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Copper, zinc and lead speciation in salt marsh sediments colonised by *Halimione portulacoides* and *Spartina maritima*

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Abstract

Total concentrations and fractionation of Cu, Zn and Pb in seven operationally defined phases (exchangeable, carbonates, manganese oxides, organic complexes, amorphous iron oxides, crystalline iron oxides and residual) were determined in sediments colonised by the halophyte species *Halimione portulacoides* and *Spartina maritima* in a Tagus estuary salt marsh (Portugal). We aimed to determine whether the speciation of these metals was different in areas colonised by each halophyte. Higher concentrations of Cu, Zn and, in particular Pb, were found in the rhizosphere of *S. maritima* than in the root sediments of *H. portulacoides*. Geochemical fractionation of Cu, Zn and Pb in sediments of the salt marsh depended upon the metal, and for Zn and Pb clearly varied with depth and with the colonising species. The higher redox potential observed in sediments colonised by *H. portulacoides* may in part explain the observed differences in the speciation of Cu, Zn and Pb.

Keywords: Bioavailability; *Halimione portulacoides*; Metal pollution; Salt marsh; *Spartina maritima*; Speciation

1. Introduction

Tagus estuary salt marshes are effective sinks of metals, as they incorporate high quantities of metals transported by tides in the sediments and, to a lesser extent, in the plants (Caçador et al., 1996a, 2000). When we consider the possible toxic effects of metals to the marsh ecosystem and also to human health, the total amount of metal is not as important as the chemical form that is present, responsible for the bioavailability to the plant uptake and consequently to the introduction in the food web. Factors affecting bioavailability of metals to the marsh plants include pH, redox potential, salinity, particle size and organic matter content (Alloway, 1990; Williams et al., 1994). Activity of plant roots can alter physical and chemical properties of the sediment, influencing geochemical fractionation of metals and thus availability to the plant (Caçador et al., 1996b, 1999; Doyle and Otte, 1997; Lacerda et al., 1997; Otero and Macías, 2002; Mucha et al., 2005). As a result, vegetated areas of the salt marsh concentrate more metals in sediments, at least in rooted depths, than non-vegetated areas (Caçador et al., 1996a,b; Doyle and Otte, 1997; Otero and Macías, 2002).

Speciation of metals in salt marsh sediments has been studied in several estuaries. Different geochemical associations of metals depending on metal nature, presence of roots, and local factors as sediment redox potential or grain size have been found (Allen et al., 1990; Caçador et al., 1996b; Mortimer and Rae, 2000; Otero et al., 2000; Sáenz et al., 2003; Almeida et al., 2004). For the Tagus estuary salt marshes it is known that sediments between the roots of the halophyte species *Spartina maritima* concentrate more metals than sediments between the roots of *Halimione portulacoides*, whereas in plant tissues the trend is the opposite (Caçador et al., 2000). These differences have interest for phytostabilisation of metals in polluted zones, as those plants with the ability to induce concentration of metals in the root sediments, and avoid the

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translocation to aboveground tissues are likely to contribute more effectively to the immobilisation of pollutant metals (Weis and Weis, 2004). However, it is essential to know the geochemical form of metals in the sediment colonised by these plant species to evaluate their utility to reduce the availability to the marsh ecosystem. In the present study we analyse the fractionation of Cu, Zn and Pb in sediments colonised by these two species in order to determine whether the higher concentrations of metals observed in the rhizosphere of *S. maritima* correspond to less available geochemical fractions than between the roots of *H. portulacoides* or not. This would let us know which salt marsh zones, in terms of colonising species, contribute more effectively to the phytostabilisation of metal pollutants in the sediment, making them less available to the marsh system.

2. Materials and methods

2.1. Site description

The Tagus estuary is situated on the Atlantic coast of Portugal, covering an area of about 320 km^2 at extreme high tide. The southern and eastern parts of the estuary contain extensive intertidal mudflat areas colonised by halophyte plant communities dominated by *S. maritima* and *H. portulacoides*. Homogeneous stands of *S. maritima* colonise the muddy low marsh, whereas *H. portulacoides* occupies the banks of creeks. The study was carried out in Corroios salt marsh (Fig. 1). This site experiences two tidal flushing a day. The salt marsh sediments consist mainly of silt (60%) and clays (38%) at all plant areas (Caçador et al., 2000), i.e., the studied plants grow at sediment with similar grain size. Sediments colonised by *S. maritima* present higher organic matter content (18–21% LOI) than sediments colonised by *H. portulacoides* (16–18% LOI) (Caçador et al., 2000). The vertical profile of total C in areas colonised by both species is similar (Caçador et al., 2004).

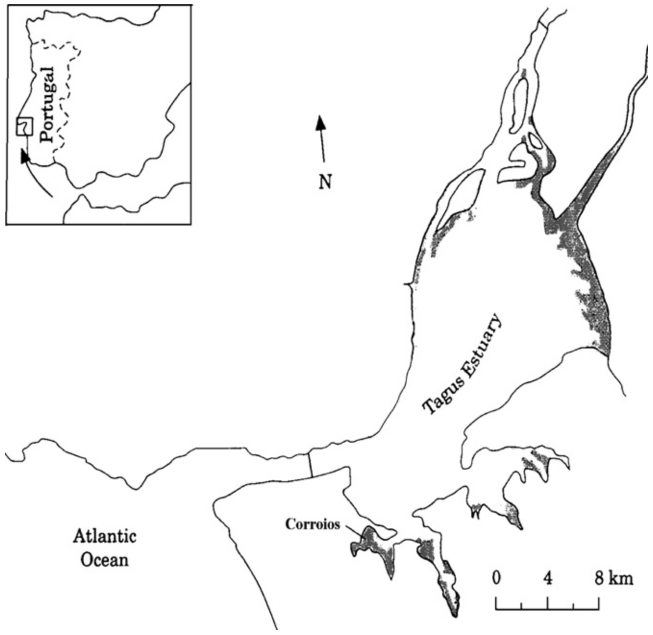

Fig. 1. Location of Corroios salt marsh in the Tagus estuary (Portugal).

2.2. Sampling

In September 2003 four sediment cores – 7 cm diameter, 60 cm depth – were collected from areas of the Corroios salt marsh, Tagus estuary, colonised by the halophytes *H. portulacoides* and *S. maritima*, respectively. Redox potential and pH of the sediment under the plant were measured *in situ* using a portable Crison pH/mV meter with a platinum electrode. Values were accepted after a 15 min period of stabilisation.

2.3. Analysis of samples

Once in the laboratory sediment was separated in 5 cm slices. One of the cores collected for each species was used to determine the depth containing the highest biomass. This was determined separating live root material from sediment under a water jet, using a sieve, and then drying to constant weight at 80 °C. For the rest three corers, sediment from the surface layer, the intermediate layer containing highest root biomass and the deepest layer, was dried to constant weight at 80 °C. Once dried, roots were removed and sediment was ground and homogenised.

For determining Cu, Zn and Pb chemical associations, 1 g of sample was subjected to the sequential extraction described in Forster (1995). This consisted of successive extractions of soil samples with the following solutions: 1 M ammonium nitrate (>99%, pa) (exchangeable fraction), 1 M ammonium acetate pH 6 (98%, pa) (carbonate-bound fraction), 0.1 M hydroxylamine hydrochloride (98%, pa) + 1 M ammonium acetate pH 6 (manganese oxides-bound), 0.025 M NH_4–EDTA pH 4.6 (99.4–100.5%) (organic complexes-bound fraction), 0.2 M ammonium oxalate pH 3.25 (99.5–101%) (amorphous Fe oxides-bound fraction), 0.1 M ascorbic acid (99.0%, pa) + 0.25 M ammonium oxalate pH 3.25 (crystalline Fe oxides-bound fraction), concentrated perchloric acid (pa) + nitric acid (65%, pa) (residual fraction).

Concentrations of Cu, Zn and Pb in these fractions were determined by graphite furnace atomic absorption spectroscopy (GFAAS) using a GBC 932 plus Atomic absorption Spectrometer with GBC PAL 3000 Autosampler and GBC GF 3000 Grafite Furnace system. All elements were measured in the absorbance and concentration calibration least squares mode using wall atomisation Zeeman background correction. Samples were analysed at $\lambda = 307.6$ nm, $\lambda = 324.7$ nm and $\lambda = 283.3$ nm for Zn, Cu and Pb respectively, using PHOTRON Graphite Furnace tubes, and a $NH_4H_2PO_4$–$Mg(NO_3)_2$ matrix modifier. Each sample was run in triplicate to guarantee that the measured absorbances were constant. After 10 samples a rescald of the highest standard for the calibaration curve was run automatically. Accuracy was assessed by the analysis of EU sludge reference material (CRM 145 and CRM 146). A total injection volume of 20 μl was used. After the determination of one element had been completed, the tube was cleaned at 2200 °C. Instrument-grade compressed Argon (BOC Gases) was used as sheath gas. Metal concentrations calculated from each replicate absorbance value, was then used to calculate an average metal concentration. Total concentrations of Cu, Zn and Pb were calculated as the sum of the seven fractions.

2.4. Statistical analysis

Statistical differences in root biomass, pH and Eh between species and between sediment depth was determined using two-way analysis of variance (ANOVA) (significance 0.05). Concentrations of Cu, Zn and Pb in the seven chemical fractions as well as total were tested for significant differences with depth and between species using two-way ANOVA (significance 0.05). Subsequent Newman–Keuls multiple comparison of means test was used when significant differences between groups were found. Normality and homogeneity of data were tested using Kolmogorov–Smirnov and Cochran–Hartley–Bartlett tests, respectively.

3. Results and discussion

3.1. Root biomass

Maximum values of root biomass in sediments colonised by *S. maritima* and *H. portulacoides* were found at 15 cm depth. In the case of *S. maritima* a second maximum appeared at 45 cm, which could not be explained (Fig. 2). Root biomass of *H. portulacoides* was higher than root biomass of *S. maritima* (51.8% difference at 15 cm), as previously found by Caçador et al. (2000).

3.2. Sediment characteristics

Redox potential (Eh) and pH values varied inversely with depth in sediments colonised by *H. portulacoides*. Variations seemed to be related to variations in root biomass, as a maximum in Eh and a minimum in pH were found at 15 cm (maximum root biomass). Therefore, sediment layers containing higher root biomass were more acidic and more oxidative than those containing lower root biomass, as a consequence of oxygen liberation and ions exchange. Values of pH varied little with depth in sediments colonised by *S. maritima* ($p > 0.05$), whereas Eh values noticeably decreased ($p < 0.05$).

Negative Eh was only found in sediments colonised by *H. portulacoides* between 35 and 45 cm depth. Sediment between the roots of *H. portulacoides* was more oxidative in the surface layers (5–25 cm) ($p = 0.050$) than sediment between the roots of *S. maritima*, probably influenced by their different locations in the salt marsh (*H. portulacoides* high marsh and *S. maritima* low marsh). Below 25 cm the trend was the opposite, i.e., Eh values were higher in areas colonised by *S. maritima* than in those areas colonised by *H. portulacoides*, although it was not significant ($p = 0.116$). Differences in sediment pH between the two species were only significant between 15 and 20 cm depth ($p < 0.05$), i.e., where root biomass was highest. There was an increase in Eh values in the deepest sediment layers colonised by both species that could not be explained by an increase in root biomass.

3.3. Total metal concentrations in sediment

Total concentrations of Cu, Zn and Pb varied with sediment depth and with the colonising species (Fig. 3). Zn was the most concentrated metal ($p < 0.05$) in sediments colonised by both species. Copper and Pb were the least concentrated metal in sediments colonised by *S. maritima* and *H. portulacoides*, respectively ($p < 0.05$). Higher concentrations of Cu, Zn and, in particular Pb, were found in sediments covered by *S. maritima* than in sediments covered by *H. portulacoides* (Fig. 3, Table 1). The 10–15 cm layer (highest root biomass) showed enrichment in metals when compared to other layers (post hoc Newman–Keuls test), except for Cu, which showed similar concentration at 0–5 cm and 10–15 cm depth. The metal enrichment of the intermediate layer (10–15 cm) was expected as this contained the highest root biomass, and thus the alteration of chemical properties of the sediment by the plant is

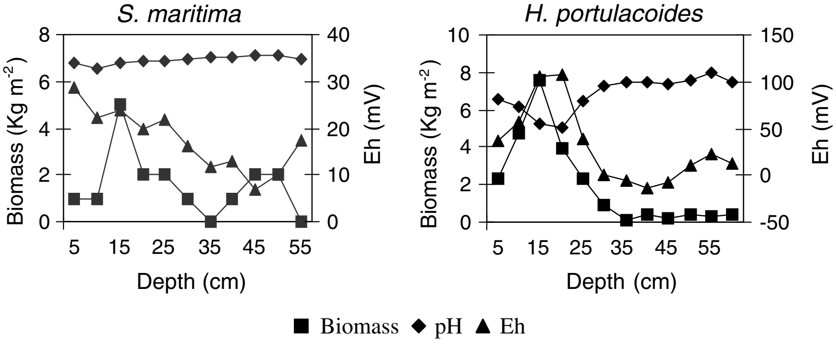

Fig. 2. Vertical profile of biomass (Kg m^{-2}), pH and Eh (mV) in sediments colonised by *Spartina maritima* and *Halimione portulacoides*.

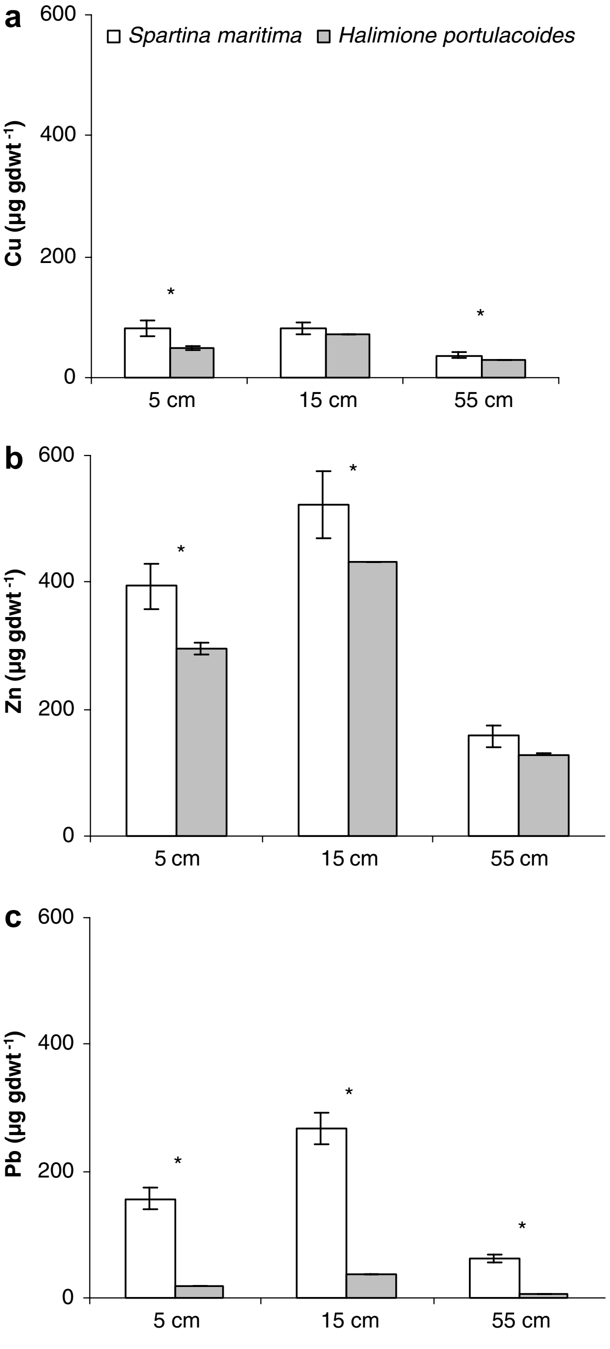

Fig. 3. Total concentrations of Cu, Zn and Pb (µg gdw^{-1}) at surface, root layer and deep layer in sediment colonised by *Spartina maritima* and *Halimione portulacoides*. Error bars denote standard deviation.

expected to be highest (Williams et al., 1994). These results are comparable with those reported in Caçador et al. (1996a,b) for vegetated zones of this salt marsh.

3.4. Speciation of metals in sediments

Table 1
Results of two-way ANOVA for total metal concentration in sediments colonised by *H. portulacoides* and *S. maritima*

Metal	Effect	*F*-value	*p*-Value
Cu	Depth	63.60	<0.001*
	Species	26.28	<0.001*
	Interaction	6.89	0.010*
Zn	Depth	231.33	<0.001*
	Species	32.83	<0.001*
	Interaction	3.07	0.083
Pb	Depth	138.07	<0.001*
	Species	583.22	<0.001*
	Interaction	73.29	<0.001*

* Indicate significant difference at $\alpha < 0.05$.

Chemical associations of Cu, Zn and Pb to the operationally defined fractions 'exchangeable', 'carbonates', 'Mn oxides', 'organic complexes', 'amorphous Fe oxides', 'crystalline Fe oxides' and 'residual' in sediments between the roots of *S. maritima* and *H. portulacoides* are shown in Fig. 4. Most fractions analysed revealed significant differences in concentration between areas colonised by different species and between sediment layers (two-way ANOVA, Table 2).

Copper was mainly bound to the residual fraction (25.9–50.7%, the strongest association of the three metals) and to organic complexes (23.0–44.4%) (Fig. 4), the percentage bound to organic complexes been greater in the intermediate (root) layer (∼45%). However, the latter (organo-metallic complexes) are probably dissolved in

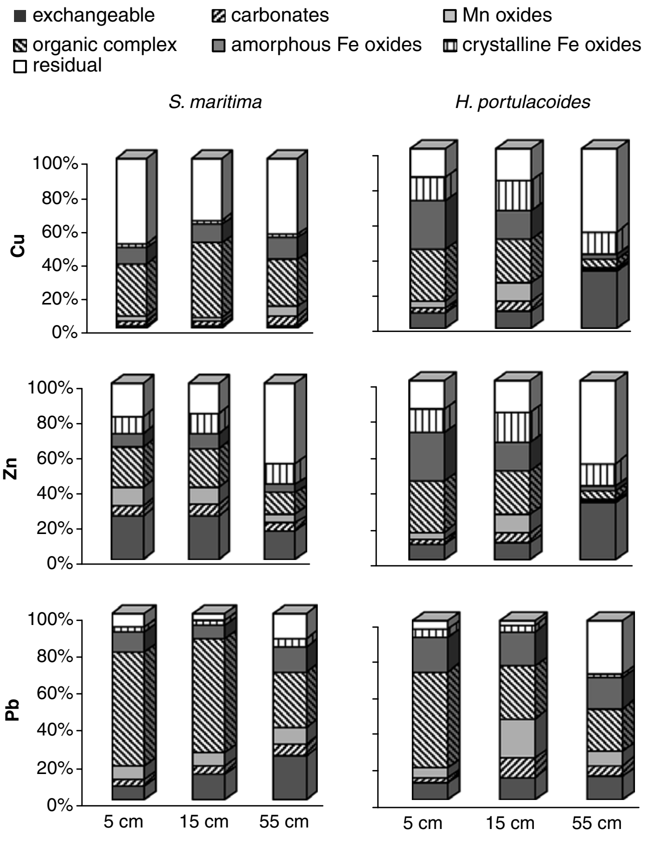

Fig. 4. Chemical associations of Cu, Zn and Pb at surface, root layer and deep layer in sediments colonised by *Spartina maritima* and *Halimione portulacoides*.

Table 2
Results of two-way ANOVA (*p*-value) for chemical associations of metals (concentration in different phases) in sediments colonised by *H. portulacoides* and *S. maritima*

Metal	Effect	Exchan.	Carbon.	Mn ox.	Org. comp.	Fe ox. (amorph.)	Fe ox. (cryst.)	Residual
Cu	Depth	<0.001*	0.764	0.004*	<0.001*	<0.001*	<0.001*	<0.001*
	Species	0.005*	0.913	0.143	<0.001*	0.191	0.792	<0.001*
	Interaction	0.247	0.101	0.104	0.065	0.293	0.344	<0.001*
Zn	Depth	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.006*
	Species	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.781	0.009*
	Interaction	<0.001*	–	<0.001*	<0.001*	<0.001*	–	–
Pb	Depth	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Species	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.526
	Interaction	<0.001*	0.002*	<0.001*	<0.001*	<0.001*	0.138	<0.001*

* Indicate significant difference at $\alpha < 0.05$.

previous steps of the sequential extraction procedure. The percentage of Cu associated to the exchangeable fraction was the lowest (1.0–2.2%). Fractionation was similar for both species, although the percentage bound to the residual fraction was higher in superficial sediments (0–5 cm and 10–15 cm) colonised by *S. maritima* (50.7% and 36.7%) than in those colonised by *H. portulacoides* (25.9% and 31.3%). Apparently Cu was the least available metal for plant uptake. However, both species are able to induce Cu mobilisation in sediments (ratio soil level/root < 1) (Reboredo, 1993). This ability seems to be stronger in *H. portulacoides*, since concentrations of metals (including Cu) are usually higher in roots of this species than in roots of *S. maritma* (Reboredo, 1993; Caçador et al., 1996a; Reboreda and Caçador, 2007).

Distribution of Zn in chemical phases was similar in the surface and root layers, which were different between the two areas (Fig. 4). In sediments colonised by *S. maritima* it was mainly bound to residual (~18%), organic complexes (~23%) and exchangeable fractions (~25%). When sediments were colonised by *H. portulacoides* Zn association to residual and organic complexes were also considerable (~17% and 26%), whereas exchangeable fraction was lower (~9%) than in *S. maritima* bearing sediments. Nevertheless, the Zn concentration in plant tissues of *H. portulacoides* is higher than in *S. maritima* (Caçador et al., 2000), in spite of the apparent lower availability for this species. Perhaps the higher uptake of Zn in *H. portulacoides* causes depletion of this element in the most available fraction (exchangeable) of the sediment between the roots of this species (Hinsinger, 2001). The main difference occurred for Fe oxides (amorphous and crystalline) bound fractions, much higher in sediments covered by *H. portulacoides* (~21% + 15%) than covered by *S. maritima* (~8% + 11%) (Fig. 4). This is in accordance with the higher redox potential found in areas covered by *H. portulacoides*, favouring the precipitation of Fe and Mn oxides (Lacerda et al., 1997). At the deepest layer (50–55 cm) the percentage associated to the residual fraction increased considerably in sediments covered by both species (up to 46%), whereas percentage associated to the exchangeable fraction increased under *H. portulacoides* (31.9%) and decreased under *S. maritima* (16.4%).

Lead was largely associated to organic complexes (50–60% in superficial sediments), reducing the percentage in this fraction with depth (20–30% at 50–55 cm) (Fig. 4). This metal is able to form stable complexes with organic ligands in the sediment (Raskin and Ensley, 2000). However, as already said for Cu, these organo-metallic complexes are probably dissolved in previous steps of the extraction. Percentages of Pb in this fraction were slightly higher in sediments colonised by *S. maritima* (29.1–61.6%) than in those sediments colonised by *H. portulacoides* (24.0–53.5%). This may be caused by different type or amount of organic exudates from the roots of these species, as composition of organic exudates under different vegetation can be different (Ryan et al., 2001). Lead bound to amorphous Fe oxides was substantial (7–19%), and for every depth considered, higher in sediments covered by *H. portulacoides* (17.1–19.0%) than in sediments covered by *S. maritima* (7.3–13.9%). At the root depth (10–15 cm) Mn oxides bound fraction was also noticeably higher in sediments covered by *H. portulacoides* (22%) than in sediments covered by *S. maritima* (7%). Again, as discussed for Zn, the differences in sediment redox potential under the two species could account for this association to Fe and Mn oxides. In addition, Mn oxides promote the degradation of organic complexes as humic substances and phenols (Tebo et al., 2004), and this probably contributes to the lower percentage of Pb bound to organic complexes in sediments between the roots of *H. portulacoides* compared to sediments between the roots of *S. maritima*.

Zinc and Pb were more bioavailable than Cu, as their percentages in the exchangeable phase were higher, although as already discussed both plant species are able to induce mobilisation of this metal. Copper and Pb showed more affinity for the organic phase than Zn, which could be due to the affinity of the latter to form complexes with chlorine ligands in the pore-water, remobilising Zn from organic complexes (Williams et al., 1994). These results differ from Mortimer and Rae (2000), who found very similar Zn and Cu fractionation in contrast with Pb and Cd fractionation.

The type of vegetal cover, i.e., the colonising plant species, affected chemical associations of Zn and Pb in the sediment, whereas apparently it did not have strong effect on Cu speciation. The higher redox potential observed in areas colonised by *H. portulacoides* may in part explain the observed differences in the speciation of Zn and Pb. Mortimer and Rae (2000) concluded that redox conditions in the salt marsh controlled the iron cycling (iron and manganese oxides) and this, in turn, controlled trace metal association. Our results also show a higher association of metals to Fe and Mn oxides in sediments with a higher redox potential, i.e., those colonised by *H. portulacoides*. Otero et al. (2000) also found different chemical associations of metals in salt marsh sediments under different redox conditions. Differences in redox characteristics of sediments between the roots of both species might be explained by two different reasons. On the one hand, the different position of these species on the salt marsh contributes to variations in chemical and physical properties between their roots, such as changes in the position of the redoxcline (limit between oxic and anoxic zone) due to different tidal immersion times. *S. maritima* is situated in the lower salt marsh and therefore immersion time of the sediment is higher than in *H. portulacoides*, limiting oxygen diffusion from the atmosphere. *H. portulacoides* establishes in this well aerated sediments because as a dicotyledon it has a poorly developed arenchyma, limiting the transport of oxygen from aerial parts to roots (Ingold and Havill, 1984; Rozema et al., 1985; Sánchez et al., 1998). *S. maritima* has a well developed arenchyma and therefore higher capacity to oxidise the rhizosphere, being able to colonise anoxic environments. On the other hand, sediment redox potential may be influenced by differences on the amount and chemical composition of root exudates of the two species.

Fractionation of Cu, Zn and Pb with depth presented some differences with the speciation reported in Caçador et al. (1996b) for the same salt marsh. Namely, they found association to residual fraction (less available) to be highest in the rooted sediments (composite samples of *S. maritima*, *H. portulacoides* and *Arthrocnemum fruticosum*), and these percentages of association were considerably higher than those reported here at 10–15 cm. This may be attributable to the use of composite samples instead of separated samples for different colonising species or to different sampling seasons in the two studies. Also, it should be taken into account that the extraction procedure was different. In the present study the percentage of Cu, Zn and Pb in the residual phase tended to increase with depth. Although a possible explanation might be the reductive reactions that lead to formation of metal sulphides and pyritic metals (Otero and Macías, 2002), these are likely to be oxidised in previous steps. Percentages of Cu, Zn and Pb bound to organic complexes decreased at 50–55 cm, as a possible consequence of degradation of organic matter in buried sediments (Allen et al., 1990). Affinity of metals to organic matter in the scavenging process (Panda et al., 1995) and association to organic matter derived from the plant may explain the higher percentages of metals bound to this phase in surface and root layers.

Thus, geochemical fractionation of Cu, Zn and Pb in sediments of Corroios colonised by *S. maritima* and *H. portulacoides* depended upon the metal, and for Zn and Pb clearly varied with depth and with the area of the salt marsh colonised by different species. Nevertheless, it could not be clearly elucidated which was the effect of the plant species alone. *H. portulacoides* seemed to have a more evident influence in chemical characteristics of the sediment than *S. maritima*, as revealed by the Eh/pH/biomass profiles with depth. Still, this was not reflected in a generalised greater (or lower) bioavailability of metals, which was quite metal specific: virtually no effect for Cu, lower bioavailability of Zn and higher bioavailability of Pb (at most rooted depth). Reboredo (1993) found that the percentage of Fe, Cu and Zn that was bidistilled water extractable (BWE) (minimum available for plant uptake) remained approximately constant in sediments colonised by these two species, regardless of total metal concentration and nature of the matrix (given by different sampling locations and different colonising species), except for one site, where differences in BWE levels were found between sediments colonised by both species. This suggests that not only the different positions of the species on the salt marsh account for the differences in bioavailability, but more factors are involved to cause differences in metal bioavailability. Maybe the influence of the plant on geochemical fractionation of metals is affected by variations in the physiological response of the plant to local factors, and thus different behaviours might occur for different salt marshes.

From the results observed in this study we concluded that, for this salt marsh, the considerable differences observed in Zn and Pb fractionation between sediments colonised by *S. maritima* and *H. portulacoides* could indicate that both species may influence the fractionation of these metals in different ways, which in addition seemed to be metal specific. These findings guarantee further research to elucidate to what extent the different positions occupied by these species in the salt marsh influence the differences in fractionation. A thorough study in different locations and salt marshes would be needed to achieve this. This would help us to determine which species and location is more appropriate to induce phytostabilisation of heavy metals in polluted salt marshes by immobilising them in low available chemical phases.

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