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Variation with depth and season in metal sulfides in salt marsh soils

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Abstract. Seasonal monitoring of metal sulfides was carried out in four soils of the Ría de Ortigueira salt marshes. Soils from the high salt marsh (with suboxic redox conditions at the surface), had low concentrations of iron sulfides (AVS and pyrite fraction) and thus a low degree of trace metal pyritization (DTMP) in surface layers (0–10 cm), but concentrations of metals associated with the pyrite fraction increased considerably at depth (27.5 cm). In the low salt marsh soils (with anoxic conditions at the surface) maximum concentrations of metal sulfides were found in the surface layers of soils colonized by *Spartina maritima*. These results are explained by the double effect exerted by roots in strongly reduced soils. On the one hand, they stimulate the activity of sulfate-reducing bacteria and on the other, they favour the partial oxidation of the soil, thus generating polysulfides with which Fe^{2+} immediately precipitates as pyrite, whereas in the deepest, permanently anoxic layers, pyrite must be formed in a reaction in which FeS is an intermediate, as follows: $FeS + H_2S \rightarrow FeS_2 + H_2$. Concentrations of metal sulfides also varied greatly with the season, with two patterns being distinguished. In soils colonized by *S. maritima* in both high and low salt marshes, the lowest concentrations were found in summer. At this time of the year there is a net loss of metal sulfides throughout the profile, presumably due to physiological activity of plants (evapotranspiration and release of oxygen from roots). In contrast, maximum concentrations of AVS and pyritic metals were found in the summer in the low salt marsh soils not colonized by vascular plants (creek bottom). In this case, the higher temperatures increased the activity of sulfur-reducing bacteria leading to synthesis and accumulation of metal sulfides in the soil.

Abbreviations: AVS – acid volatile sulfide, SEM – simultaneously extracted metals, DOP – degree of pyritization of Fe, DTMP – degree of trace metal pyritization, dw – dry weight, MHWS – mean high water springs, MLWS – mean low water springs

Introduction

In anoxic marine sediments and soils, organic material is degraded by microorganisms that are capable of using compounds other than oxygen as electron acceptors, including NO_3^-, MnO_2, FeOOH, SO_4^{2-} and CO_2 (Zehnder and Stumm 1988). The reduction of sulfate to ΣH_2S ($=H_2S+HS^-+S^{2-}$) is considered to be one of the main processes in the mineralization of organic matter in salt marsh soils (Howarth 1984). Part of the ΣH_2S can react with Fe^{2+} to form metastable Fe sulfides such as amorphous FeS, mackinawite (FeS) greigite (Fe_3S_4) and pyrrhotite ($FeS_{1.1}$) (Morse et al. 1987), which can later be transformed to pyrite (FeS_2) (Berner 1970). Current techniques do not allow quantitative separation of these forms and two categories of Fe sulfides are traditionally established in this type of study: acid volatile sulfides (AVS) and pyrite (Morse et al. 1987). The AVS fraction is extracted by distillation in acid (usually a dilute solution of HCl; see Cornwell and Morse (1987)). Pyrite resists this acid attack and is extracted by oxidation with HNO_3 or by reduction with Cr (II) (Morse et al. 1987; Huerta-Díaz and Morse 1990). Recently, both the AVS and pyrite fractions have received much attention because they can act as sinks for heavy metals and therefore control their bioavailability and toxicity (Di Toro et al. 1990, 1992; Huerta-Díaz and Morse 1992; Morse 1994). The work of Di Toro et al. (1990, 1992) was based on the idea that when the interstitial water is in equilibrium with the solid phase sulfides, very low concentrations of free divalent metal cations should be left, assuming the molar ratio of Metal (soluble in 1 or 2N HCl): AVS to be less than 1. This idea is supported by the fact that most of the divalent metal sulfides (CdS, HgS, NiS, CuS, ZnS, PbS….) have a lower solubility product (Kps) than those of the metastable Fe sulfides, so that they can displace this of its sulfur, according to reaction (1).

$$Me^{2+} + FeS(s) \rightarrow MeS(s) + Fe^{2+} \qquad (1)$$

The results obtained from laboratory toxicity tests were in accordance with the AVS model, in which bioaccumulation of trace metals by different species of benthic invertebrates took place at ratios of [SEM]:[AVS] >1 (Di Toro et al. 1990, 1992; Casas and Crecelius 1994). On the other hand, previous studies have shown that the concentration of pyrite in salt marsh soils is much higher than that of the AVS fraction (Kostka and Luther 1995) and may be accompanied by high concentrations of heavy metals (Huerta-Díaz and Morse 1990, 1992; Otero et al. 2000a). Metal sulfides (AVS and pyrite fraction) are stable and very insoluble under anoxic conditions (Krauskopf 1956). However, previous studies have shown that the geochemical conditions of salt marsh soils are subject to much spatial and seasonal variation, and range from anoxic conditions, which favour the formation of metal sulfides, to oxic conditions, which lead to their destruction by oxidation (Hines et al. 1989; Luther and Church 1988; Luther et al. 1991). It is therefore possible that metals associated with the AVS and pyrite fractions can be released into the interstitial water and become bioavailable (Morse 1994).

Few studies have been carried out on seasonal variation in AVS and metals associated with the pyrite fraction in salt marsh soils. The main objective of this research was to study the variations with season and depth in these two fractions in an intertidal zone. Monitoring was carried out at four different physiographical locations in the salt marsh, three colonized by *Spartina maritima* and one lacking vegetation.

Table 1. General characteristics of the sampling points selected for characterization of seasonal variation (after Otero (2000)).

	Soil	Type of salt marsh	Physiographical position	Dominant plant species	Characteristic Soil
ESTEIRO	EST1	Low or pioneer salt marsh	Marsh flat	*Spartina maritima*	Typic Sulfaquents
	EST3		Creek bottom	Unvegetated	Typic Sulfaquents
MERA	MERA1	High salt marsh	Marsh flat	*Spartina maritima*	Typic Sulfaquents
LADRIDO	LAD2	High salt marsh	Creek edge	*Spartina maritima*	Sodic Hydraquents

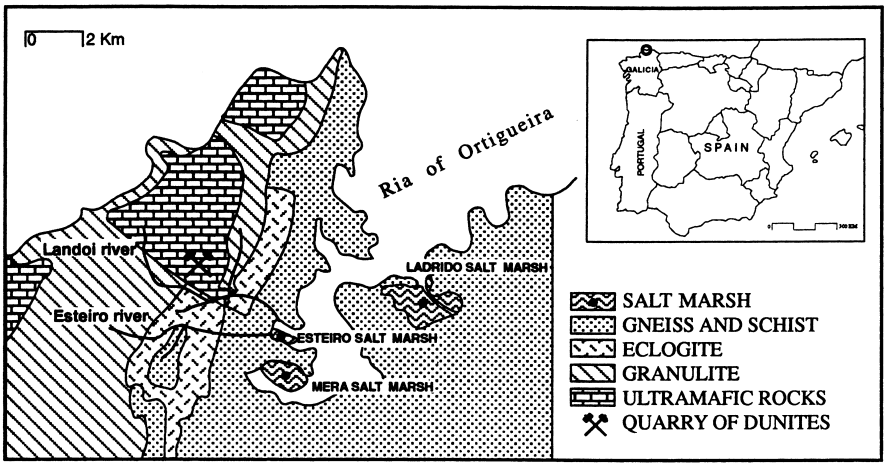

Figure 1. Map showing location and geology of the study area.

Materials and methods

Study area

The three salt marshes under study (Esteiro, Mera and Ladrido) are situated in the Ría of Ortigueira (Galicia, NW Iberian Peninsula; Figure 1). Tidal periodicity is semidiurnal with a mesotidal range (3.6 m between MHWS and MLWS). The geological substrate is comprised of gneisses and amphibolic schists as well as basic (eclogite, granulite, amphibolites) and serpentinized ultrabasic rocks (dunites), the latter of which are characterized by a high concentration of Cr and Ni (van Calsteren 1978). There are a number of dunite quarries in the area, one of which lies within the catchment area of the river Landoi, which flows into the Esteiro inlet (Figure 1). This quarry generates a waste sludge containing high levels of Cr and Ni, which is transported via the river Landoi to the Esteiro salt marsh (Otero et al. 2000a).

Seasonal monitoring was carried out at 4 sampling sites selected on the basis of their physiographical position, the presence or absence of vegetation (*Spartina maritima*) and the stand density and form (tall or short) of *Spartina*. The Esteiro salt marsh corresponds to low salt marsh and Mera and Ladrido to high salt marsh. In the low salt marsh the sampling sites were situated in the marsh flat (EST1) and on the creek bottom (EST3). The two sites were close to one another (<5 m). In the high salt marsh, one sampling site was situated in the marsh flat (MERA1) and another close to the creek edge (LAD2). All sites were colonized by *Spartina maritima*, except the creek bottom of the Esteiro salt marsh, which lacked vegetation. The density of stands of *S. maritima* varied greatly, the highest density was at LAD2 (1603 ± 286 shoots m^{-2}); these stands consisted of the short form of *Spartina*, whereas at MERA1 (956 ± 93 shoots m^{-2}) and EST1 (471 ± 131 shoots m^{-2}) the stands were of the tall forms (for more detail see Sánchez et al. (1997); Otero et al. (2000b)).

Sampling of underground biomass

The vertical distribution of underground biomass was established according to the method of Castellanos et al. (1994). At sites in each of the salt marshes where *Spartina maritima* grew, three points were selected at random and samples were collected using a PVC tube (internal diameter, 11 cm, length 25 cm). The sample was carefully extracted in the laboratory and divided into 5 cm sections. The sediment was removed by washing each section with water, through a 63 μ m seive. The live roots and rhizomes were then carefully separated from dead ones and dried at 80 °C until constant weight.

Soil sampling and analysis

Sampling was carried out in March, July and December, 1996, at low tide. The samples were collected with PVC tubes (11 cm i.d. and 35 cm in length). One core was collected from every sampling site each month; the cores were taken from within an area of 1 m radius each time, in order to avoid spatial variations. The tubes were hermetically sealed under pressure, maintained at approximately 4 °C and transported in a vertical position to the laboratory where they were frozen, cut into 5 cm sections with a carbon fiber saw and stored frozen until analyzed. We analyzed superficial samples (2.5 and 7.5 cm depth) and deep samples (27.5 cm depth i.e. below the rhizosphere zone) from each core. However, because the soil at EST3 (creek bottom) was only 20 cm deep, the deep samples from the corresponding cores were taken at 17.5 cm depth.

For the extraction of interstitial water, samples were defrosted at 4 °C. One sample ($\approx$ 35 ml) was taken from the middle of the soil samples under a flow of nitrogen (Roden and Tuttle 1993), placed in 50 ml polypropylene bottles, which were purged with nitrogen for 1 min then hermetically sealed before centrifuging at 3000 rpm for 30 min. Electrical conductivity and pH were measured immediately in these samples.

The concentration of acid volatile sulfides (AVS) was determined in triplicate according to the method of Kostka and Luther (1994). The sulfide in the AVS fraction was released from wet samples ($\approx$ 0.25–0.5 g) with 20 ml 6N HCl, previously deaerated for 40–50 min. in a gas-tight reaction flask through which nitrogen was bubbled as slowly as possible. The H_2S was carried by the flow of nitrogen to a flask containing 25 ml of 3% Zn acetate. The sulfide concentration was determined colorimetrically with a UV-VIS spectrophotometer (Vitatron, model MCP) at a wavelength of 670 nm according to the methylene blue method of Cline (1969).

The reactive-Fe, which is the Fe fraction that can react with sulfides to give pyrite (Berner 1970), was extracted by the citrate-dithionite method outlined in previous studies (see e.g. Canfield (1989); Raiswell et al. (1994)) and which extracts amorphous and crystalline oxyhydroxides of Fe and AVS. Extraction was carried out in triplicate using 0.5 g of wet sample, following the method established by Kostka and Luther (1994). The degree of pyritization of Fe (DOP), a term proposed by Berner (1970), and used to establish the percentage of reactive-Fe incorporated into the pyrite fraction, was calculated by equation (2).

$$DOP(\%) = \left[\frac{(\text{pyrite} - Fe)}{(\text{pyrite} - Fe) + (\text{reactive} - Fe)} \right] \times 100 \tag{2}$$

Trace metals associated with the reactive phase as well as Fe and trace metals associated with the pyrite phase were determined in triplicate using the sequential extraction method of Huerta-Díaz and Morse (1990). The method consists of the extraction of four operationally defined fractions: (1) the reactive fraction was extracted with 20 ml of 1N HCl after 16 h of continuous shaking, and was comprised of the metals associated with the AVS fraction, amorphous and crystalline oxyhydroxides of Fe and Mn and carbonates; (2) metals associated with aluminosilicates were then removed by dissolving them in 30 ml of HF during 16 h of continuous shaking, (3) metals associated with organic material with 10 ml of concentrated H_2SO_4 and 2 h shaking and (4) the metals associated with the pyrite fraction were extracted with 10 ml concentrated HNO_3 and 2 h continuous shaking. The degree of trace metal pyritization (DTMP), a term proposed by Huerta-Díaz and Morse (1990), and which gives an estimate of the content of a determined metal (Me) incorporated into the pyrite phase, was calculated by the following equation (3):

$$DTMP(\%) = \left[\frac{(\text{pyrite} - Me)}{(\text{pyrite} - Me) + (\text{reactive} - Me)} \right] \times 100 \tag{3}$$

The concentrations of Cr, Ni, Cu, Mn, Fe and Zn obtained from the different extracts were determined by flame absorption spectrophotometry (Perkin-Elmer, model 1100B). Laboratory and field equipment used during collection and analysis of soils was previously washed in 20 % HCl and Milli-Q water.

Statistical analysis

The seasonal differences in the concentrations of the AVS and pyrite fractions were determined by non-parametric ANOVA (Kruskal-Wallis test) using the SYSTAT 5.0 (Systat 1992) computer program. A non-parametric test was chosen because of the small number of samples involved (n=3).

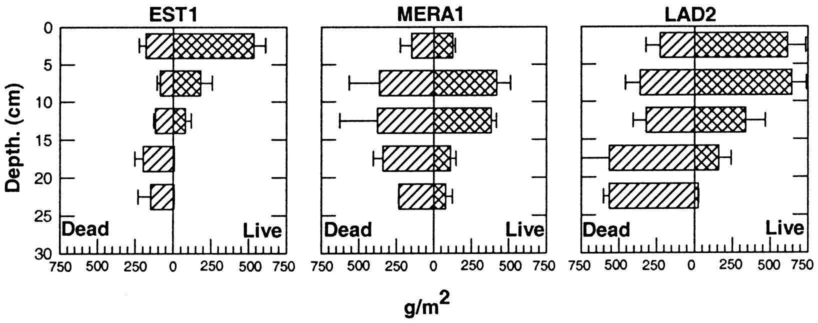

Figure 2. Vertical distribution of dead and live roots and rhizomes in soils colonized by *S. maritima* (average± 1 SE).

Results

Underground biomass

The highest density of roots and rhizomes was found in the upper 15 cm of the high salt marsh soils (Mera and Ladrido), with values ranging between 126 ± 18 and 644 ± 96 g m^{-2}. Below this depth there was a considerable decrease in the proportion of live roots and an increase in the proportion of dead roots (Figure 2). Densities found in the upper 5 cm of the low salt marsh (Esteiro) (535 ± 77 g m^{-2}) were similar to those found in the upper salt marsh, but at lower depths the amounts of both live and dead roots and rhizomes decreased considerably and were much less than in the high salt marsh.

Variation in the interstitial water: electrical conductivity and pH

The electrical conductivity of the interstitial water showed large spatial and seasonal variations (Figure 3). There was a clear pattern of seasonal variation in the surface layers (2.5 and 7.5 cm) of all the soils, with the highest values occurring in summer and the lowest in winter. The greatest difference was found in the surface of the MERA1 soil, with a variation of 23 dS m^{-1} between these seasons.

The spatial variation in pH was low with most values falling within a narrow range between 7 and 8 (Figure 3). There was little variation with depth and no clear pattern of variation. There were, however, two clear patterns of seasonal variation. In the soils with vegetation growing on them (in this study, *Spartina maritima*), the pH was low (minimum pH 4.8 in the MERA1 soil, at 2.5 cm depth), whereas in the soil from the channel bottom (EST3), the values were slightly higher in summer than in other seasons.

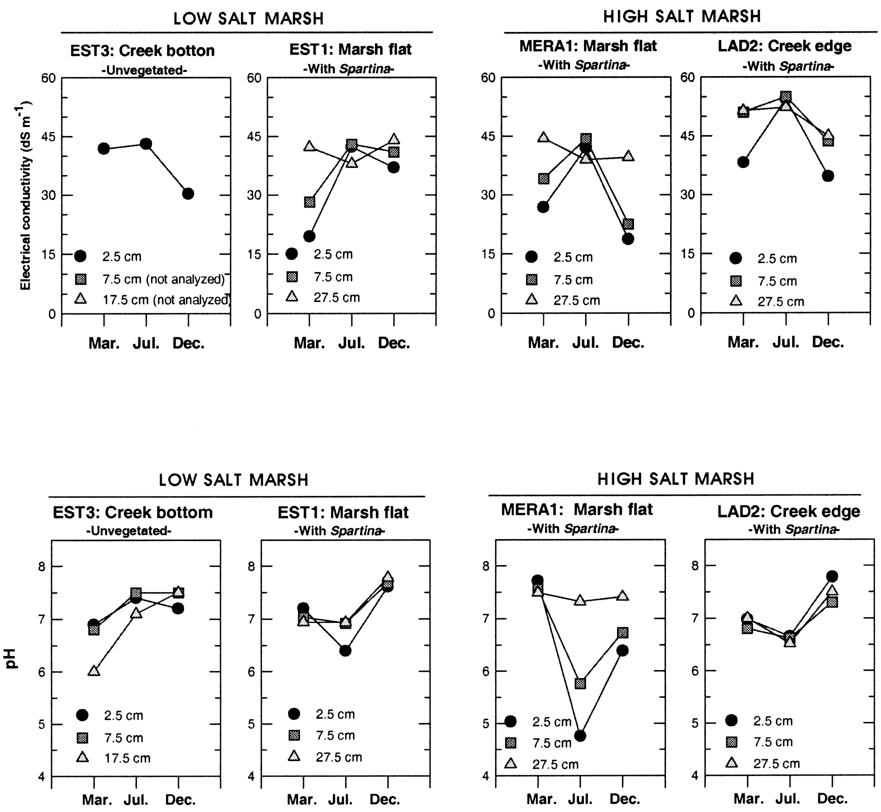

Figure 3. Electrical conductivity and pH of the interstitial water.

Variation in the AVS fraction

The highest concentrations of AVS were found in the upper layers of the low salt marsh (EST1, marsh flat: 10.51 ± 5.7 – 47 ± 5.8 μmol g^{-1} at 2.5 cm and EST3, creek bottom: 3.89±3.2 – 24.75±11.6 11.6 μmol g^{-1} at 2.5 cm; Figure 4). Levels reached no higher than 15 μmol g^{-1} in the MERA1 and no higher than 5 μmol g^{-1} in the LAD2, except in December when a maximum level of 40.8±2.3 μmol g^{-1} was reached (Figure 4). The results from the different sampling sites displayed significant seasonal differences which were especially notable in the uppermost layer (2.5 cm) of the soils, where the difference between the maximum and minimum values meant that the range of AVS concentration was greater than 90%. In general, in the soils with plant cover, the maximum concentrations were found in spring (March), whereas in the creek bottom (without vegetation) the maximum values were obtained in summer (July).

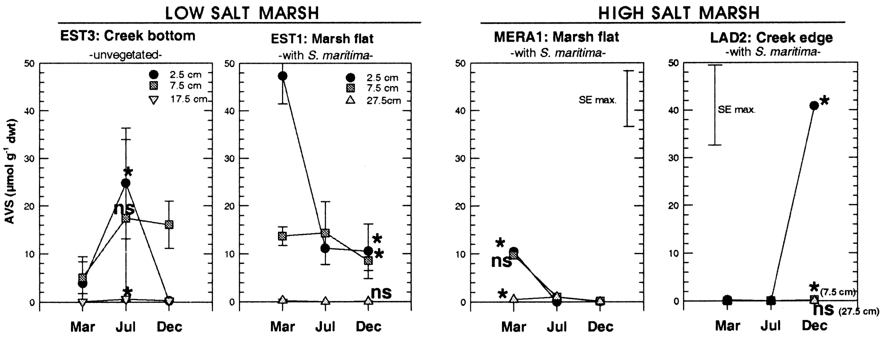

Figure 4. Concentrations of AVS fraction in salt marsh soils (SE max.: maximum standard error). For each depth, an asterix (*) indicates significant seasonal differences at a probability level of 5% and, ns indicates that differences were not significant.

Variations in reactive-Fe and pyrite-Fe fractions and degree of pyritization (DOP)

The highest concentrations of reactive-Fe were found in the upper layers of each soil, with maximum values in the high salt marsh soils (MERA1: 230–450 μmol g^{-1}) and minimum values in the low salt marsh soils (EST1: 55–260 μmol g^{-1}) (Figure 5). The concentration of reactive-Fe decreased significantly with depth (LAD2: 75–105 μmol g^{-1}; MERA1: 50–60 μmol g^{-1}, EST1: 10–30 μmol g^{-1} at 27.5 cm depth and EST3: 20–50 μmol g^{-1} at 17.5 cm depth). We also observed large seasonal differences but without a clear pattern that could be explained in terms of the variables selected (presence/absence of plants and physiographical position of the soil). Thus, in the EST1 and LAD2 soils the highest concentrations were found in summer and in the MERA1 and EST3 soils the lowest values were found in this season.

The highest concentrations of Fe associated with the pyrite fraction were found in the deepest part of the soil, except for the EST1 (Figure 5). The low salt marsh soils had levels that were clearly higher (EST3, creek bottom: 265–40 μmol g^{-1} and EST1, marsh flat: 400–75 μmol g^{-1}) than those of the high salt marsh (MERA1: 220–2 μmol g^{-1} and LAD2: 125–0.15 μmol g^{-1}). In accordance with these results, the degree of pyritization (DOP) was also higher in the low salt marsh soils, in most samples it was greater than 50% (e.g. EST1: 47–95%), which means that the concentration of pyrite-Fe was higher than that of the reactive-Fe (Figure 5). The DOP in the high salt marsh was only greater than 50% in the deepest part of the Mera soils, whereas in Ladrido, the concentration of reactive-Fe was always higher than the pyrite-Fe (Figure 5).

The concentrations of pyrite-Fe displayed significant seasonal changes that appeared to follow two different trends. In soils colonized by *S. maritima* the highest concentrations of pyrite-Fe were found in spring and winter, whereas the lowest levels were found in summer throughout the profile. In contrast, in the EST3 (with-

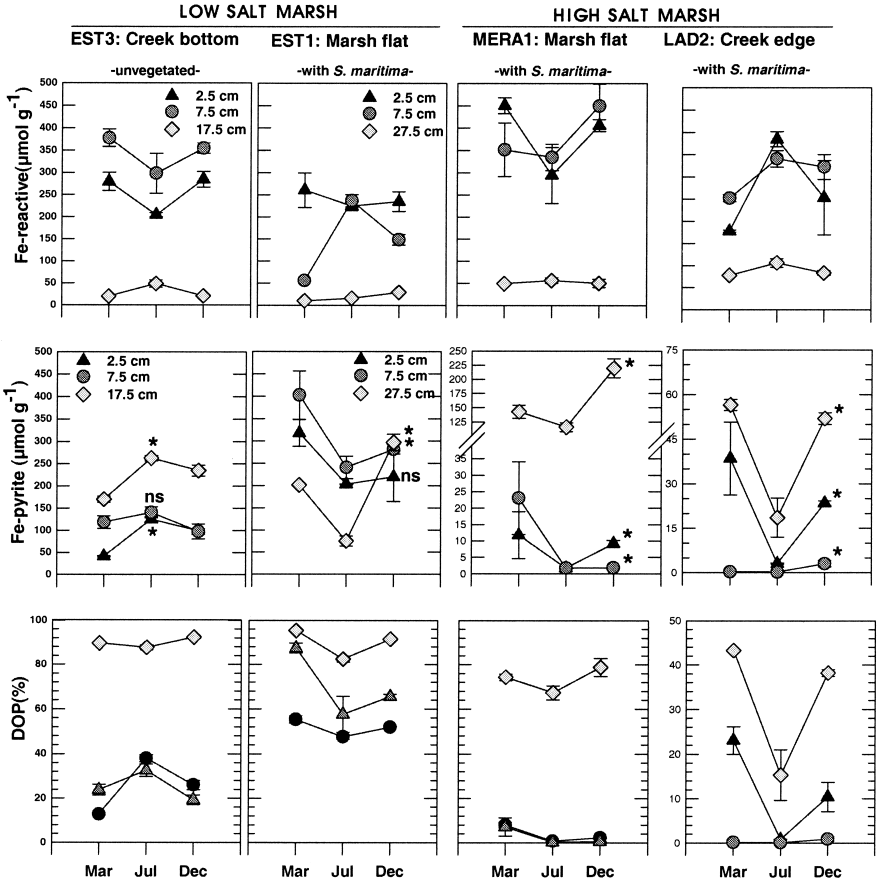

Figure 5. Concentrations of reactive-Fe, pyrite-Fe and degree of Fe pyritization (DOP) (average±1 SE). For pyrite-Fe, an asterix (*) indicates significant seasonal differences at a probability level of 5% and, ns indicates that differences were not significant.

out vegetation) the highest concentrations of pyrite-Fe were found in summer. Seasonal differences in DOP were similar to those observed for Fe-pyrite.

Variation in trace metals associated with the reactive and pyrite fractions and degree of pyritization (DTMP)

The highest concentrations of reactive-metals fraction were found in the upper 10 cm of each soil (Figure 6). Nickel was found to be highly concentrated, most notably in the Esteiro salt marsh (EST3: 4700–9300 nmol g^{-1}; EST1: 4600–6400 nmol g^{-1}; MERA1: 1600–1800 nmol g^{-1}; LAD2: 925–1750 nmol g^{-1}), probably due to fact that this salt marsh receives the sludge from a dunite quarry (Figure 1), which has been characterized by having high concentrations of total and reactive Ni and Cr (soluble 1N HCl) (Otero et al. 2000a). The Esteiro salt marsh soils also contained high levels of Cr (EST1 1100–1900 nmol g^{-1}, EST3: 800–1300 nmol g^{-1}; MERA1: 450–670 nmol g^{-1}; LAD2: 400–760 nmol g^{-1}) and Mn (EST3: 800–1300 nmol g^{-1}; EST1: 1100–1900 nmol g^{-1}; MERA1: 550–1200 nmol g^{-1} LAD2: 450–775 nmol g^{-1}). Copper and Zn were found at much lower concentrations, the highest being in the MERA1 salt marsh soil (Cu: 115–324 nmol g^{-1}; Zn: 410–870 nmol g^{-1}). These results also show large differences throughout the year in the concentrations of reactive-metals fraction, although with no clear pattern to explain these differences at the four sites.

Figure 7 shows the results obtained for trace metals associated with the pyrite fraction. The high salt marsh soils (MERA1 and LAD2) had the lowest concentrations, which tended to increase with depth, whereas the highest concentrations in the Esteiro soil were found in the upper layers (0–10 cm). The highest concentrations of trace metals-pyrite fraction in these soils corresponded to Ni (EST3: 160–1025 nmol g^{-1}; EST1: 1650–2659 nmol g^{-1}) followed by Mn (EST3: 140–450 nmol g^{-1}; EST1: 410–2500 nmol g^{-1}), Cu (EST3: 50–100 nmol g^{-1}; EST1 85–270 nmol g^{-1}), Zn (EST3: 20–160 nmol g^{-1}; EST1: 30–100 nmol g^{-1}) and Cr (EST3: 20–45 nmol g^{-1}; EST1: 30–60 nmol g^{-1}). The highest percentages of DTMP were also obtained for the EST1 soil (Figure 8), with values of more than 80% for metals such as Cu and Mn. The values for Ni ranged between 25 and 50%, for Zn between 5 and 25%, and those for Cr were never higher than 10%.

As with pyrite-Fe, the concentrations of metals associated with the pyrite phase and the DTMP both showed clear seasonal variation. There was a marked decrease in the concentration of metals associated with this phase in the soils colonized by *S. maritima* in summer. In contrast, in soils without vegetation, the highest concentrations of metals associated with the pyrite phase were found in summer.

Discussion

Variation in metal sulfides with depth

Salt marsh soils are considered to form a dynamic system in which the biogeochemical conditions depend on the interaction of a number of physical (tides, precipitations, storms) and biological (microbial activity, physiological changes in plants, etc.) factors (Hines 1991; Luther et al. 1991). A large number of relevant studies have been carried out on the effect of plants on the soils, with the genus *Spartina* being one of the most widely studied (see e.g. Howes et al. (1981); Howarth and Merkel (1984); Dacey and Howes (1984); Howes et al. (1986); Luther and Church (1988); Hines et al. (1989); Kostka and Luther (1995); Madureira et al. (1997)). Most of the studies show that soils colonized by plants display more strongly oxidizing conditions than soils without vegetation (see e.g. Howes et al. (1986); Howarth (1984); Madureira et al. (1997)). The main reason for this lies in the fact that plants facilitate the diffusion of oxygen into the soil, mainly through water loss at the soil surface by evapotranspiration (Dacey and Howes 1984; Howes et al.

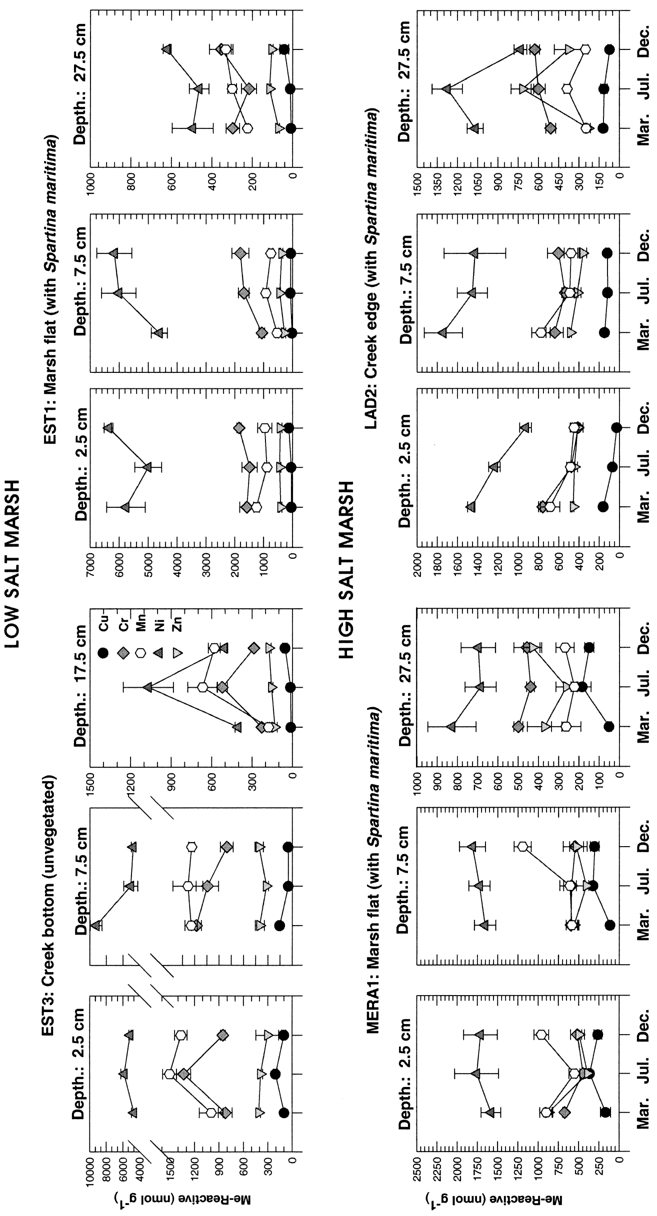

Figure 6. Concentrations of trace metals associated with the reactive fraction (average± 1 SE).

1986). Furthermore, species of the genus *Spartina* possess a well-developed arenchyma that allows the movement of oxygen from above ground to the roots, where it is released (Howarth and Teal 1979). The changes with depth in the metals associated with the pyrite phase in the high salt marsh (Mera and Ladrido) (Figures 5

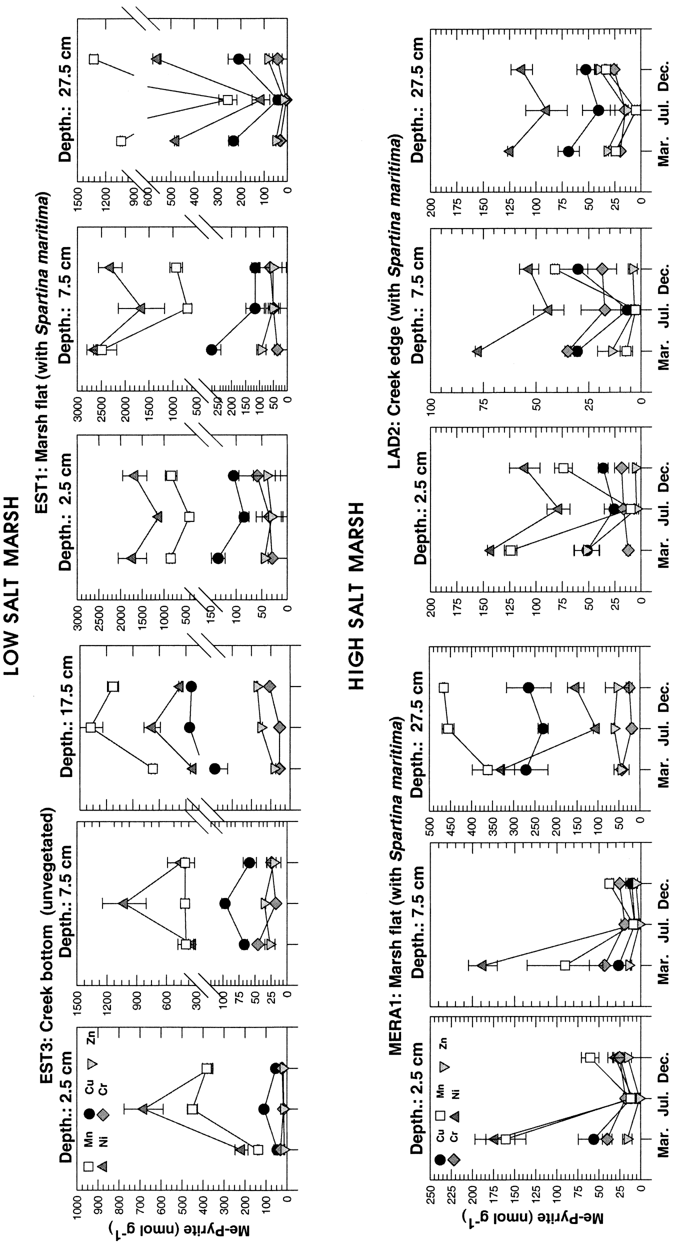

Figure 7. Concentrations of trace metals associated with pyrite fraction (average± 1 SE).

and 7) appear to fit in with this idea, as the concentrations found were relatively low in the surface layers of the soil, where suboxic conditions prevailed (MERA1, 0–10 cm Eh, 172–159 mV; LAD2, 0–10 cm. Eh, 121–127 mV, Otero et al.

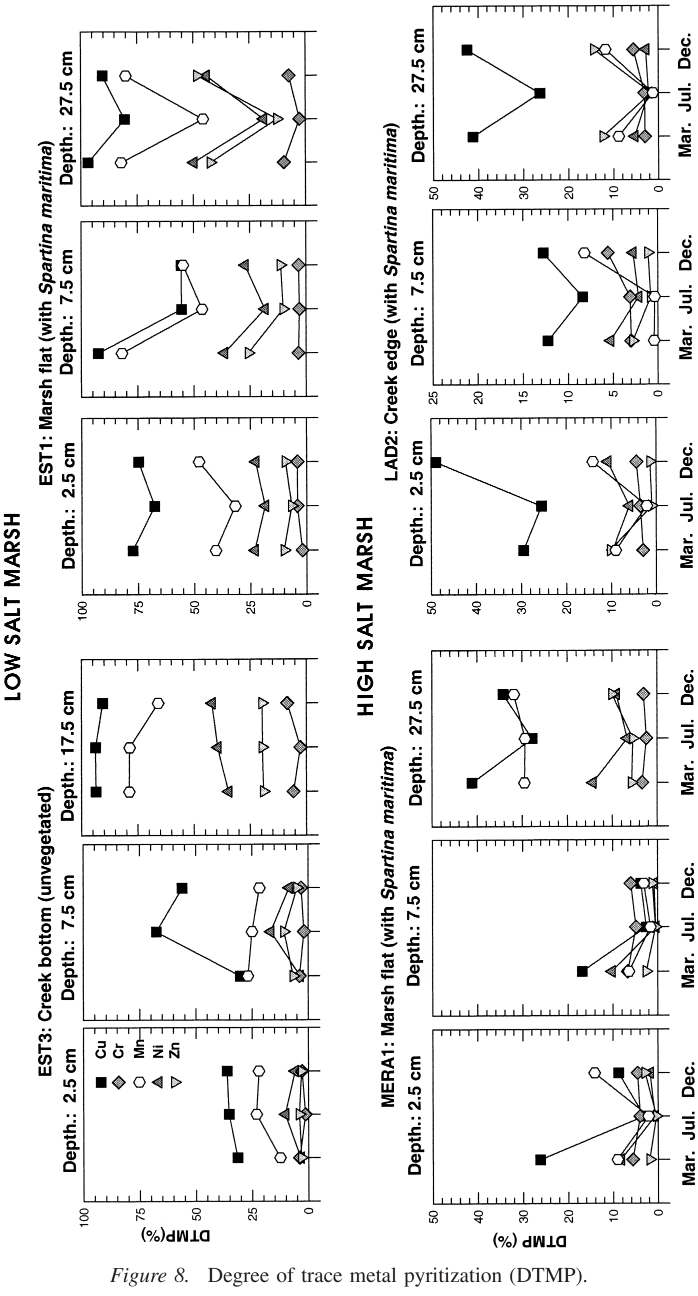

Figure 8. Degree of trace metal pyritization (DTMP).

(2000a)). This is possibly a consequence of a shorter period of flooding and the high density of roots and rhizomes present (Figure 2). The amount of live roots decreased considerably with depth and the redox conditions were more reducing

(Eh: −81 and −17 mV at 27.5 cm for MERA1 and LAD2 respectively; Otero et al. (2000a)). The concentrations of AVS in the high salt marsh were low in the surface layers and at depth, except in, for example, the MERA1 soil in spring (10.14 ± 3.66 μmol g^{-1} and 9.73 ± 9.1 μmol g^{-1} at 2.5 and 7.5 cm, respectively) and LAD2 in winter (40.8 ± 2.3 μmol g^{-1}, at 2.5 cm depth) (Figure 4). The highest concentrations of AVS were found in the surface layers of soil in the high salt marsh coinciding with the presence, in the top 10 cm, of black mottles (5BG 2/1 in wet samples), which indicate the presence of metastable Fe sulfides (possibly mackinawite, FeS, and greigite, Fe_3S_4), in an olive grey matrix (5Y 4/1), whereas at depth (27.5 cm) the color is uniformly dark grey (5Y 3/1). The presence of metastable metal sulfides in the surface layers may be due to the existence of labile forms of organic matter (e.g. root exudates), which stimulate the activity of sulfate-reducing bacteria (SRB) and create discrete anoxic microenvironments (Howarth 1984). This is in accordance with the results of previous studies in which the highest concentration of SRB in the surface layers of marine sediment corresponded with suboxic conditions (Jørgensen and Bak 1991).

The low salt marsh soils were characterized by having higher concentrations of metal sulfides than those of the high salt marsh (Figures 4, 5 and 7). These results can be explained in terms of the long periods of flooding to which these soils are subjected every day, which lead to anoxic conditions in the surface layers (e.g., EST1, at 2.5 cm, Eh: −90 mV; Otero et al. (2000a)). The highest concentrations of metal sulfides (AVS, pyrite metals) were found in the surface layers of the EST1 soil (with *Spartina*), and were also higher than those in the creek bottom (EST3, no vegetation). Similar results have been found in other studies (e.g. Giblin (1988)) and may be explained by the effect of the plants in anoxic soils. On the one hand, the root exudates favour the activity of the SRB and on the other, they facilitate the partial oxidation of the sulfides generated by the SRB, giving rise to the formation of polysulfides that allow formation of pyrite, according to reaction (4) (Rickard 1975; Giblin and Howarth 1984; Giblin 1988).

$$Fe^{2+} + S_5^{2-} + HS^- \rightarrow FeS_2 + S_4^{2-} + H^+ \tag{4}$$

Studies carried out with different macrophyte species (*Zostera noltii*, Isaksen and Finster (1996) and *Oriza sativa*, Wind and Conrad (1997)) have shown that the rates of sulfate reduction and concentrations of AVS and pyrite were higher in soils with plants than those without plants. These results can be explained by the fact that under anoxic conditions plants release fresh, low molecular weight organic compounds (e.g. ethanol, malate; Mendelssohn et al. (1981)). These can be used directly by the SRB (Hines et al. 1989) or can even serve as a substrate for fermentative bacteria that generate hydrogen and short chain fatty acids as by-products; these, along with acetate are the most important substrates for SRB (Sørensen et al. 1981; Widdel 1988; Parkes et al. 1989). However, in a previous study, Otero et al. (2000b) found that these soils did not contain high levels of sulfides (ΣH_2S), which is understandable if we assume that under weakly oxidizing conditions (e.g. entrance of air at low tides or infiltration of tidal water in the spaces surrounding roots;

see Giblin (1988)) sulfides can be oxidized to polysulfides that react with Fe^{2+} to form pyrite directly, according to reaction (4). The formation of pyrite according to reaction (4) implies that the interstitial water becomes oversaturated with pyrite but not with Fe monosulfides (AVS). This condition may occur when the concentrations of sulfides are relatively low and the pH close to 6 Howarth (1979); Giblin and Howarth (1984); Giblin (1988), a situation similar to that found in the EST1 soil in a previous study (EST1: (ΣH_2S = 1.4, 2.5 and 14.9 μM at 2.5, 7.5 and 22.5 cm, respectively, and pH 6–7, Otero et al. (2000b)). It is possibly for this reason that the concentration of pyrite in the low salt marsh soils was clearly higher than the AVS fraction (Figures 3 and 4). Furthermore, this reaction (4) allows much more rapid formation of pyrite (in days) than reaction (5), which takes place in subtidal environments and which requires long periods (years) (Berner 1970) because it involves the reaction between two solids to form pyrite. However, more recent studies have shown the importance of considering FeS as a precursor of pyrite (see e.g. Rickard and Luther (1997); Rickard (1997); Chadwell et al. (1999)). According to these authors, FeS can react with H_2S to form pyrite, as in reaction (6). The latter process is even faster than reaction (4) and is in accordance with the low concentrations of AVS and ΣH_2S found in these soils.

$$FeS_{(s)} + S^{o}_{(s)} \rightarrow FeS_{2(s)} \tag{5}$$

$$FeS_{(s)} + H_2S_{(aq)} \rightarrow FeS_2 + H_{2(g)} \tag{6}$$

The first of these processes – reaction (4) – appears to occur under partially oxic to anoxic conditions, where there is a low oxygen content, whereas the second – reaction (6) – takes place under strictly anoxic conditions (Rickard 1997). Previous studies have shown that these conditions may occur and even coexist in salt marsh soils (Hines 1991; Luther et al. 1991; Otero 2000).

The higher concentration of AVS in the surface layers than at depth appears to confirm the hypothesis of Nedwell and Abram (1978) that in anoxic environments, the sulfide generated in the deepest part of the soil diffuses towards the surface where it is trapped as FeS. This occurs because at depth, the concentration of reactive-Fe is very low (Figure 5), with only the most refractory forms remaining (Kostka and Luther 1995).

In addition, the large differences in DTMP of different metals in any one soil at a particular depth, should be noted; the degree of pyritization was, in decreasing order, Cu ~ Fe>Mn>Ni>>Zn>Cr (Figures 5 and 7). These results are in accordance with those found in previous studies (see e.g. Huerta-Díaz and Morse (1992); Morse et al. (1993)) and the explanation for each of the metals is based on kinetic and thermodynamic considerations (for more detail see Morse and Luther (1999)).

Seasonal variations in metal sulfides

There were large seasonal variations in all soils studied; this was evident in the interstitial water (Figure 3) as well as in the solid fraction (Figures 4, 5, 7 and 8).

In the interstitial water seasonal changes mainly affected the superficial layers of the soil, and there was an increase, in all soils, in electrical conductivity in summer due to the general effect of the increase in temperature on evapotranspiration and also the decrease in rainfall in summer (de Leeuw et al. 1991).

Different results were observed for the pH of the interstitial water and the pyrite fraction and two different patterns, in terms of the effect of the presence or absence of plant cover on soils, were found. The situation in the soil in the creek bottom (EST3, unvegetated) is in accordance with the effect of temperature on microbial activity. In summer, when the temperature is high, the activity of the SRB increases (see e.g. Jørgensen (1977); Abdollahi and Nedwell (1979); Roden and Tuttle (1993); Holmer and Kristensen (1996)), thus producing an accumulation of AVS and pyrite in the soil (Leonard et al. 1993; Holmer and Kristensen 1996) and an increase in the pH of the interstitial water (Berner 1970) (which should in the range between 6.9 and 8.3 – Ben-Yaakov (1973)), all of which is in accordance with our results (Figure 3). The low concentrations of metal sulfides found in winter and spring show that the destructive processes outweigh the formative processes. In these environments, the reoxidation of the metal sulfides may be due to molecular oxygen present in sea water, to Fe (III) organic complexes or to the action of chemolithotrophic microorganisms (e.g. species of the genus *Thiobacillus*) (Nordstrom 1982; Luther et al. 1986; King 1988; Luther et al. 1992; Moeslund et al. 1994).

The results obtained in the soils colonized by *S. maritima* show a contrasting trend, i.e., accumulation of metal sulfides (mainly pyrite metals) in winter and spring and a decrease in summer, which in turn leads to a large decrease in the pH of the interstitial water (Figures 3, 4, 5, 7 and 8). Similar patterns have been found in soils colonized by *S. alterniflora*, in previous studies (Giblin and Howarth 1984; Howes et al. 1986; Luther and Church 1988; Kostka and Luther 1995). The explanation for these results centres on the fact that during the growth period of *Spartina* (summer) there is net destruction of pyrite because this plant facilitates the entrance of air into the soil by the previously mentioned processes (evapotranspiration, transport of oxygen via arenchyma, etc.) leading to the oxidation of pyrite.

Evapotranspiration, which is the main factor favouring the entrance of air into these soils (Dacey and Howes 1984; Howes et al. 1986), decreases in winter and early spring mainly because of the lower temperatures found at these times, compared to that during the summer (Carballeira et al. 1983). Furthermore, according to Hines et al. (1989) sulfate reduction increases in the spaces surrounding the roots of *S. alterniflora* in spring, because during growth of aerial parts, this species releases low molecular weight organic compounds through its roots and these serve as a substrate for SRB, whereas during flowering, this process ceases, thus decreasing their activity. In our study area, flowering in this species occurs between June and September (Mayor and Díaz 1977). Thus, outside of this period, the activity of the SRB is stimulated by the exudates released through the roots. In the high salt marsh, where the period of tidal flooding is much shorter, wetting of the soil due to rainfall can favour the formation of metal sulfides during the autumn-spring period (Kostka and Luther 1995), when more than 80% of the annual rainfall occurs (Carballeira et al. 1983).

Mobilization of trace metals (Ni, Cu, Zn and Cr) by oxidation of the pyrite fraction and its relation to bioavailibility

The bioavailability and toxicity of heavy metals depend on the forms in which these metals are found associated with the different components of soils and sediments (organic material, clays, oxides and hydroxides of Fe and Mn, sulfides). One of the current problems in these studies is not being able to define the metal fraction that is biologically available in a sediment or soil (Ankley 1996). Our results showed that there were high concentrations of reactive-Cr and Ni in soils from the Esteiro salt marsh, whereas concentrations of reactive-Cu and Zn were similar in all three salt marshes (Figure 6). The Esteiro salt marsh soils contained much higher levels of trace metals associated with the pyrite fraction than the soils from Mera and Ladrido, with Ni and Cu being found at the highest concentrations (Figures 7 and 8). Furthermore, the ratio of SEM(ΣCu,Ni,Zn)/AVS in the summer was found, in a previous study, to be greater than 1 in Mera and Ladrido soils, whereas in Esteiro soils it was always less than 1 during the three months considered (Otero et al. 2000c). According to the model of Di Toro et al. (1990, 1992), this implies that in the latter salt marsh divalent metals should not be bioavailable because most of the metal sulfides present in anoxic soils are stable and rather insoluble (Krauskopf 1956), and they have a direct influence on the concentration of metals in the interstitial water of anoxic soils and sediments (Boulegue et al. 1982). However, significantly higher levels of Ni and Cu were found in a population of the marine polychaete, *Nereis diversicolor*, from the Esteiro salt marsh, than in populations from Mera and Ladrido, whereas there were no significant differences in levels of Cr (despite the high levels of reactive-Cr in Esteiro soils) (Otero et al. 2000c). These authors therefore concluded that metal sulfides may be an important source of bioavailable metals in this species, more important than reactive-trace metals and independently of the ratio of SEM/AVS. Metals associated with the pyrite fraction become bioavailable on being oxidized, and in the specific case of *N. diversicolor* this may be favored by the construction of burrows in the soil by the worms themselves.

The results of the present study show that oxidation of the pyrite fraction that occurs in spring and summer may lead to the mobilization of large quantities of trace metals. Therefore, in the surface layers of the EST1 soil, the decrease in the pyrite fraction led to the release of large amounts of Ni (943.5 nmol g^{-1}; 55.3 $\mu g\ g^{-1}$) and Cu (154.3 nmol g^{-1}; 9.80 $\mu g\ g^{-1}$) and to a lesser extent of Cr (48.1 nmol g^{-1}; 2.50 $\mu g\ g^{-1}$) and Zn (14.7 nmol g^{-1}; 0.96 $\mu g\ g^{-1}$), although the results obtained for Zn should be considered with caution because of the limitations of the method for determining the fraction of Zn associated with sulfides (for more detail see Cooper and Morse (1998); Morse and Luther (1999)). In addition, the bioavailability of these metals may be favored by the decrease in the pH of the interstitial water, caused by the acidity generated by the oxidation of metal sulfides, and this increases the solubility of the metals.

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