zn and Pb concentrations in roots, shoots, rhizomes, and in different sediment fractions, were examined for normal distribution using the Lilliefor's test. Data that did not follow a normal distribution were log_{10} transformed and their distributions reexamined. All variables were tested for homogeneity of variances using Levene's test. A two-way ANOVA was used to test significant differences in the mean metal concentration in the plants and in the sediment. All statistical analyses were performed using GENSTAT Release 7.1 (2003). Differences are reported as significant at $p < 0.05$.

Results

Sediment

The initial characteristics of sediments used in the assay are shown at Table 1. The sediments were acidic, with a high content of organic matter and nutrient supply, and they were similar to those reported by de Cabo (2001) for a background marsh with bulrush cover. The levels of Zn and Pb were similar to those for the sediments of a basin with cattle grazing land use (Rendina and Fabrizio de Iorio 2012).

The pH of the sediment rose from 4.0 to 5–5.5 in all treatments (Fig. 1). During the experiment, the trend of the pH increased over time and reached values of 6.1 at the end. Although some decrease in the pH was observed in treatments with added Zn during the first weeks, no significant

differences were found between the treatments (general mean 5.7 ± 0.3). The water pH showed similar behavior to the sediment pH in each treatment (general mean 5.9 ± 0.3) (Fig. 1). The zinc and lead concentrations in water during planting were lower than 0.1 and 0.01%, respectively, of the total added metal.

In treatments without the addition of zinc (CT, LoPb, and HiPb), Zn was associated to residual, iron and manganese oxides, and exchangeable fractions, with similar percentages (22–31%), and to a lower extent to the organic matter-sulfide and carbonate fractions (7–12%) (Fig. 2a). In treatments without added Pb (CT and TZn), the highest percentage of metal was found in the exchangeable fraction (36–42%), followed by the residual fraction (22–29%) and the organic matter-sulfide fraction (15–18%) (Fig. 2b).

The addition of Zn (TZn, Zn-LoPb, and Zn-HiPb) increased its total concentration in sediment and in all fractions ($p < 0.01$) (Table 2). Considering that an error lower than 10% is allowed, the low coefficient of variation between the results obtained for the determination of total Zn by mineralization and as a sum of fractions indicates the accuracy of the method used for this element (CV = 7–8% for treatments without Zn addition and CV = 1–9% for treatments with Zn addition).

The highest percentages of zinc were found in the exchangeable fraction (higher than 50%) followed by iron and manganese oxides (20%) and carbonate (15%) fractions. Zinc bound to iron and manganese oxides in Zn-LoPb and Zn-HiPb treatments was lower than in TZn ($p < 0.01$). Also, the addition of Pb increased its total concentration in sediment and in all fractions as a function of the added dose of Pb independently of the addition of Zn ($p < 0.01$). Considering that an error lower than 10% is allowed, the low coefficient of variation between the results obtained for the determination of total Pb by mineralization and as a sum of fractions indicates the accuracy of the method used for this element (CV = 8–9% for treatments without Pb addition and CV = 3–9% for treatments with Pb addition). The highest percentages of Pb were found in the organic matter-sulfide fraction (35–39%) followed by exchangeable (20–33%) and iron and manganese oxides (19–21%) fractions (Table 2).

Plant

There was no adverse effect on biomass production by metal exposure. Moreover, the net primary production (NPP) was highest in Zn-HiPb ($p < 0.01$) followed by the TZn treatment ($p < 0.01$). The aboveground biomass increased over time in all treatments and it reached NPP values between 203 and 346 g/m^2 (Table 3). In all treatments, the highest RGR values were found in the first week and then they declined to reach almost zero values from 47 days to the end of the experiment (Table 4).

Table 1 Initial characteristics of air-dry sediments used in the assay

pH	OM (%)	N Kj (%)	P Bray (μg/g)	CEC (cmol$_c$/kg)	Zn (μg/g)	Pb(μg/g)
4.0	55.6	1.6	124	100	102.6	48.4

OM organic matter percentage, *N Kj* Kjeldahl nitrogen, *P Bray* extractable phosphorus, *CEC* cation exchange capacity

In treatments with the addition of Zn, metal levels in whole plants increased with regard to the treatments without added Zn ($p < 0.01$) (Fig. 3a). The same occurred in treatments with added Pb ($p < 0.01$) (Fig. 3b).

The distribution of Zn within the plant followed the pattern root > rhizome > shoot for all treatments, and this distribution was more evident for treatments where Zn was added. The addition of Zn significantly increased its concentration in roots and shoots ($p < 0.01$) independently of added Pb (TZn, Zn-LoPb, and Zn-HiPb). The zinc concentration in rhizomes increased only when both metals were added together ($p < 0.01$), although the high dispersion of data in the TZn treatment could have masked the differences. The highest values of BCF for Zn were found in treatments with added Zn ($p < 0.01$) and were higher than unity in the Zn-HiPb treatment (Table 3).

In treatments with Pb addition, metal in roots increased with increasing lead concentrations in sediments ($p < 0.001$). Highest root concentrations of Pb were found in treatments with high doses (HiPb and Zn-HiPb) followed by treatments with low doses (LoPb and Zn-LoPb), TZn and CT ($p < 0.01$) (Table 3). The concentrations of Pb in rhizomes increased only in high-dose treatments (HiPb and Zn-HiPb) ($p < 0.01$). The lowest concentrations of Pb in shoots were found in CT ($p < 0.01$) and the highest in the LoPb treatment ($p < 0.05$), but no significant differences were found between the rest of the treatments. The highest values of BCF for Pb were found in treatments TZn, Zn-LoPb, and Zn-HiPb (Table 3) and Pb addition had no effect on this parameter.

The translocation factor indicates metal mobilization within the plant. Data presented here indicate that metals accumulated by *S. californicus* were largely retained in the belowground tissues, mainly in the roots, as shown by the general TF values lower than unity (Table 3). The translocation factor decreased when the concentration of Zn and Pb in soils increased. The highest Zn concentration in roots was associated with the lowest TF values for Zn ($p < 0.05$). In treatments HiPb and Zn-HiPb, the roots presented the highest Pb concentration and were associated with the lowest TF value for Pb independently of the Zn addition ($p < 0.05$). The same occurred for treatments with a low addition of Pb (LoPb and Zn-LoPb) with TF intermediate values and differed significantly from the others ($p < 0.05$). Conversely, the lowest concentrations of metals in the roots were associated with the highest TF values for both metals ($p < 0.05$).

Discussion

The trend of increasing pH to values near to neutrality is a common feature in wetland sediments (Ponnamperuma 1972; Sahrawat 1998). The overall effect of submersion is to increase the pH of acid soils. Thus, submersion makes the pH values converge to 7 due to the important reduction reactions that occur in nature involving the consumption of H$^+$ ions (Ponnamperuma 1972). Besides, the increase of about one unit of pH in rewetted air-dry soils was also reported by Haynes and Swift (1989), and they attributed this to a rapid ammonification, which results in the release of 1 mol of hydroxyl ion per mole of ammonium formed. The acid-base buffering ability of organic matter (Klavins and Purmalis 2013; Baidoo et al. 2014; Jayalath et al. 2016) contributes to maintain the relatively constant pH in sediment after the fifth week. Other biogeochemical processes, such as nutrient uptake, particularly calcium and magnesium, and high water
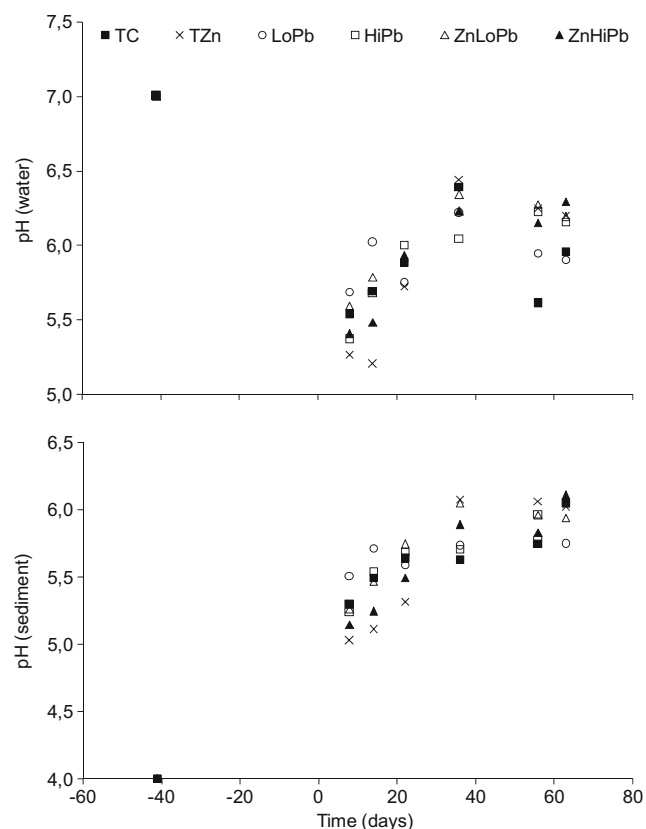
Fig. 1 Variations of pH in water and sediment over time for the different treatments

Fig. 2 Partitioning of Zn (**a**) and Pb (**b**) between the different sediment fractions expressed as percentages (%) as sum of fractions: exchangeable, carbonate, oxides of iron and manganese, organic matter-sulfide, and residual

□ Exchangeable □ Carbonates ▦ Oxides of Fe and Mn ▨ Organic matter-sulfide ■ Residual
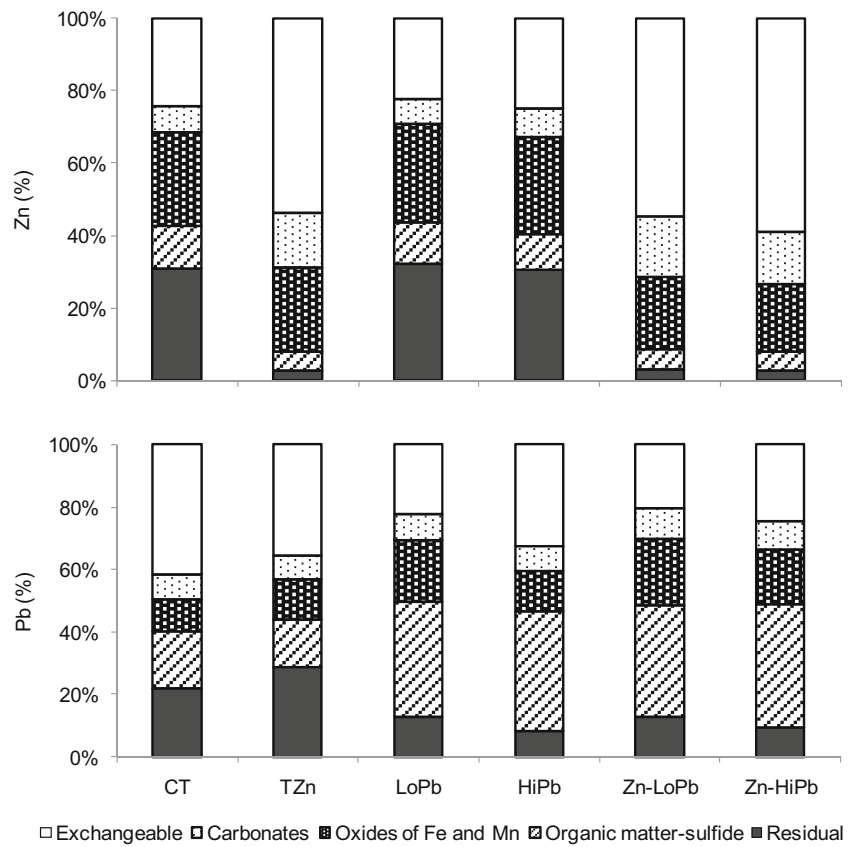
concentrations of carbonate and (bi)carbonate, can contribute to more alkaline conditions in water (Reid and Mosley 2016).

The distribution of metals in the different geochemical phases reflects their mobility, bioavailability, and their potential risk to the biota (Ashraf et al. 2012; Fan et al. 2014). The association of metals with a particular geochemical phase depends on several factors, such as their chemical affinity, physical and chemical conditions (pH, Eh, ionic strength), the supply of mineral fraction and organic matter offering binding sites, competence with other metals, kinetic of reactions in

Table 2 Zinc and Pb concentrations (μg/g air-dry sediment) for different sediment fractions

		CT	TZn	LoPb	HiPb	Zn-LoPb	Zn-HiPb
Zn	Exchangeable	25.1 ± 0.7 a	1095 ± 67 b	22.8 ± 1.0 a	28.5 ± 8.9 a	1021 ± 79 b	1134 ± 70 b
	Carbonate	7.5 ± 0.6 a	304 ± 23 b	7.1 ± 0.7 a	9.0 ± 3.0 a	307 ± 42 b	274 ± 45 b
	Fe and Mn oxides	27.0 ± 1.2 a	477 ± 26 b	27.9 ± 1.1 a	31.1 ± 7.7 a	373 ± 131 c	358 ± 20 c
	OM-sulfides	12.4 ± 1.2 a	105 ± 26 b	11.7 ± 1.1 a	11.4 ± 7.7 a	104 ± 39 b	100 ± 33 b
	Residual	32.2 ± 3.0 a	58.5 ± 0.4 b	33.1 ± 1.5 a	35.1 ± 5.4 a	60.0 ± 2.5 b	57.3 ± 2.5 b
Pb	Exchangeable	20.1 ± 0.7 a	20.1 ± 0.9 a	80.6 ± 6.0 b	469 ± 20 c	70 ± 20 b	304 ± 110 c
	Carbonate	3.9 ± 0.7 a	4.1 ± 0.5 a	31.4 ± 2.3 b	115 ± 24 c	33.1 ± 6.1 b	110 ± 13 c
	Fe and Mn oxides	5.0 ± 0.5 a	7.3 ± 0.7 a	70.4 ± 6.4 b	183 ± 95 c	73 ± 11 b	217 ± 110 c
	OM-sulfides	8.8 ± 0.4 a	8.6 ± 1.1 a	134 ± 6 b	551 ± 30 c	121 ± 14 b	480 ± 53 c
	Residual	10.6 ± 2.8 a	16.0 ± 4.7 a	47.0 ± 3.7 b	118 ± 14 c	43.8 ± 3.1 b	118 ± 12 c

Different letters denote significant differences between treatments for each fraction

Table 3 Net primary production (NPP) (g/m^2) at 63 days, zinc and lead concentrations in shoot, rhizome and root (μg/g) and bioconcentration factor (BCF: metal concentration ratio of plant roots to sediment) and translocation factor (TF: metal concentration ratio of plants shoots to roots) for each metal in *Schoenoplectus californicus*

	CT	TZn	LoPb	HiPb	Zn-LoPb	Zn-HiPb
NPP	208 ± 87 ab	271 ± 34 b	212 ± 55 ab	206 ± 24 a	203 ± 8 a	346 ± 16 c
Zn in root	66.0 ± 3.9 a	1594 ± 322 b	66 ± 25 a	53 ± 14 a	1814 ± 1023 b	2432 ± 415 b
Zn in rhizome	41 ± 18 a	207 ± 96 a	40 ± 19 a	33 ± 11 a	681 ± 171 b	435 ± 193 ab
Zn in shoot	26.7 ± 5.2 a	59 ± 13 b	38 ± 18 a	38 ± 19 a	56 ± 26 b	73 ± 22 b
BCF Zn	0.63 ± 0.05a	0.78 ± 0.16b	0.65 ± 0.26a	0.44 ± 0.01a	0.97 ± 0.54b	1.36 ± 0.10b
TF Zn	0.41 ± 0.10a	0.04 ± 0.02b	0.56 ± 0.09a	0.93 ± 0.70a	0.032 ± 0.008b	0.030 ± 0.004b
Pb in root	8.3 ± 3.0 a	31.8 ± 5.5 b	94 ± 36 c	425 ± 94 d	148 ± 16 c	597 ± 212 d
Pb in rhizome	4.6 ± 1.1 a	4.6 ± 2.5 a	13.4 ± 3.7 a	54 ± 30 b	22 ± 16 a	85 ± 39 b
Pb in shoot	5.3 ± 0.8 a	9.8 ± 1.0 ab	17.6 ± 2.7 c	12.5 ± 3.9 b	10.1 ± 2.9 b	12.8 ± 6.8 b
BCF Pb	0.17 ± 0.07a	0.57 ± 0.10b	0.26 ± 0.11a	0.31 ± 0.08a	0.44 ± 0.04b	0.57 ± 0.18b
TF Pb	0.68 ± 0.24a	0.32 ± 0.09b	0.21 ± 0.08c	0.04 ± 0.02d	0.07 ± 0.02c	0.03 ± 0.02d

Different letters denote significant differences between treatments

which they are involved, and the time of exposure (de Iorio 2010; Rendina and Fabrizio de Iorio 2012; Fan et al. 2014).

The association of Zn with the iron and manganese oxides of sediments has been widely recognized, showing that the Zn adsorption onto the oxides has high stability constants. Several authors (Shuman 1988; McBride 1989; Barrow 1993; Jain and Ram 1997; Kabata-Pendias and Mukherjee 2007; de Iorio 2010; Ashraf et al. 2012) denote the strong affinity of zinc for iron and manganese oxides, whose union was established by adsorption processes and occlusion. Also, zinc can be bound to the exchangeable fraction by means of electrostatic unions (Barak and Helmke 1993) as a function of its ionic potential. The addition of zinc produces an increase of its association with this fraction. The high CEC values found in these sediments could favor the adsorption of metals in the exchangeable fraction (Carrillo González et al. 2005). Twardowska and Kysiol (2002) found that zinc binds to organic matter mainly through electrostatic forces, indicating that an important fraction of the organic matter-bound metal is determined in the exchangeable fraction. The joint addition of both Zn and Pb resulted in a lower binding of Zn to iron and manganese oxides, in relation to the TZn treatment, which

could be explained by the greater affinity of Pb for amorphous Fe hydroxides than other metals like Zn (McBride 1989; Tessier 1992; Jain and Ram 1997; Abuchacra et al. 2015). The affinity of Pb for organic matter and for iron and manganese oxides of sediments has been widely recognized. Many studies have reported the preferential association of Pb with iron and manganese oxides due to the strength of covalent unions of the inner sphere complex formed with this fraction (McBride 1989; de Iorio 1999; Al-Degs et al. 2006; Rendina and Fabrizio de Iorio 2012). The high content of organic matter in this sediment explains the higher association of Pb with the organic matter-sulfide fraction (nearly 40%). The tendency for Pb to sorb in discrete patches onto the organic matter, where adsorption and surface precipitation processes are combined (Schneider et al. 2001), would confer more stability to these bonds. Therefore, modifications in Pb concentrations in all sediment fractions resulting from the addition of Zn were not observed. The prevailing association of Pb with organic matter-sulfide fraction could prevent the dispersal of this metal in the environment. Metals in the organic fraction are not considered to be very mobile or available due to their association with humic substances of high molecular weight

Table 4 Relative growth rate (RGR) (mg g^{-1} day^{-1}) for each treatment over the time

Days	CT	TZn	LoPb	HiPb	Zn-LoPb	Zn-HiPb
8	702 ± 615	943 ± 210	382 ± 661	742 ± 644	1279 ± 38	1198 ± 86
14	108 ± 95	289 ± 141	731 ± 811	113 ± 1549	15 ± 103	239 ± 56
22	601 ± 625	341 ± 87	495 ± 253	581 ± 586	186 ± 53	215 ± 41
36	120 ± 16	76 ± 20	96 ± 32	98 ± 40	71 ± 42	53 ± 15
47	31 ± 29	41 ± 5	31 ± 17	38 ± 19	32 ± 28	18 ± 5
56	17 ± 12	8 ± 11	26 ± 8	26 ± 13	19 ± 10	19 ± 3
63	12 ± 7	7 ± 7	11 ± 6	16 ± 12	13 ± 4	8 ± 7
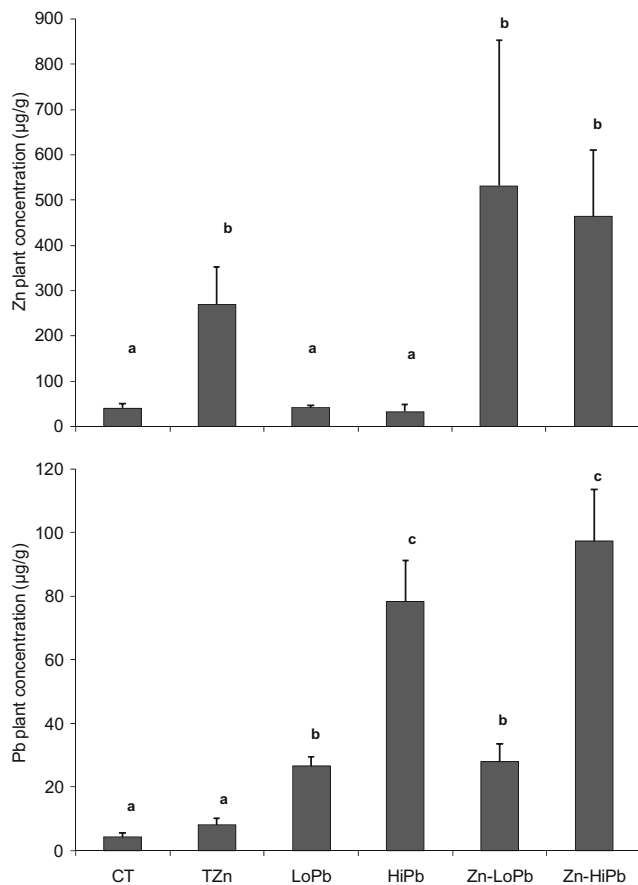
Fig. 3 Mean ± SD of Zn (**a**) and Pb (**b**) concentrations in plant (μg/g) for the different treatments. Different letters denote significant differences between treatments

(Tordoff et al. 2000; Rendina and Fabrizio de Iorio 2012; Abuchacra et al. 2015), so this favors the reestablishment of vegetation on contaminated sites.

Plant

Uptake of Zn and Pb by plants increased in the treatments contaminated with metals. The artificial enrichment of sediments with metals results in greater proportions of the metals in the most mobile fractions and with higher bioavailability compared to sediments without metal addition (McLaren and Clucas 2001; Kandpal et al. 2004). Nevertheless, the aging of contaminated sediments leads to the formation of more stable bonds and therefore their association to less mobile geochemical phases, usually decreasing their bioavailability over time (Zhong and Wang 2006; Fan et al. 2014). Although high levels of metals are reported in wetland sediments, their low bioavailability and limited transfer to the water column determine that macrophytes of the littoral zone typically exhibit relatively low concentrations of these contaminants (de Cabo et al. 2015).

The presence of high levels of Zn and Pb in sediment did not affect the growth of *S. californicus*. Both RGR and NPP did not show significant differences with respect to control.

Concentrations of Zn in the roots of *S. californicus* increased with the addition of this metal to the sediment, independently of Pb addition, and Zn concentrations in roots were higher than in rhizomes. MacFarlane and Burchett (2000) showed that the root endodermis of *Avicennia marina* acts as a barrier to prevent Zn intake, probably restricting symplastic transport and reducing its translocation to the shoot and rhizome. Under acidic or slightly acidic conditions, more salts are dissolved; even metallic oxides can be mobilized under reducing and acidic conditions (Ashraf et al. 2012). On the other hand, Armstrong (1967) reports that oxidation of the immediate root environment prevents the uptake of toxic components. Metals mobilized from the reduced sediments can precipitate with Fe and Mn (oxy)hydroxides on the root surface ("root plaque") (Gries et al. 1990), resulting in high root metal concentrations. Auguet et al. (2015) reported that roots of *Schoenoplectus californicus* plants grown in Zn-contaminated sediments show a visible iron plaque with high concentrations of immobilized Zn. Then, these mechanisms could act to immobilize the metal in belowground biomass and to prevent its translocation to aerial parts.

The bioconcentration factor is one of the most widely used factors for evaluating the metal uptake capacity of macrophytes (Olguín and Sánchez-Galván 2012). There is great variation among the reported BCF values for the different species of emergent macrophytes and heavy metals, especially related to its essentiality. Also, several forms to calculate BCF have been used in the bibliography. The BCF derives from the ratio of metal concentration in plant biomass or tissue to metal concentration in the substrate (sediment or water) depending on which is the main structure of accumulation in the plant and the growth substrate (Yoon et al. 2006; Zhuang et al. 2007; Ladislas et al. 2012; Ali et al. 2013; Mendoza et al. 2015; Oyuela Leguizamo et al. 2016; Türker et al. 2016; Bonanno et al. 2017). Due to emergent macrophytes that mainly accumulate metals in roots (de Cabo et al. 2015) and in all cases TF was lower than unity and more than 99% of added metals were associated to sediments, the BCF was calculated in this work as the ratio of metal concentration in roots to the concentration of metal in sediment. For zinc, the BCF values obtained in this study increased when the Zn concentration of the sediment also increased, and it was always close to unity. Other emergent macrophytes living in a wide range of Zn sediment concentrations—from 28.5 to 5400 μg Zn/g—also show similar behavior (de Cabo et al. 2015).

The zinc concentrations in rhizomes in the treatments with addition of both metals were higher than the concentrations resulting from the rest of the treatments. It is likely that high levels of Pb in roots compete with Zn for binding sites and

favor the Zn accumulation in rhizomes, not being observed this effect on shoots.

Although the addition of Zn increased its concentration in shoots, these concentrations did not reach the toxic level estimated by Deng et al. (2004) for aboveground biomass in wetland plants.

Concentrations of Pb in the roots of *S. californicus* increased with the addition of metal to the sediment and did not observe interaction effect when both metals were added. However, the concentration of Pb in rhizomes only increased in treatments with high doses of this metal, a feature associated with the restricted intake of nonessential metals in organisms (MacFarlane and Burchett 2000). On the other hand, Silver (1996) supports that a specific mechanism of avoiding nonessential metal absorption results in a higher metabolic penalty than efflux systems. *S. californicus* has developed strategies for Pb exclusion from aerial portions, retaining it in roots and allowing it to migrate to rhizomes only when surrounding concentrations are extremely high (HiPb and ZnHiPb). Furthermore, physiological adaptations also control toxic metal accumulations by sequestering metals in the roots (Guilizzoni 1991), preventing its translocation to the aerial biomass. In the same way, Auguet (2014) described the rhizodermis and central cylinder, in particular the Caspary strip, as preferential tissues of Pb accumulation in the roots of *S. californicus* exposed to high concentration of Pb. Our results point to a tolerance strategy in the presence of high concentrations of Pb in sediment limiting the movement of this metal into the plant, as was reflected by the reduction of TF in treatments with the metal addition. Usually, wetland plants are not hyperaccumulators and, in any case, the harvest of plants, including rooted plants, would be destructive to wetlands. Therefore, for wetland plants, storing metals belowground is the preferred alternative (Weis and Weis 2004).

For nonessential metals like Pb, the BCF values reported are generally lower than those for essential ones over a wide range of concentrations in the sediment—from 10 to 4100 µg Pb/g—(de Cabo et al. 2015). In this study although the BCF of Pb was always lower than those of Zn, unexpectedly, it only increased when zinc was added, even in the treatment in which only zinc was added. Since doses of added Zn were very high, this metal could act displacing Pb of binding sites of the TZn sediment favoring their mobilization and subsequent uptake.

Although the Zn and Pb concentration levels in roots were high, they were not sufficient to reduce biomass growth in plants. *S. californicus* might use metal detoxification mechanisms (Clemens et al. 2002; Hall 2002; Cheng et al. 2012; Lewis et al. 2001). All of these processes might be possible in an environment in which there is no restriction for nutrients, as in this assay, and they offer the possibility of a good development of biomass. High tolerance to Pb and Zn, good adaptation to wetland conditions, and wide distribution in these environments make *S. californicus* an excellent case study for metal tolerance mechanisms.

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