Effects of Zinc on the Growth of Bulrush (*Schoenoplectus californicus*) and Its Distribution Between Different Sediment Fractions

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During the last decades the discharge of heavy metals into water bodies has increased dramatically. There, they interact with organic and inorganic ligands thus precipitating and accumulating in the sediments; mainly adsorbed on the fine grain components (Greger and Kautsky 1991). The sediments of the littoral zone represent a sink of heavy metals, which in certain conditions might be released or recycled by the aquatic macrophytes. A portion of the total content of the metal in the sediment may not be easily available to plants (for example: that bound to carbonates, organic matter or sulphurs, or occluded in iron and manganese oxides). However, changes in the physical or chemical environment of the sediment can increase metal availability (Jackson et al. 1993). It is a well known fact that wetlands have an important nutrient retention capacity that contributes to attenuate the impact of anthropogenic activities on the aquatic environments. As a consequence, wetlands have been considered as an alternative way for the treatment of wastes from different sources. In spite of the extensive knowledge of many aspects of the behavior of nutrients in wetlands, information on the behavior, retention, final destination, and bioaccumulation of metals is less well known (Masscheleyn et al. 1992). The potential use of this approach in our region would require, as a previous condition, the identification of those macrophytes presenting suitable ecophysiological characteristics to be effectively applied.

The water bodies of the industrial belt arround Buenos Aires receive high inputs of contaminants, resulting in high concentrations of metals in their waters (García et al. 1998). *Schoenoplectus californicus* is an elongated, slender (2-3 m height and 1-2 cm diameter) and rhizotomas emergent macrophyte indigenous from South America through Central America to southern USA (Lange et al. 1998) which attains dense stands in muddy river banks and in coastal areas of lakes and estuaries. Its presence in the margins of rivers and channels where metal concentrations are high in Buenos Aires suggests a great tolerance to metals, the main local xenobiotic agents. The present contribution aims at assessing zinc toxicity to *S. californicus* under laboratory conditions, by evaluating its tolerance, effects on growth, absorption, metal distribution in shoot and rhizomes and in different sediment fractions.

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MATERIALS AND METHODS

The experiment was carried out from February to May 1996 (12 weeks). Sediments and rhizomes of *S. californicus* were collected from an area of comparatively low metal content within the suburban basins surrounding Buenos Aires city. Two treatments with different doses of zinc (treatment 1: 500 µg Zn g^{-1} dry sediment and treatment 2: 2500 µg Zn g^{-1} dry sediment) were assayed and compared with a control without zinc addition. Three kilograms of dry sediment were placed in plastic containers (21-cm diameter and 21 cm deep) and mixed with a known quantity of zinc chloride and water in order to obtain a homogeneous mixture of sediment, metal and water. Several rhizomes containing ten buds were then transplanted into each plastic container. A constant 10-cm water layer was maintained throughout the experiment with rainwater. All the treatments received natural light amounting roughly 13 hours of light per day. All three treatments were carried out in triplicate. The height of each shoot was measured weekly. Shoot heights were converted to shoot biomass using a height-weight curve obtained at the end of the experiment by harvesting the shoots and drying them at 70°C to constant weights. Total shoot biomass per pot was calculated as the sum of shoot mass in each pot.

Water conductivity and pH, and sediment pH were measured four times throughout the experiment (2nd, 3rd, 5th and 12th week). The floodwater in each container was sampled at the end of the experiment for zinc, soluble reactive phosphorus (SRP), bicarbonate, chloride and calcium determinations. The SRP concentration was determined using ascorbic molybdate, bicarbonate by helianthine titration, chlorides by silver nitrate titration, and calcium by EDTA titration, according to APHA (1992). The zinc content in water was determined through acid mineralization and atomic absorption spectrophotometry, using a Buck Scientific 210 VGP device.

Sediment samples were taken by duplicate at the end of the experiment and kept in the freezer until analysis. They were then conditioned to determine the zinc bound to the following sediment fractions: exchangeable, carbonates, iron and manganese oxides, organic matter and residual, according to the sequential technique proposed by Tessier et al. (1979). This method consists of sequential extractions with NaOAc at pH 8.2; NaOAc at pH 5, HOAc; NH$_2$OH.HCl 25% (v/v) of HOAc; HNO$_3$, H$_2$O$_2$, and NH$_4$OAc; HF-HClO$_4$, respectively.

The dry shoot and rhizomes were washed with water and EDTA 0.01M after removing from the sediments and digested in concentrated nitric acid, concentrated perchloric acid and H$_2$O$_2$ and their zinc content were determined by atomic absorption spectrophotometry, using a Buck Scientific 210 VGP device. Total phosphorus in dry shoot was determined according to Mackereth et al. (1978), after ignition.

Zinc content in water, plant tissue and different sediment fractions of each treatment were compared by means of one-way analysis of variance (ANOVA).

The same analysis was used to compare the chemical variables in the floodwater of each treatment. Total shoot biomass per pot over time in each treatment was compared by means of analysis of covariance (ANCOVA). Tuckey HSD test was used to reveal which treatments were significantly different. All variables were tested for normality and homogeneity of variances using the Lilliefors and Levene´s tests, respectively. Data, which did not conform to the assumption of normality and homogeneity of variances, were transformed to logarithms (Sokal and Rohlf 1969).

RESULTS AND DISCUSSION

Total shoot biomass per pot throughout the experiment is shown in Figure 1. Total shoot biomass showed significant differences over time between control and treatment 2 and between treatments 1 and 2 (***P<0.001). Treatment 2 showed very poor growth without any measurable increase in biomass since the 4th week. These shoots showed loops in the apical zone, similarly as observed by Fergusson (1990) for cadmium toxicity. Mean zinc concentrations in shoots were 5 and 23 times higher in treatments 1 and 2 than in the control (Table 1). Differences were significant to a P=0.054. Our results are consistent with the range of toxicity (300 - 1000 µg Zn g^{-1} dry plant) suggested by Chaney (1993).

The zinc content in rhizomes increased with the zinc dose, obtaining significant differences among treatments (**P<0.01), but this increase was not proportional. Zinc concentrations in rhizome were 4 times higher than in shoots in the control and 2 times higher in treatments 1 and 2 (Table 1). These results suggest an increasing translocation from rhizome to shoots at high zinc levels.

Table 1. Concentrations of zinc and phosphorus in plant tissue in each treatment (mean ± SD; n=3). The same letter denotes not significant differences between treatments (P<0.05).

	Control	Treatment 1	Treatment 2
Total Zn in shoot (mg Zn kg^{-1} dry shoot)	38 ± 6 a	210 ± 70 a	881 ± 599 a
Total Zn in rhizome (mg Zn kg^{-1} dry rhiz.)	148 ± 24 a	430 ± 102 b	1647 ± 506 c
Total P in shoot (mg P kg^{-1} dry shoot)	1609 ± 85 a	1522 ± 117 a	1688 ± 476 a

Rooted emergent macrophytes incorporate metals mainly from the sediment (Agami and Waisel 1986). The absorption of metals by plants is associated not only with their total quantity within the sediments but also with their bioavailability. However, increasing doses of zinc in the sediment resulted in an increase of roughly the same magnitude in shoot concentrations. High concentrations in shoots may result in decreased incorporation of iron, which in turn can interfere in the synthesis of chlorophyll, thus originating chlorosis and reduced growth (Chaney 1993). Boawn and Rasmussen (1971) showed lower shoot phosphorus content in plants that suffered phytotoxicity. On the contrary, our results show that the high zinc concentrations in shoots did not seem to affect the phosphorus uptake and translocation to the shoots, since the phosphorus levels in shoots were not significantly different (Table 1).
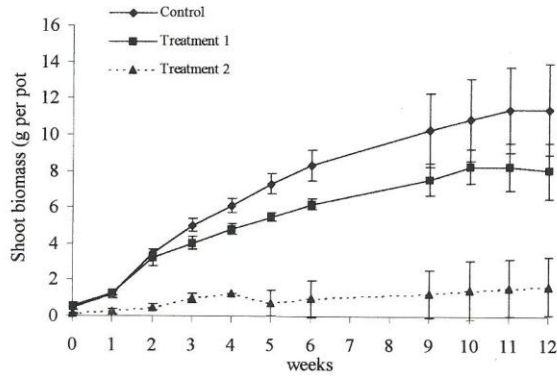
Figure 1. Shoot biomass of *Schoenoplectus californicus* in each treatment throughout the experiment. Error bars represent standard deviation.

The levels of soluble reactive phosphorus and bicarbonates in water (Table 2) were lower at the highest zinc level (treatment 2) (*$P<0.05$), whereas the concentrations of calcium and chlorides significantly increased as the zinc content in water increased (***$P<0.001$). Sediment pH decreased (*$P<0.05$) and conductivity increased (***$P<0.001$) at the highest zinc level. This trend was followed throughout the experiment (data not shown). The levels of chlorides in water increased together with zinc addition because of the chemical form supplied ($ZnCl_2$). Similarly, higher water conductivities would mainly be determined by the increasing levels of Ca^{+2}, Zn^{+2} and Cl^- in water.

Table 2. Physicochemical variables measured in the floodwater at the end of the experiment, (mean ± SD; n=3). The same letter denotes not significant differences ($P<0.05$).

	Control	Treatment 1	Treatment 2
pH (sediment)	7.75 ± 0.13 a	7.77 ± 0.06 a	7.35 ± 0.23 b
pH (water)	7.91 ± 0.05 a	7.96 ± 0.11 a	7.62 ± 0.31 a
Conductivity (water) (mS cm^{-1})	0.84 ± 0.25 a	1.19 ± 0.06 a	3.41 ± 0.20 b
Total Zn in water (mg Zn L^{-1})	0.46 ± 0.16 a	0.55 ± 0.07 b	10.94 ± 2.26 c
SRP (µg l^{-1})	283 ± 126 a	258 ± 76 ab	44 ± 9 b
Cl$^-$ (mg l^{-1})	36 ± 19 a	227 ± 6 b	1115 ± 30 c
HCO$_3^-$ (mg l^{-1})	500 ± 85 a	368 ± 74 a	156 ± 70 b
Ca^{+2} (mg l^{-1})	71 ± 3 a	125 ± 12 b	429 ± 24 c

The concentrations of zinc in the different sediment fractions (Table 3) were higher than in the control. The relative constancy of the zinc concentration in the residual fraction may be attributed to the fact that this metal forms part of highly resistant crystalline nets of minerals (Tessier et al. 1979). The added zinc was mainly retained in the fractions associated to carbonates and to iron and manganese oxides. These fractions represent 37 and 27% of the total in treatment 1, and 32 and 49% of the total in treatment 2, as compared to 17 and 38% of the total in the control (Figure 2). Since zinc was added as a soluble salt ($ZnCl_2$), its retention in the sediment carbonate system should follow the precipitation of zinc carbonate, consistent with the bicarbonate decrease in floodwater and the subsequent pH decrease (Table 2). It seems likely that the high amounts of soluble zinc added in both treatments compete with calcium for the exchange sites within the sediments causing desorption of calcium to the water as evidenced by the higher calcium concentration in floodwater at the end of the experiment. The significant decrease in dissolved phosphates as zinc concentrations in water increased, might be due to coprecipitation with zinc carbonate.

At higher concentrations of zinc, the association of this metal with the organic matter increased owing to the formation of complexes. Higher concentrations of zinc determine an increase in the exchangeable fraction, by the exchange of Ca^{+2} by Zn^{+2}, or because of zinc capacity as a function of its ionic potential to become adsorbed to the sediment by means of electrostatic unions (Barak and Helmke 1993).

Table 3. Mean and range of total zinc content and its distribution within sediment fractions (mg Zn kg^{-1} dry sediment) (n=2).

	Control		Treatment 1		Treatment 2	
	Mean	Range	Mean	Range	Mean	Range
Exchangeable	6.2	(5.8-6.7)	18.1	(14.5-21.8)	78.7	(81.4-76.0)
Carbonate	54.3	(54.0-54.5)	209.2	(159.2-259.1)	624.8	(608.7-640.8)
Organic matter	39.8	(36.8-42.8)	78.5	(75.2-81.7)	146.4	(137.8-155.0)
Fe and Mn oxides	125.7	(121.0-130.4)	149.6	(125.7-173.5)	948.2	(898.3-998.2)
Residual	102.0	(99.8-104.2)	105.9	(99.0-112.8)	148.5	(145.1-151.9)
Total Zn in sediment (sum of fractions)	328.1	(324.9-331.3)	561.2	(473.5-648.9)	1946.6	(1872.7-2020.6)

Because of their wide distribution and its tolerance to high zinc levels, *S. californicus* can be considered as a promising organism to be used in constructed wetlands for effluent treatment. Although zinc was incorporated in the form of a soluble salt, only 0.05% of the added metal finally remained in water. Therefore, wetlands of *S. californicus* would very efficiently improve the water quality of effluents and/or contaminated water courses. Natural *S. californicus* stands retain metals mainly in the sediments (Villar et al. 1999a). Coastal marshes typically attain an above ground biomass 2-3 kg dry weight per sq meter (Villar et al. 1996) and a
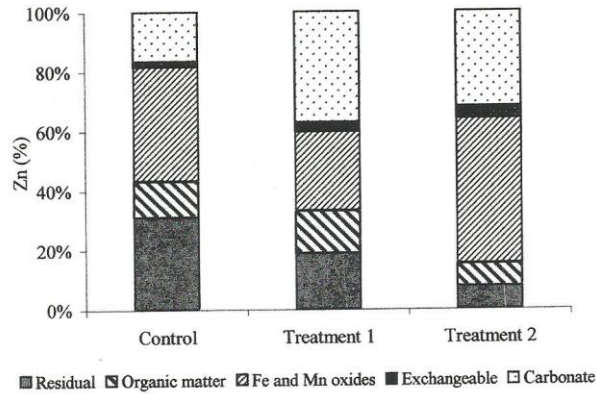
Figure 2. Mean relative content of zinc in each sediment fractions.

below ground biomass of 15 kg dry weight per sq meter (Villar et al. 1999b). Since high zinc concentrations may be attained in shoots (210 $\mu g\ g^{-1}$, treatment 1) and rhizomes (430 $\mu g\ g^{-1}$, treatment 1) without any discernible effect in the biomass, constructed wetlands would also retain high amounts of zinc in plant tissue.

Since zinc quantities in the aerial part of the plant were proportional to the sediment concentration, *S. californicus* may also be used as a good indicator of zinc contamination.

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