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Juan Santos-Echeandía, Carlos Vale, Miguel Caetano, Patrícia Pereira, Ricardo Prego. Effect of tidal flooding on metal distribution in pore waters of marsh sediments and its transport to water column (Tagus estuary, Portugal). *Marine Environmental Research*, 2010, 70 (5), pp.358. 10.1016/j.marenvres.2010.07.003 . hal-00631692

HAL Id: hal-00631692

<https://hal.science/hal-00631692>

Submitted on 13 Oct 2011

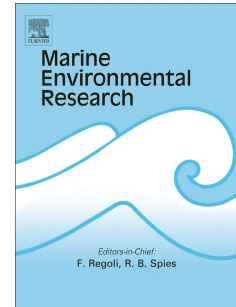
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Accepted Manuscript

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PII: S0141-1136(10)00112-1

DOI: [10.1016/j.marenvres.2010.07.003](https://doi.org/10.1016/j.marenvres.2010.07.003)

Reference: MERE 3465

To appear in: *Marine Environmental Research*

Received Date: 1 June 2010

Revised Date: 16 July 2010

Accepted Date: 18 July 2010

Please cite this article as: Santos-Echeandía, J., Vale, C., Caetano, M., Pereira, P., Prego, R. Effect of tidal flooding on metal distribution in pore waters of marsh sediments and its transport to water column (Tagus estuary, Portugal), *Marine Environmental Research* (2010), doi: 10.1016/j.marenvres.2010.07.003

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1 **Effect of tidal flooding on metal distribution in pore waters of marsh**
2 **sediments and its transport to water column (Tagus estuary, Portugal)**

3
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20
21 **Abstract**

22
23 Sediment cores and flooding water were collected at 0, 5, 10 and 50 minutes of tidal inundation in two
24 sites of the Rosário salt marsh located in the proximity of a heavy industrialised zone of Tagus estuary
25 colonised by pure stands of *Spartina maritima* (low marsh) and *Sarcocornia fruticosa* (high marsh). The
26 cores were sliced in 5-cm layers and sediment solids, pore water, and belowground biomass were
27 separated in order to measure Fe, Mn, Zn, Cu, Pb and Cd. The pore waters and sediments colonised by
28 *S. fruticosa*, as well as belowground biomass presented high concentrations of Zn, Cu, Pb and Cd.
29 Belowground biomass exceeded in one order of magnitude the metal levels in sediments. Abundant
30 belowground biomass and small dimension of *S. fruticosa* roots facilitates the root-sediment interactions
31 and presumably the metal retention in the higher marsh. The novelty of this work is the result of tidal
32 inundation on pore water concentrations of metals in salt marsh sediments and their exportation to the
33 water column. Concentrations in pore waters varied at minute scales, but 50 minutes after inundation
34 levels were comparable to the initial values. The metal levels in flooding water increased abruptly during
35 the first 10 to 20 minutes of inundation. The concentration peaks (Fe=60 µM, Mn=7.5 µM, Zn=1.7 µM,
36 Cu=550 nM, Pb=100 nM, Cd=1.7 nM) reached one to two orders of magnitude above the values found in
37 subsequent periods of inundation. The advective transports during the 50-min inundation during two daily
38 pulses of inundation were: Fe (9520 and 1640), Mn (24), Zn (220 and 82), Cu (74 and 16), Pb (13 and 15)
39 and Cd (0.3 and 0.08) for *S. maritima* and *S. fruticosa*, respectively. These quantities exceeded 3 to 4
40 orders of magnitude of the corresponding predicted diffusive fluxes (Fick 1st law) on a daily basis. This
41 work emphasizes the importance of tidal flooding over salt marsh sediments to the metal exportation to the
42 water column.

44 **Keywords:** Salt marsh; metals; pore-waters; Tagus estuary; advective; diffusive; fluxes; tidal flooding.

45

46 **1. Introduction**

47

48 The post-mobilisation of deposited iron, manganese and trace elements has been extensively studied in
49 deep and coastal sediments (e.g., review Sundby, 2006). On the basis of vertical properties of sediment
50 pore water (pH, redox potential, dissolved oxygen, sulphur compounds, nutrients and metals) and of metal
51 concentrations in solid sediments, it was proposed a sequence of reactions for the oxidation of the labile
52 sedimentary organic matter that reaches the sediment surface (Aller, 1977; Froelich et al., 1979). Oxygen
53 diffused from the water column into the upper sediment layer is the most favourable oxidant, and as
54 concentration decreases with the depth other oxidants are consumed to degrade the organic matter.
55 Reactions of similar free energy like nitrate and manganese oxide may overlap, (Burdige, 1993; van
56 Capellen and Yang, 1996). The chemical zonation in the upper sediments is characterised by gradients
57 with the depth, concentrations in pore waters exceeding largely the levels in overlying water. These
58 differences result in diffusive fluxes of solutes from sediments to water column (Berner, 1980). The
59 presence and activity of macroinvertebrates, causing perturbation and irrigation in the upper sediments,
60 enhances the sediment-water exchanges of solutes (Brune et al., 2000). The pore waters of inter-tidal
61 permeable sediments are renewed as tidal water floods the area causing exports of solutes by pulses
62 (Falcão and Vale, 1990; Caetano et al, 2007).

63

64 The salt marsh sediments have specificities with relevance to early diagenesis and sediment-water
65 exchanges such as: deliver of oxygen into subsurface layers of sediments by plant roots (Clothier and
66 Green, 1997; Caetano and Vale, 2002); supply of organic matter as roots dye (Caçador et al, 2004;
67 Pereira et al, 2007); presence of a mosaic of small channels in the upper sediments where tidal water
68 excurses during flooding. Works have reported two situations: anaerobic conditions in the rooting
69 sediments, meaning that delivered oxygen was insufficient to oxidise the large amount of degradable
70 organic matter (Haines and Dunn, 1985; Mendelssohn et al., 1995; Sundby et al., 2003); oxic to suboxic
71 conditions suggesting an excess of oxygen supplied to the sediment for the requirements. Various studies
72 have been focused on the influence of root-sediment interactions on sediment chemistry (Sundby et al.,
73 2003; 2005; Taillefert et al., 2007; Koretsky et al., 2008a,b) and response of plants (Windham et al., 2001;
74 Jacob and Otte, 2003; Weis and Weis, 2004; Caetano et al., 2007; 2008). However, sediment-water
75 exchanges in salt marsh are poorly documented (Taillefert et al., 2007).

76 Several studies showed that Tagus salt marshes, including Rosário, incorporate large quantities of
77 anthropogenic metals into the colonised sediments (e.g. Caçador et al., 1993; 1996; 2000; Sundby et al.,
78 1998; Caetano et al., 2007). Retention of contaminated particles from the anthropogenic sources (Vale,
79 1990) and root-sediment interactions appear to contribute to the metal enrichment of colonised sediments
80 and belowground biomass (Caçador et al., 1993; 1996). Recent studies in Rosário have shown that as
81 root dye the accumulated and sorbed metals are redistributed in the sediments (Pereira et al, 2007). Few
82 works have been focused on Tagus marshes pore waters. Reboreda and Caçador (2007) have reported
83 high concentrations of trace metals in bulk pore waters of rooting sediment. By using an amalgam
84 voltammetric microelectrode Sundby et al. (2003; 2005) proved that dissolved oxygen, Fe, Mn and Pb
85 concentrations in pore waters of Rosário salt marsh sediments vary seasonally.

86

87 This paper reports the changes of Fe, Mn, Zn, Cu, Pb and Cd concentrations in pore water and flooding
88 water during the first 50 minutes of the tidal inundation over sediments of the Tagus salt marsh (Rosário)
89 colonised by *Spartina maritima* and *Sarcocornia fruticosa*. On the basis of the registered concentrations it
90 was estimated the diffusive fluxes between sediments and overlying water during submerged periods. In
91 addition, it was calculated the amounts exported from the colonised sediments during the first 50 minutes
92 of tidal inundation. The magnitude of the two contributions to the water column is discussed.

93

94 **2. Material and methods**

95

96 **2.1. Study area**

97 The 340 km² of the Tagus estuary represent one of the largest transitional systems in Europe. Almost 40%
98 of the estuary is composed by inter-tidal mudflats and its southern and eastern shores contain extensive
99 areas of salt marshes colonised by *Sarcocornia fruticosa*, *Sarcocornia perennis*, *Halimione portulacoides*
100 and *Spartina maritima*. The marsh selected for this study (Rosário) is located in the southern shoreline of
101 the estuary (Figure 1). It covers an area of 200 ha (Crespo, 1993), being characterized by homogeneous
102 stands of *S. maritima* as a pioneer species in the lower part, pure stands of *H. portulacoides* across the
103 20-50 cm elevation transect, and *S. fruticosa* and *S. perennis* in the higher salt marsh. The marsh is fully
104 inundated twice a day by tidal action (2-4m of tidal amplitude) through a highly branched system of
105 channels that cross the elevation transect. The channels have 0.5 to 1.5 m depth promoting the inundation
106 of the higher marsh even at low amplitude tides. The study area is located in the proximity of a heavy
107 industrialised zone that includes several chemical plants. During the past four decades these industries
108 discharged effluents enriched in various contaminants into the estuary that were transported towards the
109 Rosário marsh by the semi-diurnal tidal flooding.

110

111

111 **2.2. Sampling strategy**

112 Sediment cores of 30-cm long were collected, as well as flooding water over the short period of tidal
113 inundation in the two sites of the Rosário salt marsh colonised by pure stands of *S. fruticosa* (high marsh)
114 and *S. maritima* (low marsh) and separated by less than 5 meters. Sampling took place in March 2008. At
115 low tide, four sediment cores were collected at each site, when sediment was exposed to the atmosphere,
116 and water retained in the main channel of the marsh was sampled by hand into acid pre-cleaned syringes.
117 One of the cores was sampled at each site to measure temperature, dissolved oxygen and salinity all
118 along its depth. When tidal water starts to flood each site, two additional sediment cores and flooding
119 water were collected at each time of inundation: 5, 10 and 50 minutes. Flooding water was sampled 1 cm
120 above the sediment surface directly into acid pre-cleaned syringes. All water samples were acidified with
121 suprapure HCl (pH < 2). The cores were sliced immediately after sampling in 5-cm layers, prepared
122 composite samples for each layer of the cores collected at each site, and material stored in acid
123 pre-cleaned HDPE vials avoiding air presence inside. Sampling took place in less than three minutes. Core
124 material consisted of dense rooting sediments with no evidence of burrowing worms, crabs or bivalves.
125 The water and sediment samples were kept in refrigerated boxes and immediately transported to the
126 laboratory. A more detailed description of these sampling procedures can be found in previous works
127 (Caetano et al, 2007).

128

129 **2.3. Sample treatment**

130 Pore waters were separated from the sediment layers by centrifugation at 10160 rcf $\times g$ for 30 minutes at
131 +4° C and filtered through 0.45 μ m cellulose acetate membranes. Filtration and subsequent manipulation

of the samples were carried out in a glove box under argon atmosphere in order to avoid alteration of the initial conditions (Caetano et al., 2007; Santos-Echeandia et al., 2009). Pore-water samples were acidified with suprapure HCl (pH < 2). The remaining solid material of each sediment layer was washed with Milli-Q water (18.2 M Ω .cm) smoothly using a teflon sieve of 212- μ m mesh size and tweezers to separate belowground biomass from sediment particles. Sediment particles and belowground biomass of each layer was oven dried at 40 °C and homogenised with an agate mortar. Procedures were done in order to subsequent determinations of total Fe, Mn, Zn, Cu, Pb and Cd concentrations in water, sediment and biological samples.

2.4. Methods

Dissolved oxygen was measured in sediment layers using a Diamond Electro-Tech needle electrode following the method described in Brotas et al. (1990). Temperature was measured with a Pt100 sensor. The salinity of flooding and pore waters was measured using a conductivity electrode. Each composite sample was divided in three subsamples and analyzed independently for trace metals. Total dissolved Mn and Fe in overlying, flooding and pore waters were determined by graphite furnace atomic absorption spectrometry (Varian SpectrAA Zeeman 220). Concentrations were determined using the interpolation method in a previously designed calibration curve. Detection limits (defined as three times the standard deviation) were 2.6 nM for Fe and 1.9 nM for Mn. The reproducibility, measured as the RSD was always lower than 5%. The rest of total dissolved metals (Zn, Cu, Pb and Cd) were determined by means of stripping voltammetry using a Metrohm VA-694 equipped with a hanging mercury drop electrode (HMDE) as the working electrode, Ag/AgCl as the reference electrode, and a Pt wire as the counter-electrode. Prior to determination, samples were diluted (when necessary) and UV-digested for 1 h using UV-Digester equipped with a high-pressure mercury lamp of 200 W (Achterberg and Van Den Berg., 1994). The simultaneous determination of Zn, Cu, Pb and Cd in the dissolved phase was carried out using the method of standard additions by means of differential pulse anodic stripping voltammetry (DPASV). One blank was run every five samples and results were blank-corrected. The accuracy of the analytical procedure was assessed by the analysis of three different certified reference materials (CASS-4, SLEW-3 and SLRS-4), obtaining good agreement with the certified concentrations (Table 1). The detection limits of the analytical procedure, defined as three times the standard deviation of the blanks, were 0.5 nM for Zn, 0.7 nM for Cu, 22 pM for Pb and 20 pM for Cd. The precision of the analysis measured as relative standard deviation was: 5–10% for Zn (at 20 nM), 5–10% for Cu (at 10 nM), 5–10% for Pb (at 300 pM) and 25–40% for Cd (at 60 pM).

Approximately 100 mg of sediment was mineralized completely with 6 cm³ of HF (40%) and 1 cm³ of Aqua-Regia (HCl-36%:HNO₃-60%; 3:1) in closed Teflon bombs at 100°C during 1 hour. The bomb contents were evaporated to near dryness in Teflon vials, redissolved with HNO₃, heated for 20 min at 75 °C and diluted to 50 cm³ with Milli-Q water (Caetano et al., 2008). Plant material (\approx 200 mg) was digested with a mixture of HNO₃ (60%) and H₂O₂ (30%) in Teflon bombs at 60 °C for 12 hours and at 100 °C for 1 hour. Two procedural blanks were prepared using the same analytical procedure and reagents, and included within each batch of 20 samples. Iron, Mn and Zn were analysed by flame atomic absorption spectrometry (Perkin Elmer AA100) with an air-acetylene flame. Metal concentrations were determined with the standard additions method. Concentrations of Cu, Pb and Cd were determined using a quadrupole ICP-MS (Thermo Elemental, X-Series) equipped with a Peltier Impact bead spray chamber and a concentric Meinhard nebulizer. Coefficients of variation for metal counts (n=5) varied between 0.5 and 2%. The precision and accuracy of each metal concentration measurements, determined through repeated analysis of certified references materials (MESS2, PACS2 and MAG1 for sediment and BCR60

177 and BCR61 for plant material) (Table 1), using In as internal standard, were 1-4% and 2-5%, respectively
 178 (data not shown). Procedural blanks always accounted for less than 1% of the total metal concentrations in
 179 samples.

180

181

182 **2.5. Estimation of sediment-water exchange**

183 Different levels of trace metals between pore waters of upper sediment layers and overlying water imply
 184 their export/import to/from the water column. Since inter-tidal sediments are alternatively covered with
 185 water and exposed to the atmosphere, two types of fluxes should be considered: diffusive fluxes during the
 186 submerged period and advective transport associated with the tidal flooding.

187 *Diffusive flux.* On the basis of the measured concentration profiles, the benthic fluxes of trace metals are
 188 predicted by Fick's first law of diffusion (Berner, 1980):

$$189 F_{\text{dif}} = \phi D_s (C_0 - C_p) / \Delta x \quad (1)$$

190 where ϕ is the porosity, D_s is the diffusion coefficient, $C_0 = [\text{Me}]$ in pore water of the topmost sediment
 191 layer, $C_p = [\text{Me}]$ in overlying water and x is the space co-ordinate, negative into sediment and origin at
 192 sediment-water interface. Sediment porosity (ϕ) was calculated from the following equation:

$$193 \phi = m_{\text{PW}} / (m_{\text{PW}} + m_s / 2.65 + m_r / 1.2) \quad (2)$$

194 where m_{PW} represents the pore water mass, m_s the sediment solids mass and m_r the roots mass. The
 195 2.65 value is the density of the sediment considering to be formed by pure mud (Berner, 1980) and the 1.2
 196 is the density of the roots. The diffusion coefficient (D_s) was calculated from the following equation:

$$197 D_s = \phi^2 D_0 \quad (3)$$

198 where D_0 represents the diffusion coefficient of a metal at a given temperature (calculated from Li and
 199 Gregory, 1974).

200 *Advective flux.* As tidal water floods over inter-tidal sediments the pore water solutes tends to be exported.
 201 The associated pressure difference and tidal water movement are the driven mechanism for this export. By
 202 using the temporal variation of trace metals concentrations in the flooding water the advective transport (T)
 203 to the water column are calculated using the following expression (Caetano et al., 2007):

$$204 T = \sum [(C_{t+1} - C_t) / 2 - C_i] (h_{t+1} - h_t) \quad (4)$$

205 where C_{t+1} and C_t are $[\text{Me}]$ in the flooding water at times $t+1$ and t and h_{t+1} , C_i is the residual concentration
 206 (lowest value obtained for the flooding water) and h_t are the water depth at the same times. It was
 207 observed that water depth during the field measurements increased on the average 0.5 and 1 cm each
 208 minute of inundation in *S. fruticosa* and *S. maritima* areas, respectively. The transport of trace metals is
 209 calculated for the first 50 minutes of inundation. Since inter-tidal sediments are inundated twice a day, the
 210 advective daily flux (T) was multiplied by a factor of two.

211

212 **2.6. Statistics**

213 Statistical software (Statistica 7.0) was used for statistical analyses. Considering the number of samples,
 214 analysis was performed by the non-parametric Kruskal–Wallis test in order to detect metal concentrations
 215 differences in sediment solids, belowground biomass and pore water. Correlations between parameters
 216 were searched by Spearman rank correlations ($p < 0.05$).

217

218

219 **3. Results**

220

221 **3.1. Metal concentrations in rooting sediments**

222 The rooting sediments from the two sampling sites contained between 10.2 and 12.3% of Al, a proxy for
223 the preponderance fine grain particles (Windom et al, 1989). The sediments colonised by *S. fruticosa*
224 presented significantly higher levels of Pb ($p<0.05$), Zn, Cu and Cd ($p<0.1$) than by *S. maritima* (table 2).
225 Maximum concentrations reached 290, 2408, 83 and $4.1 \mu\text{g g}^{-1}$, respectively. The depth variation of the
226 metal concentration differed in the two sampling sites. Whereas the first 15-cm depth of sediments
227 colonised by *S. maritima* exhibited elevated concentrations of Cu and Pb, the 15-25 cm layers of
228 sediments colonised by *S. fruticosa* showed enhanced levels of Cd and Zn.

229

230 **3.2. Plant belowground biomass**

231 Belowground biomass of the two plants was concentrated in the upper 15-cm depth, decreasing uniformly
232 with the depth (table 3). The belowground biomass of *S. fruticosa* 3873 g m^{-2} , calculated as the amount
233 present the upper 25-cm, exceeded 1292 g m^{-2} found for *S. maritima*. The roots of *S. maritima* were
234 thicker (maximum diameter of 3 mm) than the roots of *S. fruticosa* that were usually $<1 \text{ mm}$. These
235 observations combined with biomass point to a higher specific surface area of the belowground biomass of
236 *S. fruticosa*.

237

238 **3.3. Metal concentrations in belowground biomass**

239 The concentrations of Cu, Cd and Pb differed significantly ($p<0.05$ and $p<0.1$) in the belowground biomass
240 of the two plants (Table 3). The metal concentration varied with the depth, although levels were not
241 correlated to the quantity of roots presented at each layer. In fact, roots in the first 5-cm sediment layer
242 contained generally lower metal concentrations than in deeper layers with elevated biomass. The most
243 noticeable aspect is the accumulated levels of metals (Cu, Pb and Cd) in roots being one order of
244 magnitude above the concentrations found in the corresponding rooting sediments (tables 2 and 3). The
245 maximum concentrations ($\mu\text{g g}^{-1}$) in belowground biomass of *S. maritima* and *S. fruticosa* were: 3571 and
246 11017 (Zn), 212 and 334 (Cu), 1528 and 2739 (Pb) and 19 and 30 (Cd,) respectively.

247

248 **3.4. Pore water characteristics of sediments under air-exposed conditions**

249 Temperature of air-exposed sediments in the sampling period (early morning) ranged within a narrow
250 interval ($14\text{-}15 \text{ }^\circ\text{C}$) and showed no substantial differences between higher and lower marsh, and with the
251 depth. Table 4 presents the maximum and minimum values of parameters in pore waters of sediments
252 colonised by the two plants under air-exposed conditions. Salinity varied from lower ($32.4\text{-}33.6\%$) to higher
253 marsh ($28.5\text{-}31.8\%$), reflecting freshwater drainage to the upper area colonised by *S. fruticosa*. In both
254 cores dissolved oxygen remained $< 2 \mu\text{M}$ at all depths. Maximum values of dissolved Fe and Mn in
255 sediments colonised by *S. maritima* and *S. fruticosa* were in the same order of magnitude (12.7 and 65.3
256 μM of Fe and 34.4 and $14.5 \mu\text{M}$ of Mn, respectively). On the contrary, dissolved levels of the analysed
257 trace elements were one order of magnitude higher in the area colonised by *S. fruticosa*. The differences
258 had higher significance for Zn, Cu and Pb ($p<0.001$) than for Cd ($p<0.05$). Concentrations in pore waters of
259 *S. maritima* and *S. fruticosa* reached: 1.2 and $20.6 \mu\text{M}$ (Zn), 28 and 201 nM (Cu), 15 and 268 nM (Pb), 1.4
260 and 75 nM (Cd), respectively.

261

262 **3.5. Time variation of metal concentrations in pore waters**

263 Figures 2 and 3 compare the depth metal concentrations in pore waters of sediments at air-exposed
264 conditions ($t=0$), 5, 10 and 50 minutes after the tidal flooding of the marsh. Vertical concentrations at these

265 periods of time varied with the analysed elements at the two surveyed sites. It is remarkable that pore
266 waters at t=5 min. of the surface sediment layers in the marsh colonised by *S. maritima* exhibited lower
267 metal concentrations than at t=0 (Figure 2). The differences were less clear for the higher marsh covered
268 with *S. Fruticosa* (Figure 3), meaning that tidal inundation had a lower effect on the modification of pore
269 water chemistry. The profiles at 10 and 50 minutes inundation pointed to establishing conditions towards
270 those registered at air-exposed period. This tendency was better observed in the lower marsh. The shape
271 of profiles differs in the two sites. Whereas pore waters from the upper layers of the lower marsh showed
272 higher metal concentrations, sub-surface maxima were found the pore waters of sediments covered by *S.*
273 *fruticosa*.

274

275 **3.6. Metal concentrations in flooding water**

276 The levels of Fe, Mn, Zn, Cu, Pb and Cd in the dissolved fraction of flooding water over the two sites of the
277 marsh increased abruptly in the first 2 to 5 minutes of inundation (figure 4). The sharp concentration peaks
278 (Fe=60 µM, Mn=7.5 µM, Zn=2.5 nM, Cu=550 nM, Pb=100 nM, Cd=1.7 nM) reached one to two orders of
279 magnitude above the values found in subsequent periods of inundation. Furthermore, these maximum
280 values are within the range of levels registered in pore waters (table 4). The maximum of Fe, Mn, Cu and
281 Cd concentrations in the area colonised by *S. fruticosa* exceeded the values observed in lower marsh (*S.*
282 *maritima*).

283

284 **4. Discussion**

285

286 A noticeable remark of the present work is the consistent differences of belowground biomass and Zn,
287 Cu, Pb and Cd concentrations in solid fine sediments, roots and pore waters between sites colonised by *S.*
288 *maritima* and *S. fruticosa*. Higher values were registered in the sediment column of the upper marsh
289 covered by *S. fruticosa*. Despite the daily flooding of the full marsh by the tide, differences of metal
290 concentrations in pore waters stand.

291

292 **4.1. The influence of belowground biomass on metal retention**

293 The sediments of the Rosário marsh and adjacent inter-tidal flats consist of fine particles contaminated by
294 discharges of the industrial complex localised in the southern part of the Tagus estuary. Various works
295 have pointed to the retention of these contaminants in inter-tidal flats (Vale, 1990) and in the adjoining salt
296 marshes (Caçador and Vale, 2001; Caetano et al., 2007). However, the levels of anthropogenic metals in
297 marsh sediments exceed largely the values found in the non-vegetated sediments (Caçador et al., 1993,
298 1996a,b, 2000). The enrichment is confined to the rooting sediments and varies with the plant species that
299 cover the marsh sediment. The differences found on metal concentrations between sediments colonised
300 by *S. maritima* and *S. fruticosa* corroborate the previous findings.

301 The iron-oxides plaque found on roots of plants in northern European and US marshes (Otte et al., 1989;
302 Mendelssohn et al., 1995; St-Cyr and Campbell, 1996; Cambrollé et al., 2008) increases the thickness in
303 Tagus marshes to form hollow cylinders around the roots (Vale et al., 1990). The growth of these
304 structures results from the reaction between O₂, which is delivered by roots during most time of the year,
305 and Fe (II) in pore waters that diffuses towards the roots (Sundby et al., 1998)). As observed in other
306 marshes (St-Cyr and Crowder 1990, Otte 1991, St-Cyr and Campbell 1996), trace elements are
307 incorporated in the iron-oxides (Vale et al., 1990; Caetano and Vale, 2002; Vale et al., 2003). However,
308 significant relationships between trace elements and Fe concentration in the bulk rooting sediments of the
309 two sites were not found (p<0,05), suggesting that Fe precipitates are not the major driver to retain trace

310 elements in sediments colonised by *S. fruticosa* and *S. maritima*. Enhanced levels of total Fe and Mn in
311 the 0-5 cm layer may indicate the precipitation of oxides by the O₂ diffused from the surface rather than
312 delivered by roots. Since these plants have a short or absence of dormancy in winter (Catarino and
313 Caçador, 1981) it is unlike a stop on the O₂ deliver. Therefore the lack of oxygen observed in the sediment
314 column may indicate that compounds, like ammonium and sulphide, have consumed the supplied O₂.
315 Reduced species of Fe and Mn are also oxidised by root-derived oxygen, although the higher background
316 concentration do not allow differentiating until oxygen is in excess. Nevertheless, it appears that oxygen
317 delivered by the roots was not used to oxidise substantial amounts of Fe(II) in Fe(III) and of Mn(II) in
318 Mn(IV) and enhance the Fe and Mn in solid sediments, since sediment layers of higher belowground
319 biomass did not present increases of these elements.

320 Previous works on the Tagus marshes indicated a correlation between metal concentration in rooting
321 sediments and loss on ignition (Caçador et al., 1999). These relationships pointed to the importance of
322 organic matter, which at these layers derives mainly from the decomposing roots, on retention of trace
323 elements (Jones, 1998; Weis and Weis, 2004). This effect may be emphasised in marshes with abundant
324 belowground biomass, like the Rosário site colonised by *S. fruticosa*. Because belowground biomass of
325 this plant was found to have an elevated specific area (Sundby et al., 2005), sediment-pore water
326 interaction is expected to be higher than in the sediments covered with *S. maritima*. The enhancement of
327 this interaction, among others related to the different strategies of the two studied plants (Groenendijk and
328 Vink-Lieavaart, 1987; Caetano et al., 2008), may induce higher levels of trace elements found in the roots
329 of *S. fruticosa* in comparison to the *S. maritima*. A study of metal release from decomposing root litter in
330 Tagus marshes over a 7-month period pointed to complex behaviour of the surveyed metals. Litter in
331 spring may act as a cation exchanger adsorbing Cu and Pb from sediments, while Zn and Cd stocks
332 decreased progressively in plants (Pereira et al, 2007; Caçador et al., 2009). As metals exchange with the
333 decomposing litter the metal concentrations in pore waters may enhance. The litter decomposition and the
334 release of exudates from live roots are a continuous source of organic matter that has direct impact on
335 metals availability (Sundby et al., 2005; Pereira et al, 2007). In a previous study over the annual growth
336 cycle dissolved sulphide was absent in rooting sediments, even in periods of lack of oxygen (Sundby et al.,
337 2003). Assuming similar conditions in the sediment, no substantial amounts of metals in pore waters are
338 expected to precipitate as metal sulphides. In the absence of this effective removal, the peak
339 concentrations of dissolved Cu, Cd, Pb and Zn in pore waters are presumably linked to organic complexes
340 stabilized in solution.

341

342 **4.2. Metal exchange between sediment and overlying water at submerged period**

343 Vertical pore water profiles differed in the two studied sites. At 50-min after inundation of the marsh and at
344 air-exposed conditions, *S. maritima* sediments exhibited in general higher levels of Fe, Mn, Zn, Cu, Pb and
345 Cd in pore waters of the upper layers. Pore waters of *S. fruticosa* sediments showed a sub-surface
346 maximum of Zn, Pb and Cd concentrations below the layers of higher root biomass (table 3) and of Cu
347 levels at layers of higher root density. The different metal concentration between pore water of 0-5 cm
348 layer and overlying water induce a diffusive flux estimated by the Fick's 1st law (equation 1). The similarity
349 of concentrations at t=50 min. of inundation and at air-exposed conditions (t=0) suggests that no
350 exchanges are foreseen by molecular diffusion after that period of inundation. The best estimation for pore
351 water concentrations under submerged period is, thus, the average between concentration at t=50 min and
352 t=0 (assumed as representative of the final submerged period). It was considered that sediments covered
353 by *S. maritima* and *S. fruticosa* are submerged 6 and 4 hours per tide, respectively. Table 5 shows the
354 predicted amounts (nmol m⁻² d⁻¹) of metals exchanged daily with the overlying water. Sediments covered

355 by *S. maritima* imported Cu and Zn (-16 and -137 nmol m⁻² d⁻¹) and exported Fe, Mn, Pb and Cd (105,
356 4800, 0.4 and 0.04 nmol m⁻² d⁻¹, respectively). The sediments colonised by *S. fruticosa* exported larger
357 amounts of Zn, Mn, Cu and Cd (570, 290, 80 and 8, respectively) and imported Fe and Pb (-510 and -4,
358 respectively). In these calculations it was considered the value of porosity (ϕ) in equation (2) commonly
359 used for fine sediments. However, the presence of micro-channels around the belowground plant parts
360 near the sediment-water interface may facilitate the exchange of solutes by molecular diffusion. Therefore
361 exchanges of metals between sediment-water interfaces driven by diffusive gradients are underestimated.

362

363 **4.3. Effect of tidal flooding on metal concentration in pore waters and overlying water**

364 The decrease of metal concentrations in pore water during the first 5 and 10 minutes of inundation (figures
365 2 and 3) clearly indicates that tidal flooding modifies the chemical equilibrium in pore water of the upper
366 sediment layers. These results are in line with studies performed in Ria Formosa (SW Europe) showing the
367 modification of nutrients, Cu and Cd profiles in pore water as permeable inter-tidal sediments are flooded
368 (Falcão and Vale, 1990; Caetano et al, 2007). During inundation overlying water containing lower metal
369 concentrations mixes with pore waters of the upper sediment layers. Alterations associated with
370 precipitation/dissolution and sorption/desorption during this period should not also be excluded (Caetano
371 et al., 1997; Heuttel et al., 1998). In general, profiles registered 50 minutes after the inundation were closer
372 to the initial conditions suggesting that equilibrium have been nearly achieved. In the first minutes of tidal
373 flooding all determined elements increased considerably their concentrations in water that inundates the
374 sediments from both sites. The abrupt increases are in line with the decreases of metal concentrations in
375 pore water pointing to a substantial pulse of solutes exported to the water column. Although different
376 trends on pore water concentration were found between both plants during the inundation the overall
377 process induced by tide was similar in *S.maritima* and *S. Fruticosa*.

378

379 **4.4. Advective transport of metal associated to tidal flooding**

380 The increase of metal concentrations in overlying waters during the first minutes of tidal flooding is
381 coherent with the decrease on the pore waters and points to the escape of metals from pore water of salt
382 marsh sediments (figure 3). On the basis of these measurements one may calculate the advective
383 transport to the water column using the equation (4). Table 4 presents the amounts of Fe, Mn, Zn, Cu, Pb
384 and Cd on a daily basis, which correspond to the sum of two pulses associated with the two tidal flooding
385 periods. The most remarkable aspect is that quantities are three to four orders of magnitude above those
386 predicted by Fick's 1st law. For example, the diffusive flux of Pb was between -4 and 0.4 nmol m⁻² d⁻¹ while
387 advective transport reached 13-15 x 10³ nmol m⁻² d⁻¹. Previous works on permeable inter-tidal sediments
388 have shown the importance of pulse mechanisms associated with the tidal flushing on sediment-water
389 exchanges (Falcão and Vale, 1990; Caetano et al, 2007). The current work permits to extend the
390 relevance of this mechanism to salt marsh sediments covered with *S. maritima* and *S. fruticosa*, two plants
391 commonly found in south European salt marshes.

392 Although sediments of Rosário marsh consist of fine-grained sediments, the micro-channels formed by the
393 root mosaic have probably a major role on water and solutes circulation driven by the increase of pressure
394 on sediments as tide inundates the area. The amounts exported by this mechanism were higher in *S.*
395 *maritima* than in *S.fruticosa* area, although the metal concentration in pore waters of sediments covered by
396 this plant exceeded largely that found in *S. maritima*. The most plausible explanation for this apparent
397 discrepancy is the smaller size of the *S. fruticosa* roots and their random and intricate distribution which
398 create more obstacles to pore water flushing during the first moments of tidal flooding. Otherwise, *S.*
399 *maritima* has thicker tillers and roots promoting increased exchanges between pore water and flooding

400 waters. These results are important from an ecological point-of-view since marshes subjected to tidal
401 inundations also act as a source of dissolved metals from sediment to the water column being available for
402 macrobenthic organisms and small fishes. These findings also support a previous works that showed the
403 transfer of trace metals to marine environment across the detritus generated by salt marsh plants (Pereira
404 et al, 2007; Caçador et al., 2009). Considering the magnitude and the periodicity of advective export,
405 successive tidal inundation would deplete the pool of metals in the salt marsh sediment if not balanced by
406 equivalent inputs. Conservation of mass requires an import during the submerged period by settling of
407 suspended particles, advection of water, deposition of particles during ebb tide and exchanges of metals
408 with belowground biomass.

409

410 **5. Conclusions**

411 This work emphasizes the importance of tidal flooding over salt marsh sediments to the metal exportation
412 to the water column. The accumulation of metals in the sediments close to the plant roots, together with
413 the arriving of the tide results in an important exportation of metals from the pore water of salt marsh areas
414 to the overlying waters. More than the diffusive flux, the advective transport has been demonstrated to be
415 an important contribution to the overall flux. This type of fluxes should be taken into account when
416 assessing trace metal contamination problems and remediation in salt marsh areas. Furthermore, one may
417 speculate that inundation of coastal areas associated with the climatic changes, either to increase of water
418 levels or floods will result in additional fluxes of trace elements from intertidal areas to the water column.

419

420

421 **Acknowledgments**

422 The authors wish to thank the colleagues Rute Cesário (IPIMAR) and Ana García-Blanco (IIM-CSIC) for
423 the field work and technical assistance. This article is a contribution to the Spanish-Portuguese Actions of
424 references 2007PT0021 and FCT/CSIC9/CSIC/08. Dr. Santos-Echeandía would like to thank the Basque
425 Government for the financial support (post-doctoral contract).

426

427

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Table 1. Certified values and measured average concentrations of Fe (μM), Mn, Zn, Cu, Pb and Cd (nM) in the reference materials in SLEW-3 (estuarine water), CASS-4 (nearshore seawater) and SLRS-4 (river water); number of replicates in brackets.

	Values	Fe (μM)	Mn (nM)	Zn (nM)	Cu (nM)	Pb (nM)	Cd (nM)
SLEW-3	Obtained			2.72 \pm 1.18 (n=3)	27.0 \pm 0.07 (n=3)		0.386 \pm 0.07 (n=3)
	Certified			3.07 \pm 0.57	24.39 \pm 1.89		0.427 \pm 0.036
CASS-4	Obtained			5.8 \pm 1.0 (n=13)	9.4 \pm 0.5 (n=6)	0.068 \pm 0.012 (n=13)	0.236 \pm 0.033 (n=13)
	Certified			5.8 \pm 0.9	9.3 \pm 0.8	0.047 \pm 0.017	0.231 \pm 0.027
SLRS-4	Obtained	0.23 \pm 0.02 (n=5)	63.5 \pm 2.3 (n=5)				
	Certified	0.18 \pm 0.09	61.3 \pm 3.3				

Table 2 – Concentrations and standard deviation of Fe (%), Mn, Zn, Cu, Pb and Cd ($\mu\text{g g}^{-1}$) in sediment solids colonised by *Spartina maritima* and *Sarcocornia fruticosa* from Rosario salt marsh. Probability (p) was provided by Kruskal-Wallis test considering the data of all depths.

Colonised sediment			
	Depth (cm)	<i>S. maritima</i> (lower marsh)	<i>S. fruticosa</i> (higher marsh)
Fe ° (%)	0 - 5	4.3±0.3	4.7±0.4
	5 - 10	3.9±0.1	3.8±0.3
	10 - 15	4.0±0.1	4.8±0.2
	15 - 20	4.0±0.2	6.8±1.5
	20 - 25	4.7±0.1	5.6±0.5
Mn ° ($\mu\text{g g}^{-1}$)	0 - 5	311±26	234±44
	5 - 10	178±11	190±22
	10 - 15	172±1	189±28
	15 - 20	170±17	210±28
	20 - 25	181±14	247±14
Zn * ($\mu\text{g g}^{-1}$)	0 - 5	482±24	333±22
	5 - 10	501±36	465±61
	10 - 15	513±13	890±183
	15 - 20	596±101	1075±304
	20 - 25	728±2	2408±131
Cu * ($\mu\text{g g}^{-1}$)	0 - 5	65±18	71±12
	5 - 10	71±4	50±22
	10 - 15	65±1	79±10
	15 - 20	27±3	83±33
	20 - 25	41±2	83±36
Pb ** ($\mu\text{g g}^{-1}$)	0 - 5	187±31	290±89
	5 - 10	166±13	168±98
	10 - 15	136±19	266±13
	15 - 20	24±4	257±13
	20 - 25	56±1	264±127
Cd * ($\mu\text{g g}^{-1}$)	0 - 5	1.2±0.4	0.80±0.2
	5 - 10	0.30±0.1	1.4±0.9
	10 - 15	0.50±0.1	2.5±1.9
	15 - 20	1.1±0.2	3.1±2.8
	20 - 25	1.6±0.7	4.1±2.8

* - $p < 0.1$; ** - $p < 0.05$; ° not significant

Table 3 – Dry weight belowground biomass (g m^{-2}), concentrations and standard deviation of Fe (%), Mn, Zn, Cu, Pb and Cd ($\mu\text{g g}^{-1}$) in belowground biomass of *Spartina maritima* and *Sarcocornia fruticosa* from Rosario salt marsh. Probability (p) was provided by Kruskal-Wallis test considering the data of all depths. Roots were inexistent in 20-25-cm layer.

	Depth (cm)	Belowground biomass	
		<i>S. maritima</i> (lower marsh)	<i>S. fruticosa</i> (higher marsh)
Biomass ** (g m^{-2})	0 – 5	282	1001
	5 -10	425	1058
	10 – 15	346	641
	15 – 20	238	829
	20 – 25	-	344
Fe ° (%)	0 – 5	2.8±0.5	3.3±0.3
	5 -10	9.8±4.2	1.7±0.1
	10 – 15	4.4±0.7	4.4±0.3
	15 – 20	4.5±0.2	6.0±1.0
	20 – 25	-	5.2±0.6
Mn ° ($\mu\text{g g}^{-1}$)	0 – 5	211±235	47±18
	5 -10	210±80	44±5
	10 – 15	99±10	89±44
	15 – 20	90±34	176±40
	20 – 25	-	190±9
Zn ° ($\mu\text{g g}^{-1}$)	0 – 5	2923±1888	576±156
	5 -10	2468±1439	3313±1355
	10 - 15	2538±764	5116±585
	15 - 20	3571±740	5875±585
	20 - 25	-	11017±1693
Cu ** ($\mu\text{g g}^{-1}$)	0 - 5	104±59	227±123
	5 -10	149±73	305±41
	10 - 15	190±49	334±22
	15 - 20	212±106	293±3
	20 - 25	-	236±4
Pb * ($\mu\text{g g}^{-1}$)	0 - 5	522±218	599±221
	5 -10	1528±243	1196±398
	10 - 15	1123±222	2739±186
	15 - 20	453±88	2106±186
	20 - 25	-	2113±74
Cd ** ($\mu\text{g g}^{-1}$)	0 - 5	19±14	13±7
	5 -10	9±9	27±5
	10 - 15	11±3	23±2
	15 - 20	17±3	23±2
	20 - 25	-	30±4

* - $p < 0.1$; ** - $p < 0.05$; ° not significant

Table 4 – Ranges of temperature, salinity and Fe, Mn, Zn (μM), Cu, Pb and Cd (nM) pore water concentrations in sediments (0-25 cm) at the air exposure situation ($t=0$), colonised *Spartina maritima* and *Sarcocornia fruticosa* from Rosario salt marsh. Probability (p) was provided by Kruskal-Wallis test considering the data of all depths.

		Pore waters	
		<i>S. maritima</i> (lower marsh)	<i>S. fruticosa</i> (higher marsh)
Temperature	$^{\circ}\text{C}$	14-15	14-15
Salinity		32.4 – 33.6	28.5 – 31.8
Fe	$^{\circ}$	0.32 – 13	0.17 – 65
Mn	μM	5.1 – 34	5.8 – 14
Zn	**	0.33 – 1.2	1.2 – 14
Cu	**	0.99 – 28	27 – 201
Pb	nM	1.0 – 15	29 – 268
Cd	**	0.10 – 1.4	0.27 - 75

** - $p < 0.05$; $^{\circ}$ not significant

Table 5 – Estimated diffusive fluxes and advective transport of Fe, Mn, Zn, Cu, Pb and Cd in Rosário sediments colonised *Spartina maritima* and *Sarcocornia fruticosa* from Rosário salt marsh. Positive fluxes indicate net exportation of metals from the sediment to the overlying waters.

	Diffusive flux (nmol m ⁻² d ⁻¹)		Advective flux (10 ³ x nmol m ⁻² d ⁻¹)	
	<i>S. maritima</i> (lower marsh)	<i>S. fruticosa</i> (upper marsh)	<i>S. maritima</i> (lower marsh)	<i>S. fruticosa</i> (upper marsh)
Fe	105	-510	9520	1640
Mn	4800	290	24	-17
Zn	-140	570	220	82
Cu	-17	27	74	16
Pb	0.4	-4.0	13	15
Cd	0.04	3.0	0.3	0.08

FIGURE CAPTIONS

Figure 1 – Location of the Rosario salt marsh in the Tagus estuary.

Figure 2 - Depth metal concentrations in pore waters of sediments at air-exposed conditions (t=0), 5, 10 and 50 minutes after the tidal flooding of the lower marsh colonised by *S. maritima*. Standard deviation bars of the three replicates are included.

Figure 3 - Depth metal concentrations in pore waters of sediments at air-exposed conditions (t=0), 5, 10 and 50 minutes after the tidal flooding of the higher marsh colonised by *S. fruticosa*. Standard deviation bars of the three replicates are included.

Figure 4 – Time course evolution of Fe, Mn, Zn, Cu, Pb and Cd concentrations in the dissolved fraction of flooding water in the areas colonised by *S. fruticosa* and *S. maritima* of the Rosario marsh. Standard deviation bars of the three replicates are included.

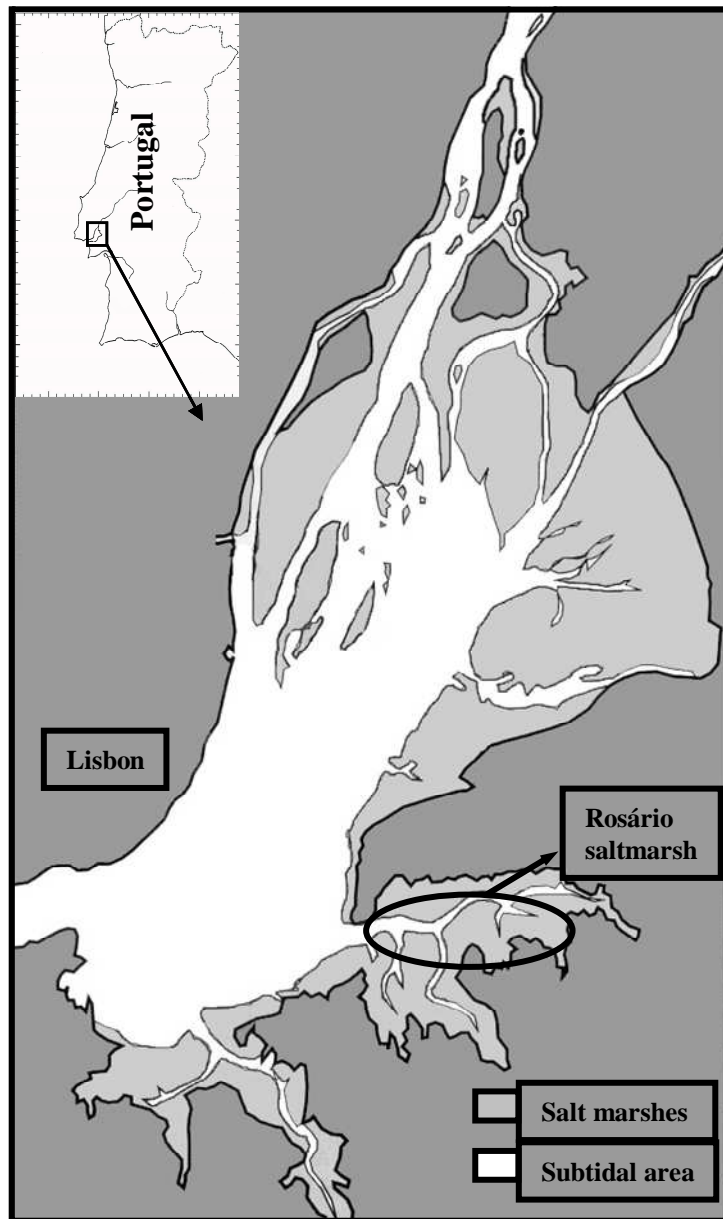


Figure 1

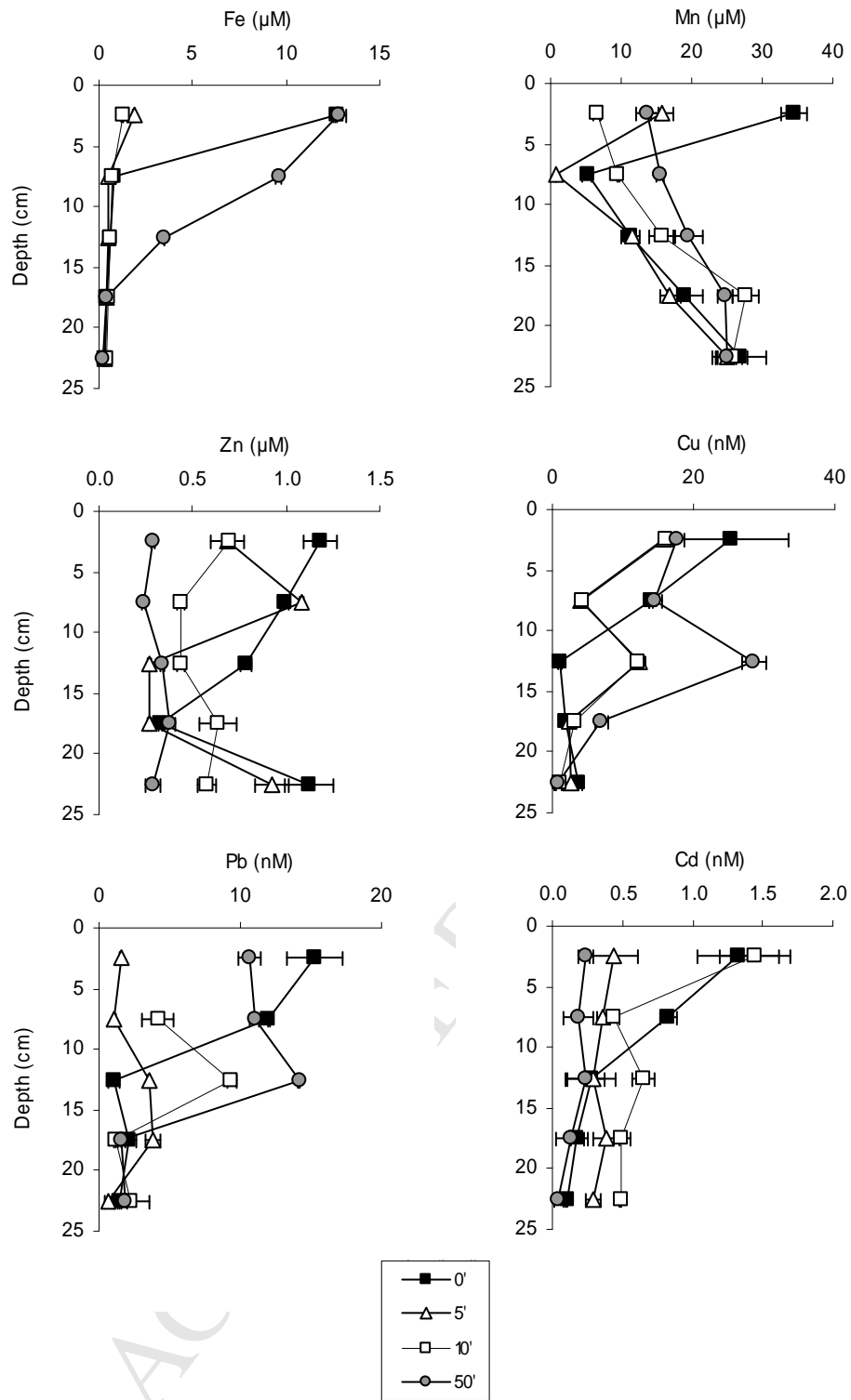


Figure 2

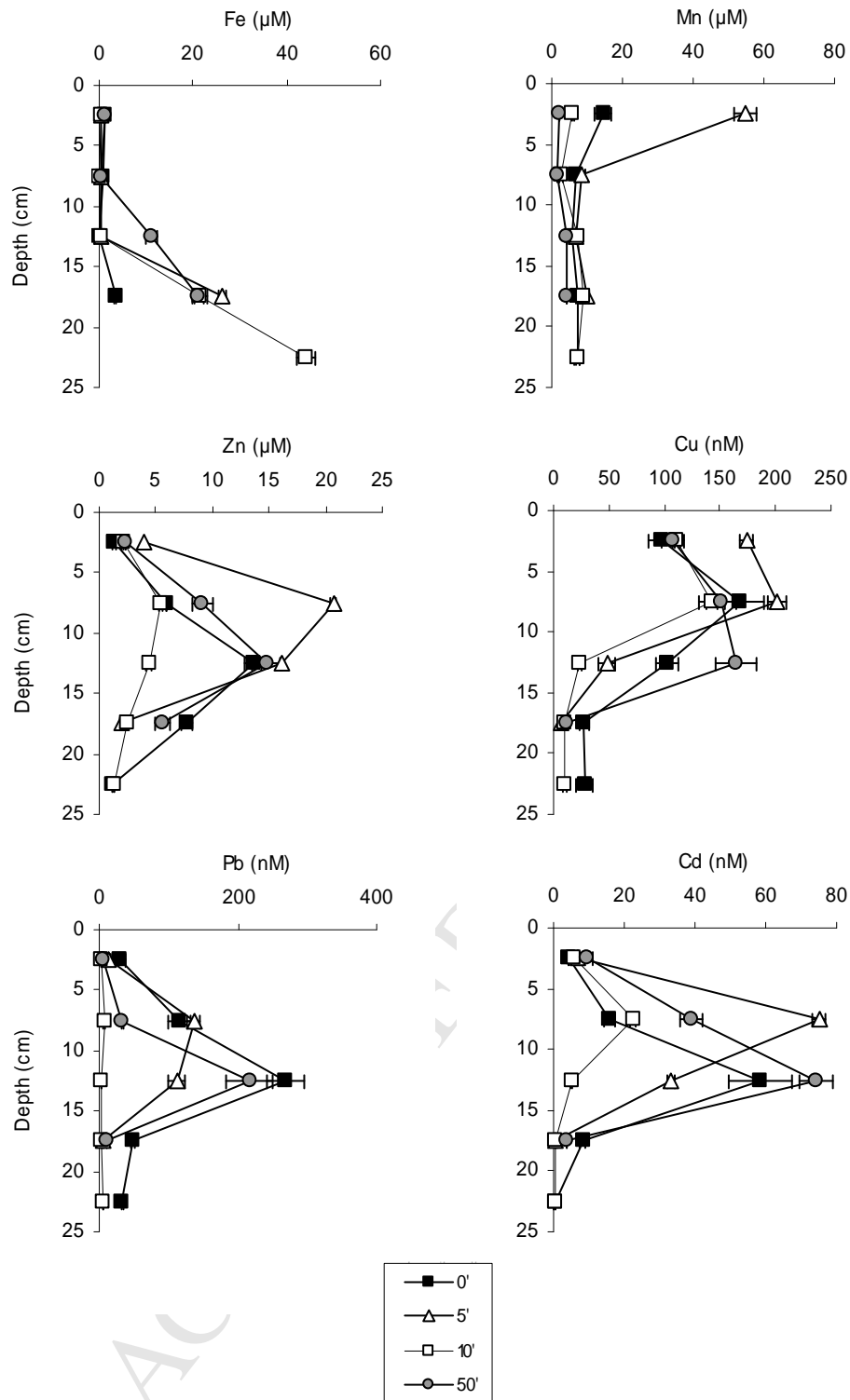


Figure 3

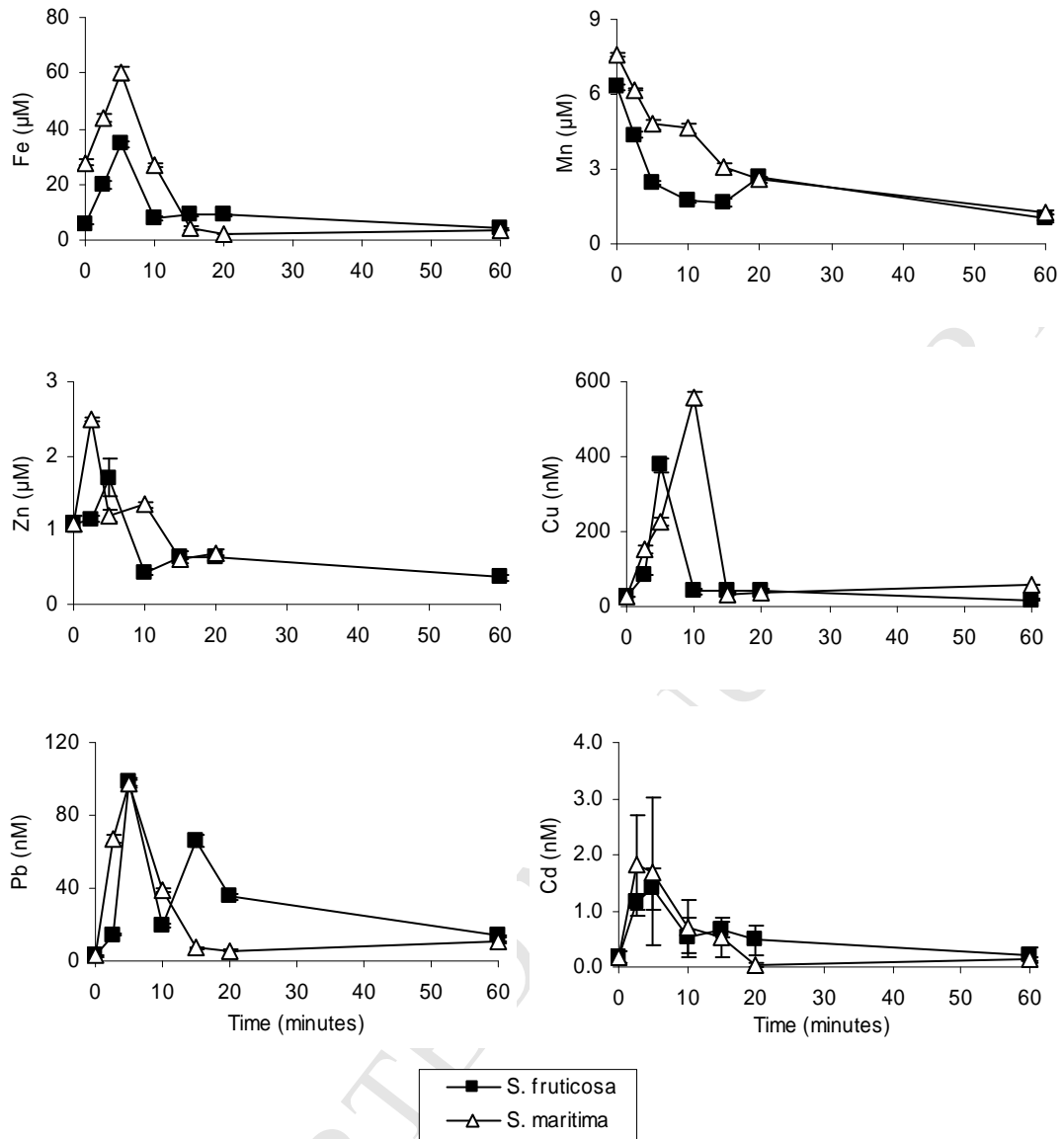


Figure 4