

Exudation of organic acids by a marsh plant and implications on trace metal availability in the rhizosphere of estuarine sediments

Ana P. Mucha^a, C. Marisa R. Almeida^a,
Adriano A. Bordalo^{a,b}, M. Teresa S.D. Vasconcelos^{a,c,*}

^a CIIMAR, Rua dos Bragas, 177, 4050-123 Porto, Portugal

^b Laboratory of Hydrobiology, Institute of Biomedical Sciences, University of Porto, Largo Professor Abel Salazar, n° 2, 4099-003 Porto, Portugal

^c Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007, Porto, Portugal

Received 5 January 2005; accepted 10 June 2005

Available online 26 July 2005

Abstract

The aim of this work was to identify a variety of low molecular weight organic acids exuded by the sea rush *Juncus maritimus* collected at two locations with different sediment characteristics (sandy and muddy) and to examine whether specific differences in physico-chemical sediment characteristics influenced plant exudation. Just after collection, plant roots were rinsed and put in contact with deionised water for 2 h. In the obtained solution the organic acids, exuded by the plants, were determined by high performance liquid chromatography. *Juncus maritimus* was shown to be capable of releasing malonate and oxalate. Sediments and rhizosediments (sediment in contact with the plant roots and rhizomes, corresponding to the area of higher belowground biomass) from the areas where the plants had been collected were characterised in terms of physical and chemical composition, including acid volatile sulphide and total-recoverable metals (Pb, Cr, Cu, Zn, Ni and Cd). It was found that the extent of exudation varied markedly between sites. The identified organic acids were used as extractants of metals from sediments and rhizosediments and the results were compared with those provided by a very commonly used sequential extraction approach, which was carried out in parallel. This work demonstrates that *J. maritimus* can release organic compounds that can act as complexing agents of trace metal and therefore organic exudates should be accounted for when dealing with estuarine environment quality.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: root exudates; organic acids; trace metals; *Juncus maritimus*; estuarine sediments

1. Introduction

In urban estuaries, salt marshes often receive variable pollutant loads, including discharges of trace metals from industrial and transportation activities. When accumulated in marsh sediments, metals can be adsorbed and absorbed by plant roots and translocated to their above-

ground biomass. Plant uptake of dissolved metals can reduce the input of metals into estuarine waters from sediments, and metal sequestration in plant tissues may provide a long term sink if metal-laden tissues are buried (Windham et al., 2003). The uptake of metals is affected by a number of environmental characteristics of sediments, namely the pH and redox potential (Burke et al., 2000).

The presence of vegetation and intense microbial activity at the root–sediment interface alters the concentration of O₂, CO₂ and organic matter (Otero and Macias, 2002), in such a way that those environments are

* Corresponding author. Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007, Porto, Portugal.

E-mail address: mtvascon@fc.up.pt (M.T.S.D. Vasconcelos).

considered as independent subsystems in the sediment, commonly referred as rhizosphere (Varenes, 2003). Differences in metal concentration and their chemical speciation in the vicinity of the roots of salt marsh plants have also been demonstrated (Caçador et al., 1996; Almeida et al., 2004).

The processes by which the plants modify metal speciation in the rhizosphere include mineral precipitation/dissolution (Sundby et al., 1998), changes in redox conditions (Madureira et al., 1997; Jacob and Otte, 2004a,b) and exudation of organic substances capable of complexing metals and changing their bioavailability (Parker et al., 2001; Berkelaar and Hale, 2003). This latter process is only well documented for plants with agricultural interest (Jones, 1998 and references therein). Organic acids, like malic, citric and oxalic acids, have been proposed to be involved in processes operating in the rhizosphere, including nutrient acquisition (Neumann et al., 1999), metal detoxification (Ma et al., 2001), alleviation of anaerobic stress in roots, mineral weathering and microbial attraction (Jones, 1998). Although it is assumed that salt marsh plants release exudates, knowledge about their nature, concentration and role on trace metals bioavailability in the rhizosphere is still scarce.

The aim of this work was to identify a variety of organic acids exuded by the sea rush *Juncus maritimus* and to examine whether specific differences in physico-chemical sediment characteristics influenced plant exudation. In addition, we studied the influence of the organic acids exuded by the plant on trace metal bioavailability in estuarine sediments, by using them as extractants, and the information provided was compared with that provided by a very commonly used sequential extraction (SE) procedure (Rauret et al., 1999), which was carried out in parallel.

2. Material and methods

2.1. Material and reagents

All reagents used were *pro analysis* grade or equivalent, except concentrated nitric acid, which was supra-pure. Filtered bi-deionised water (conductivity $<0.1 \mu\text{S cm}^{-1}$) was used.

Standard solutions were prepared daily from the stocks, in polyethylene tubes, by weighing.

To prevent contamination, all materials used for sampling and sample treatments and all labwares were soaked in 20% (v/v) nitric acid solution for at least 24 h, rinsed several times with deionised water and dried in a Class 100 laminar flow hood. To collect the samples only plastic devices were used. The sample manipulation was carried out in a clean room with Class 100 filtered air.

2.2. Samples collection

Juncus maritimus is widely distributed in estuarine salt marshes of the NW Portuguese coast. Samples were collected in July 2003 during low tide, in the lower mesotidal Douro estuary (41°N ; 08°W), at two sites with different sediment characteristics: one site located inside a small area of salt marsh, presenting high organic content muddy sediment, and the other located upstream the salt marsh, presenting low organic content sandy sediment. More detailed information on this estuary can be found elsewhere (Mucha et al., 2003a). Green plants without a senescent appearance and with similar size were collected at both sites, being carefully washed in seawater to remove debris and dead root and shoot material. Concomitantly, sediment and rhizosediment (sediment in contact with the plant roots and rhizomes, corresponding to the area of higher belowground biomass) were collected between 10 and 20 cm depth, which correspond to the roots depth. Each sample was individually packed in a plastic bag by using plastic shovels (rhizosediment and sediment) or plastic gloves (for plants) and carried to the laboratory within 30 min.

At the laboratory, plant roots were rinsed thoroughly with deionised water and then rinsed in deionised water containing the antimicrobial agent Micropur[®], to stop microbial degradation of the exudates during the collection. In preliminary tests, two antimicrobial agents, Micropur[®] and sodium hypochloride, were used in parallel, as well as rinsing only with deionised water. No significant differences were observed in the results obtained for plants washed with Micropur[®] and with only water, but sodium hypochloride significantly increased exudation, and was considered aggressive to plant roots. Despite the absence of differences for plants washed with deionised water and with Micropur[®], we decided to use the latter in order to assure the absence of microbial degradation.

2.3. Organic acids exudation

Six groups (3 replicates per site), each with 10 freshly collected plants, were prepared and the respective roots were immersed in 100 ml of deionised water for 2 h. This method has been recently used for collection of root exudates (e.g., Fan et al., 1997) from plants grown in nutrient solution, and allows the identification of possible exudates, but not the quantifications of exudation rates due to possible osmotic effects on the roots. The dry weight of the roots per group was determined as being 0.79 ± 0.20 g. The length of the exposure time was selected from a set of previous experiments with exposure times of 1, 2, 3 and 24 h. No significant differences were observed in the solution composition obtained after 1, 2 and 3 h of exposure, whereas for the 24 h experiment a marked decrease in the volume of solution was

observed, eventually due to solution evaporation and/or plant absorption/transpiration. In order to identify and quantify compounds exuded by the plant, the obtained solutions were immediately filtered through 0.45 μm cellulose nitrate membranes (Millipore) and analysed for a variety of organic acids (citric, acetic, formic, maleic, malic, malonic, oxalic and succinic acids). Aliquots of the filtered solutions were also acidified (1% nitric acid) for metal (Cd, Cu, Cr, Ni, Pb and Zn) determinations.

Low molecular weight organic acids exuded by the plants were determined by an Ion Chromatograph (DIONEX series 4000i) provided with a conductivity detector and equipped with a HPIC AG5A pre-column, a HPIC AS5A anion separator column and an AMMS anion micro-membrane suppressor (all from DIONEX). For analysis of acetic, formic, maleic, malic, malonic, oxalic and succinic acids a mixture of 2.8 mM sodium bicarbonate and 3.3 mM sodium carbonate was used as eluent, whereas for citric acid the eluent was 100 mM sodium hydroxide. The flow rate was 1 ml min⁻¹ and the injection loop 50 μl . The suppressor regeneration reservoir was pressurized with nitrogen at 0.5 atm. to maintain constant flow (ca. 5 ml min⁻¹) of regenerant (12 mM sulphuric acid). Measurements were made on the 30 $\mu\text{S cm}^{-1}$ scale.

2.4. Sediment characterization

Organic matter content (OM) was determined in dry sediments (at 60 °C) by loss on ignition (4 h at 500 °C). Grain size analysis was performed by wet (fraction <0.063 mm) and dry (other fractions) sieving (CISA Sieve Shaker Mod. R.P.08) of sediments previously treated with 30% hydrogen peroxide solution. The sediments were divided in seven fractions: silt and clay (<0.063 mm), very fine sand (0.063–0.125 mm), fine sand (0.125–0.25 mm), medium sand (0.25–0.5 mm), coarse sand (0.5–1 mm), very coarse sand (1–2 mm) and gravel (>2 mm). Each fraction was weighed and expressed as percentage of the total dry weight.

For the determination of total-recoverable metal content, sediments were first dried in an oven (at 30 °C) to constant weight. Sediments were then fractioned and homogenized by sieving through nylon net of 2 mm mesh, in order to remove large stones and dead roots. Aliquots of about 0.25 g were digested with concentrated nitric acid using a high-pressure microwave system (MLS-1200 Mega, Millestone) in closed PTFE vessels. This digestion, despite not providing a complete dissolution of the sample, has the advantage of being a strong acid digestion that dissolves all elements that could become “environmentally available”.

The method described by Allen et al. (1993) was used for extraction of acid volatile sulphide (AVS) and simultaneous extracted metals Cd, Cu, Ni, Pb and Zn

(SEM). The sulphide liberated was determined spectrophotometrically at a wavelength of 670 nm (Unicam). The acidified sediment suspension was filtered through a 0.45- μm pore size cellulose nitrate membrane before measurement of SEM in solution (see below).

The influence of the low molecular weight organic acids exuded by the plant on trace metal mobility and availability from sediments was estimated by using representative acids as extractants. For this purpose, aliquots of 0.5 g of sediments were treated with 20.0 ml of oxalic, malonic and citric acids individually, as well as a mixture (1:1:1) of the three acids (0.11 M total concentration in all cases). In all cases the final concentration of the extractant, 0.11 M, was the same as the acetic acid solution used in the first step of a very commonly used sequential extraction (SE) procedure (method established by the Measuring and Testing Program of the European Community Rauret et al., 1999), which was also carried out in the present study to estimate metal availability. Specifically, aliquots of 0.5 g of sediments were treated sequentially with: (1) 20.0 ml of 0.11 M acetic acid, pH = 2.6 (to extract the exchangeable and bound to carbonates fraction); (2) 20.0 ml of 0.5 M hydroxylammonium chloride, pH = 1.5 (metal fraction bound to Fe/Mn (hydr)oxides); (3) and 25.0 ml of 1.0 M ammonium acetate solution, pH = 2.0, after digestion with 30% hydrogen peroxide solution (metal fraction bound to organic matter and sulphide). Each soluble fraction was obtained after 16 h of extraction on a lateral shaker (JP Selecta) at 22.0 °C, being afterwards separated by centrifugation at 3000 rpm (JP Selecta, MIX TASEL). A fourth fraction, the residual fraction, was calculated as the difference between total-recoverable elemental concentrations (determined in a different portion of dry sample) and the sum of metal contents extracted in the three SE fractions. Recent studies (Wang et al., 2001; Baeyer et al., 2003) have pointed out that, for evaluating metal availability through SE, wet sediments were more suitable than dry ones. Therefore, a comparison of the results obtained for wet and dry sediments was carried out. Since no consistent conclusions could be drawn (results not shown), in the present study dry sediments were chosen.

2.5. Metal determinations

The concentrations of metals extracted from sediment, and in the exudate solutions, were analysed by atomic absorption spectrophotometry, either with flame atomisation (PU 9200X, Philips), or with electrothermal atomisation provided with a Zeeman background correction (4100 ZL, Perkin–Elmer coupled to an AS-70 auto sampler), depending on the metals' levels in the sample. Aqueous matched standards were used for external calibrations.

The reference material Estuarine Sediment BCR 277 certified for total metal content, and the reference

material BCR 701 certified for the extractable metal content in freshwater sediment following a sequential extraction procedure, both from the BCR registered trademark of Community Bureau of Reference, were also analysed to check the accuracy of both the extraction and analytical procedures. For BCR 277, statistically identical results were obtained for Cd, Ni, Pb and Zn and recovery percentages of 90% Cu and 80% Cr. Some lower values were expected as the total-recoverable metals instead of total contents were measured. For BCR 701 statistically identical results for Cd, Cr, Ni, Pb (only in the first and second fractions) and Zn and significantly lower results for Cu in all fractions (10–20% lower) and Pb in the third fraction (ca. 50% lower) were observed. The lower values obtained for Cu and Pb were taken into consideration for the interpretation of the results of this work and were shown not to compromise any relevant conclusions.

Blank solutions were prepared for each type of sample following the respective sample treatment. Three sample replicates were prepared and analysed and, after blank subtraction, the mean and respective standard deviations were calculated. Statistically significant differences among samples were evaluated through *t*-paired tests ($P < 0.05$).

3. Results and discussion

3.1. Sediment characteristics

In order to characterize the environment from which plants were collected, sediments and rhizosediments were analysed for OM, total-recoverable metals, AVS and SEM (Table 1) and grain size distribution (Fig. 1).

Sediments from the site upstream the salt marsh were sandy, coarse and displayed low OM. The rhizosediment characteristics were quite similar to those of the

sediment. On the contrary, sediments from the site inside the salt marsh were muddy, finer and richer in OM, and significant differences between the sediment and the rhizosediment were found. Rhizosediment was finer-grained and had twice the OM content of the surrounding sediment.

In terms of total-recoverable concentrations of trace metals, Table 1 shows that the muddy sediment typically exhibited higher metal contents than the sandy sediment, except for Cd and Cr levels which were similar, and Cu, which was higher at the sandy sediment, probably due to anthropogenic contamination (some copper wire was found near by the sandy site). The observed differences are likely due to the different grain size distribution and composition of sediments, because the presence of clays and organic matter contents, as well as Fe and Mn (hydr)oxides surfaces (in oxic sediments), are determinant for sorbing metals. Correlations between decreasing grain size and increasing metal concentrations have been reported (Chapman and Wang, 2001 and references therein). At the muddy site, rhizosediment displayed much higher (up to 7 times) metal contents than sediment, while at the sandy site small but statistically significant differences (higher values at rhizosediment) were only observed for Pb and Zn.

AVS was not detected at the sandy site, thus sediment was oxic. Therefore the potential bioavailability of Cd, Cu, Ni, Pb and Zn, which can form relatively insoluble sulphides, was not controlled by sulphide at sandy site. These processes were probably controlled mainly by sorption at Fe and Mn (hydr)oxides surfaces and/or organic compounds binding. At the muddy site sediment, the metals were mainly stabilized in sulphide forms ($SEM - AVS = -0.5 \mu\text{mol g}^{-1}$). In contrast, the rhizosediment from the muddy site displayed much lower AVS ($SEM - AVS = 2.4 \mu\text{mol g}^{-1}$). Therefore, *Juncus maritimus* could oxidize its rhizosphere resulting in a change of trace metal speciation and availability.

Table 1

Concentrations^a of organic matter (OM), total-recoverable metals, acid volatile sulphide (AVS), simultaneously extracted metals (SEM) and the difference between the last two parameters ($SEM - AVS$) measured in sediments of different characteristics

	Sandy		Muddy	
	Rhizosediment	Sediment	Rhizosediment	Sediment
OM (%)	1.2	0.9	12	5.3
Cd ($\mu\text{g g}^{-1}$)	0.07 (0.02)	0.07 (0.02)	0.52 (0.01)	0.07 (0.01)
Cr ($\mu\text{g g}^{-1}$)	12 (2)	10 (3)	57 (4)	11.4 (0.8)
Cu ($\mu\text{g g}^{-1}$)	13.3 (0.6)	20 (8)	26.2 (0.8)	8.4 (0.3)
Ni ($\mu\text{g g}^{-1}$)	9 (1)	6 (2)	21 (2)	10.0 (0.7)
Pb ($\mu\text{g g}^{-1}$)	33 (3)	24 (5)	173 (7)	32 (3)
Zn ($\mu\text{g g}^{-1}$)	56 (7)	33 (10)	152 (4)	59 (7)
SEM ^b ($\mu\text{mol g}^{-1}$)	1.2 (0.4)	0.4 (0.2)	2.6 (0.4)	2.3 (0.3)
AVS ($\mu\text{mol g}^{-1}$)	<0.012 ^c	<0.012 ^c	0.13 (0.07)	2.7 (0.5)
SEM – AVS ($\mu\text{mol g}^{-1}$)	1.2 (0.4)	0.4 (0.2)	2.4 (0.3)	–0.5 (0.2)

^a Mean and standard deviation (in brackets, $n = 3$).

^b Sum of concentrations of Cd, Cu, Ni, Pb and Zn extracted simultaneously with AVS (Allen et al., 1993).

^c Limit of detection.

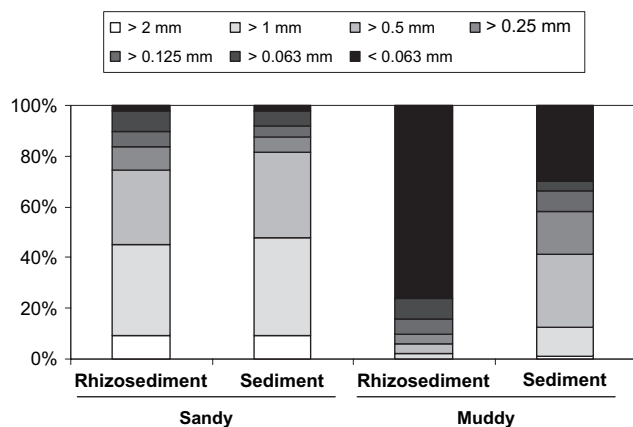


Fig. 1. Grain size distribution in rhizosediment and sediment from the studied sites.

Sulphide oxidation by wetland plants has been reported previously (Lee et al., 1999; Maricle and Lee, 2002), for instance for *Spartina alterniflora*, which has a well-developed aerenchyma system that facilitates the transport of oxygen from the atmosphere to the roots.

3.2. Plant exudation

In order to identify low molecular weight organic acids exuded by the *Juncus maritimus*, roots were placed in deionised water for 2 h and the obtained solution was analysed as described above. Among the eight organic ligands investigated, only malonate and oxalate could be found (Fig. 2). Fig. 2 shows that the plants collected at sandy site exuded, on average, about 3 times less malonate but about 7 times more oxalate than the plants collected at muddy site. It is known that the composition of root exudates is dependent on plant age and physico-chemical environment (Jones, 1998). Although plants of approximately the same size and similar aspect had been selected for this study, this does not guarantee that all the plants were of the same age and/or physiological state. Variations in these factors may explain the relatively high variability in exudation

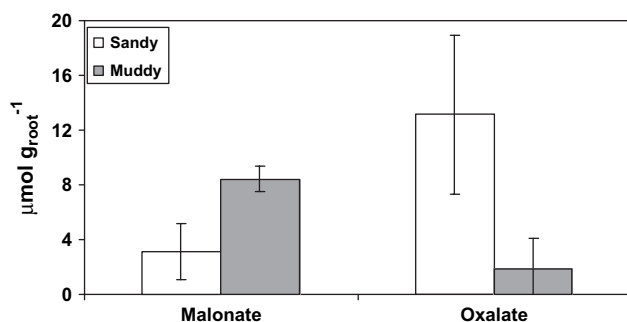


Fig. 2. Organic acids exuded by plants from the two sites under study. Mean values and standard deviation ($n = 3$) are shown.

observed among replicates (high standard deviations, see Fig. 2). Even so, statistically significant differences were registered between the levels of each exudate released by the plants from the sandy ($3.1 \pm 2.1 \mu\text{mol g}_{\text{root}}^{-1}$ for malonate and $13.1 \pm 5.8 \mu\text{mol g}_{\text{root}}^{-1}$ for oxalate) and the muddy ($8.4 \pm 0.9 \mu\text{mol g}_{\text{root}}^{-1}$ for malonate and $1.9 \pm 2.2 \mu\text{mol g}_{\text{root}}^{-1}$ for oxalate) sites. Such findings suggest that the different characteristics of the two sediments conditioned the response of the plants. Beyond the above-described characteristics it should also be referred that at sandy site plants are more exposed to desiccation, due to the higher water permeability of the sandy sediments that retain less water than the muddy sediments at the low tide. In a previous study with the same plant collected at the same sandy site during a different season (January 2003) citrate was found in addition to malonate and oxalate. Promotion of exudation of citrate, oxalate and/or malate, has been observed and described as response to nutritional deficiency (e.g., P, Neumann et al., 1999 and Fe, Jones, 1998) or metal detoxification (e.g., Al, Ma et al., 2001). Malate and citrate have been the organic ligands most often reported as plant exudates (Jones, 1998 and references therein). However, most studies on organic acids exudation have been performed with crop plants, e.g., cultivars of wheat (Parker et al., 2001) and buckwheat (Ma et al., 2001), and previous data on exudation by estuarine plants were not found.

It must be mentioned that the method used for organic acids determination (anionic chromatography) only quantified those present in the solution as free anions. Complexes with trace metals might also be present, but were not measured, which means that the malonate and oxalate concentrations may be underestimated. Nevertheless, the levels of total metal released into solution concomitantly with organic ligands were as follows ($\text{nmol g}_{\text{root}}^{-1}$): 143 (Cu, sandy), 6 (Cu, muddy), 20 (Zn, sandy), 33 (Zn, muddy), and Cd (<0.4), Cr (<1.9), Ni (<2.2) and Pb (<0.5). The values given for the last four metals are at the detection limit of the analytical method. Based on these data, the stability constants (Academic Software, 2001) and the pH of the solution, it could be estimated that the fractions of the organic acids present in complexed forms account for less than 10% of the total amount exuded, which is within the variability observed for the replicates (Fig. 2).

Finally, it deserves to be mentioned that exposure of plants to deionised water, a method that has been recently used for collection of root exudates (e.g., Fan et al., 1997), constitutes a methodological limitation in the present study, in terms of quantification of the released ligands. This is because *Juncus maritimus* comes from an ecosystem with salinity variations (salinity measured as 3 in the interstitial water at low tide), leading to a possible overestimation of exudation due to ionic unbalancing.

3.3. Organic acids as metal extractants

In order to obtain information about the influence of the low molecular weight organic acids exuded by the plant on trace metal mobility and availability from sediments, solutions of oxalic, malonic and citric acids were used as extractants either individually or mixed. In all cases the final concentration of the extractant was 0.11 M, that is, the same as the acetic acid solution used in the first step of a very commonly used SE procedure (Rauret et al., 1999). The extractant concentrations used may not be realistic, but there were no data available about organic acid concentration in the rhizosphere of salt marsh where plants were collected. The pH of the extractant solutions were as follows: 1.1 oxalic acid; 1.6 malonic acid; 1.7 citric acid; 1.5 mixture of acids and 2.6 acetic acid. Therefore, owing to the lower pH values, the extractant efficiency of the exudates of *Juncus maritimus* could be over-estimated in the present study.

All solutions of exudates had pH values lower than that of acetic acid, which has been assumed to be capable of extracting the fraction of metals exchangeable and bound to carbonates. Therefore, it was expected that the organic acid solutions used could also extract at least the same metal fraction that acetic acid does.

For comparison purpose, a detailed metal fractionation in the samples was obtained by application of the SE procedure (Rauret et al., 1999): (1) exchangeable and bound to carbonates, which gives the fraction that is more weakly bound to the sediment; (2) bound to Fe and Mn (hydr)oxides; (3) bound to organic matter and sulfide and (4) residual, which corresponds to that more intensively bound to sediment. It should be stressed that this SE scheme also provides only operationally defined fractionation and cannot directly determine the chemical speciation of metals in sediments.

The results obtained for the extraction of the different metals with the organic acid solutions, expressed as percentage of the total-recoverable metal concentration, as well as the SE procedure, are shown in Fig. 3. Comparing the extraction efficiency of the different organic acid solutions, it emerges that the oxalic acid solution extracted the highest metal fraction from sediments in most cases. The percentages were significantly higher than those obtained with malonic and citric acid solutions in, respectively, 63 and 58% of the samples, considering the different metals and the different sediments. When oxalic acid was used in combination with malonic and citric (mixture of acids) it extracted the same or slightly less metal than when it was used alone (no significant differences in 58% of the cases). Malonate and citrate solutions presented no significant differences from each other as extractants, with only one exception (Cd at sandy rhizosediment). In many cases, particularly for Pb and Zn, the extractant power increased with the concentration of protons in

solution (higher pH), preventing to distinguish whether the efficiency of the extraction was due to a complexing effect of the organic acid or to the concentration of the protons in solution. Nevertheless, in some cases the role of the complexing capacity of the organic acids emerged as fundamental for the interpretation of the results.

For Cr and Ni, the least available metals among those studied, the oxalic acid solution was, in most cases, significantly more efficient in solubilising them than the set of three extractants of the SE. The only exceptions were Cr and Ni at sandy sediment (no significant differences were observed) and Cr at muddy rhizosediment (SE was significantly more efficient). The relatively low efficiency of oxalate extraction of Cr in muddy rhizosediment may be due, among other phenomena, to the effective competition for the metal by organic ligands of different nature present in rhizosphere, which was very rich in OM. In a separate study (Mucha et al., 2003b), up to 800 nM organic ligands (measured in equivalents of Cu) capable of forming very stable metal complexes were found in elutriates of sediments from the same estuarine area. The mixture of acids was also more efficient in solubilising Cr from sandy rhizosediment and Ni from sandy rhizosediment and muddy sediment than the set of three extractants of the SE. As the pH of the mixture of acids is equal to that of the second extractant solution of SE (pH = 1.5), the liberation of Cr and Ni from some sediments may have been promoted by the oxalate.

Fig. 3 shows very irregular patterns for Cu and Cd. The power of the different extractants was very much dependent on metal nature, sediment characteristics and presence of plants. As concerns Cu, oxalic acid solution was able of extracting almost all of the total-recoverable metal present in both sediment and rhizosediment from the sandy site. On the contrary, oxalic acid solution could extract only 30% (sediment) and 10% (rhizosediment) of the total-recoverable Cu from the muddy site. Compared with the SE non-residual fraction, the oxalic acid exhibited statistically identical power, except for the muddy rhizosediment where it was 5 times less efficient. The mixture of organic acids could extract significantly less Cu from sandy and muddy rhizosediment than the three steps of SE did. Therefore, other effect beyond pH conditioned the extraction power of the organic acid solutions used. The behaviour observed for Cu could be related to the presence of adsorption and absorption sites at the surface of the rhizosediment particles, which can compete effectively for Cu with the organic acids, but can be destroyed during the SE. For Cd similar phenomena may cause the observed results. The very different patterns observed for the four studied samples indicate that the predominant chemical forms of Cd (speciation) varied markedly with sediment and rhizosediment characteristics. It must be noted that, in contrast to that observed for the other metals and

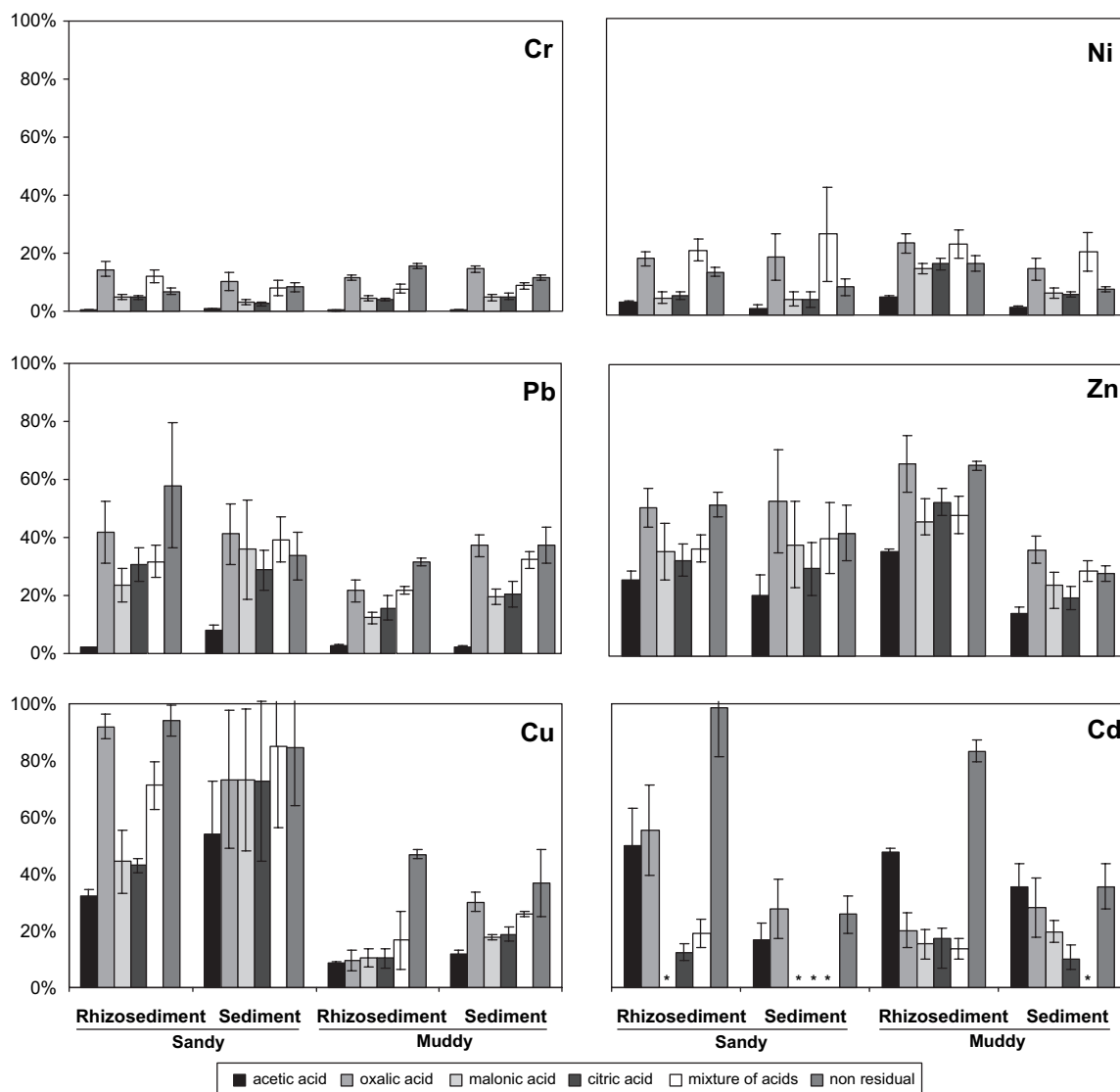


Fig. 3. Percentages (mean values and standard deviation, $n = 3$) of the different metal solubilised by different extractants from rhizosediment and sediment from the two studied sites. Acetic acid (0.11 M) is the extractant used in the first step (exchangeable and bound to carbonates fraction) of the SE (Rauret et al., 1999); Mixture of oxalic, malonic and citric acids (0.11 M total concentration), as well as each of these acids individually (0.11 M), were also used as extractants; Non-residual fraction is the sum of the three SE fractions (exchangeable and bound to carbonates, bound to Fe/Mn (hydr)oxides and bound to organic matter and sulphides). Asterisk (*) in the graphic of Cd indicates that the value was below the detection limit of the analytical technique.

would be expected attending to the pH of the solutions, malonic and citric acid solutions, and even oxalic acid solution in muddy rhizosediment, extracted significantly less Cd than the acetic acid solution (the SE metals fraction exchangeable and bound to carbonates). The cation Cd^{2+} is, among the studied metals (M) in M^{2+} oxidation state, the softest cation according to the Hard and Soft Acids and Bases (HSAB) classification (Hay, 1984) (Cu^+ is also a soft cation), whereas carboxylate is a hard anion, which displays low affinity to soft cations. Affinity of carboxylic acids for Cd^{2+} lower than for the other studied M^{2+} is compatible with the lower extraction power of the exuded organic acids observed for Cd. However, this factor does not explain why

malonic and citric acids extracted less metal than the acetic acid, aspect that remains not understandable.

The presence of low molecular weight metabolites, like citrate, has been shown to be capable of enhancing the availability of Cd (Errecalde et al., 1998; Errecalde and Campbell, 2000) and Zn (Errecalde and Campbell, 2000) to a micro-alga. Therefore, there is the possibility that the studied exudates enhance not only the transfer of trace metals to interstitial water but also their bioavailability. However, further studies, including comparison of sediment extraction with the different extractants at fixed pH, and concentrations as realistic as possible (pre-determined in sediment pore water) are required to clarify these aspects.

4. Conclusions

Juncus maritimus, collected during summer, was shown to be capable of releasing malonate and oxalate. The extent of exudation varied with the estuarine sediment characteristics from the area where plants were collected. The studied exudates, particularly oxalate, exhibited capacity to promote the solubilisation of some trace metals, particularly Cr and Ni and to reduce the desorption of Cd. Therefore, *J. maritimus* can change metal mobility and availability not only by changing oxidative conditions of sediments but also by the exudation of organic acids. As marsh plants can change drastically the metal speciation of the surrounding environment, they should be accounted for when dealing with estuarine sediment quality.

Acknowledgements

This work was partially funded by Fundação para a Ciência e Tecnologia, Portugal, through fellowships awarded to A. P. Mucha (SFRH/BPD 7141/2001) and C. M. Almeida (SFRH/BPD 9430/2002). The authors acknowledge Carlos Gomes (Chemical Department, Faculty of Sciences, University of Porto) for his help with chromatography, and João Honrado and Paulo Alves (Botanical Department, Faculty of Sciences, University of Porto) for plant identification.

References

- Academic Software, 2001. IUPAC Stability Constants Database. (5.15). Academic Software, Otley, Yorkshire, UK.
- Allen, H.E., Fu, G., Deng, B., 1993. Analysis of acid-volatile sulfide (AVS) and simultaneously extracted metals (SEM) for the estimation of potential toxicity in aquatic sediments. *Environmental Toxicology and Chemistry* 12, 1441–1453.
- Almeida, C.M., Mucha, A.P., Vasconcelos, M.T., 2004. Influence of the sea rush *Juncus maritimus* on metal concentration and speciation in estuarine sediment colonized by the plant. *Environmental Science and Technology* 38, 3112–3118.
- Baeyer, W., Monteny, F., Leermaker, M., Bouillon, S., 2003. Evaluation of sequential extractions on dry and wet sediments. *Analytical and Bioanalytical Chemistry* 376, 890–901.
- Berkelaar, E., Hale, B.A., 2003. Accumulation of cadmium by Durum Wheat roots: bases for citrate-mediated exceptions to the free ion model. *Environmental Toxicology and Chemistry* 22, 1155–1161.
- Burke, D.J., Weis, J.S., Weis, P., 2000. Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. *Estuarine, Coastal and Shelf Science* 51, 153–159.
- Caçador, I., Vale, C., Catarino, F., 1996. The influence of plants on concentration and fractionation of Zn, Pb and Cu in salt marsh sediments (Tagus Estuary, Portugal). *Journal of Aquatic Ecosystem Health* 5, 193–198.
- Chapman, P.M., Wang, F., 2001. Assessing sediment contamination in estuaries. *Environmental Toxicology and Chemistry* 20, 3–22.
- Errecalde, O., Seidl, M., Campbell, P.G.C., 1998. Influence of a low molecular weight metabolite (citrate) on the toxicity of cadmium and zinc to the unicellular green alga *Selenastrum capricornutum*: an exception to the free-ion model. *Water Research* 32, 419–429.
- Errecalde, O., Campbell, P.G.C., 2000. Cadmium and zinc bioavailability to *Selenastrum capricornutum* (Chlorophyceae): accidental metal uptake and toxicity in the presence of citrate. *Journal of Phycology* 36, 473–483.
- Fan, T.W.-M., Lane, A.N., Pedler, J., Crowley, D., Higashi, R.M., 1997. Comprehensive analysis of organic ligands in whole root exudates using Nuclear Magnetic Resonance and Gas Chromatography–Mass Spectroscopy. *Analytical Biochemistry* 251, 57–68.
- Hay, R.W., 1984. *Bio-Inorganic Chemistry*. Wiley, New York, 211 pp.
- Jacob, D.L., Otte, M.L., 2004a. Long-term effects of submergence and wetland vegetation on metals in a 90-year old abandoned Pb–Zn mine tailings pond. *Environmental Pollution* 130, 337–345.
- Jacob, D.L., Otte, M.L., 2004b. Influence of *Typha latifolia* and fertilization on metal mobility in two different Pb–Zn mine tailing types 333, 9–24.
- Jones, D.L., 1998. Organic acids in the rhizosphere – a critical review. *Plant and Soil* 205, 25–44.
- Lee, R.W., Kraus, D.W., Doeller, J.E., 1999. Oxidation of sulfide by *Spartina alterniflora* roots. *Limnology and Oceanography* 44, 1155–1159.
- Ma, J.F., Ryan, P.R., Delhaize, E., 2001. Aluminium tolerance in plants and the complexing role of organic acids. *Trends in Plant Science* 6, 273–278.
- Madureira, M.J., Vale, C., Simões Gonçalves, M.L., 1997. Effect of plants on sulphur geochemistry in the Tagus salt-marshes sediments. *Marine Chemistry* 58, 27–37.
- Maricle, B.R., Lee, R.W., 2002. Aerenchyma development and oxygen transport in the estuarine cordgrasses *Spartina alterniflora* and *S. anglica*. *Aquatic Botany* 74, 109–120.
- Mucha, A.P., Vasconcelos, M.T., Bordalo, A.A., 2003a. Macrobenthic community in the Douro estuary: relations with trace metals and natural sediment characteristics. *Environmental Pollution* 121, 169–180.
- Mucha, A.P., Leal, M.F.C., Bordalo, A.A., Vasconcelos, M.T., 2003b. Comparison of the response of three micro-algae species exposed to elutriates of estuarine sediments, based on growth and chemical speciation. *Environmental Toxicology and Chemistry* 22, 576–585.
- Neumann, G., Massoneau, A., Martinoia, E., Römheld, V., 1999. Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. *Planta* 208, 373–382.
- Otero, X.L., Macias, F., 2002. Variation with depth and season in metal sulfides in salt marsh soils. *Biogeochemistry* 61, 247–268.
- Parker, D.R., Pedler, J.F., Ahnstrom, Z.A.S., Resketo, M., 2001. Reevaluating the free-ion activity model of trace metal toxicity toward higher plants: experimental evidence with copper and zinc. *Environmental Toxicology and Chemistry* 20, 899–906.
- Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., Quevauviller, Ph., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of the new sediment and soil reference materials. *Journal of Environmental Monitoring* 1, 57–61.
- Sundby, B., Vale, C., Caçador, I., Catarino, F., Madureira, M.J., Caetano, M., 1998. Metal-rich concretions on the roots of salt marsh plants: mechanism and rate of formation. *Limnology and Oceanography* 43, 245–252.
- Varennes, A., 2003. *Produtividade dos Solos e Ambiente*. Escolar Editora, Lisboa, 490 pp.
- Wang, Z., Shan, X., Zhang, S., 2001. Comparison of speciation and bioavailability of rare earth elements between wet rhizosphere soil and air-dried bulk soil. *Analytica Chimica Acta* 441, 147–156.
- Windham, L., Weis, J.S., Weis, P., 2003. Uptake and distribution of metals in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). *Estuarine, Coastal and Shelf Science* 56, 63–72.