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**Stock and losses of trace metals from salt marsh plants**

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1 **ABSTRACT**

2 Pools of Zn, Cu, Cd and Co in leaf, stem and root tissues of *Sarcocornia fruticosa*, *S.*  
3 *perennis*, *Halimione portulacoides* and *Spartina maritima* were analysed for a Tagus  
4 estuary (Portugal) salt marsh. Pools of Cu and Cd in the salt marsh were higher in  
5 spring/summer indicating a net uptake of these metals during the growing season.  
6 Standing stocks of Zn, Cu, Cd and Co in leaf and stem biomass of *S. fruticosa*, *S.*  
7 *perennis* and *H. portulacoides* showed a strong seasonal variation, with higher values  
8 recorded in autumn. The metal-containing leaves and stems that shed in the autumn  
9 become metal-containing detritus. The amount of this material washed out from the  
10 total marsh area (200 ha) was estimated as 68 kg of Zn, 8.2 kg of Cu, 13 kg of Co and  
11 0.35 kg of Cd. The high tidal amplitude, a branched system of channels and semidiurnal  
12 tidal cycle greatly favour the export of the organic detritus to adjoining marsh area.

13

14

15 **Keywords:** halophytes, metals, detritus, salt-marshes, metal-containing litter

## 16 **1. Introduction**

17 Salt marsh plants are widely recognized to influence the transfer of metals between the  
18 aquatic compartments (Rozema et al., 1985; Crowder, 1991). These metals may be  
19 immobilized and stored in belowground biomass, sediment or in aboveground tissues  
20 (Jacob and Otte, 2003; Weis and Weis, 2004). Several plant species have the remarkable  
21 ability to concentrate metals in their tissues and are therefore being used for  
22 phytoremediation purposes (Meagher, 2000; Williams, 2002). Plants included in that  
23 category have been widely studied and their capacity to accumulate metals in roots is  
24 well documented (Almeida et al., 2004; Cundy et al., 2005; Deng et al., 2006).  
25 Since most salt marsh plants show low translocation of metals to their aboveground  
26 tissues, they have been considered as a long-term sink (McFarlane et al., 2003;  
27 Fitzgerald et al., 2003). Nevertheless, some studies on natural marsh plants have shown  
28 that a small portion of the accumulated metals may be released back to the aquatic  
29 environment through salt glands in living leaves, according to the physiological and  
30 morphological properties of plants (Burke et al., 2000; Weis et al., 2002). Furthermore,  
31 during the seasonal life cycle of salt marsh plants aboveground plant portions die and  
32 fall to the sediment becoming organic-rich detritus (Giblin et al., 1980; Valiela et al.,  
33 1985; Weis and Weis, 2004). This detritus can be retained and/or exported out from the  
34 marsh according to the flooding regime, elevation gradient, marsh topography and wind  
35 (Halupa and Howes, 2004; Neckless and Neill, 2004). Flooding and wind increase the  
36 rate of mass loss from aboveground litter (Dame, 1982; Bouchard et al., 1998;  
37 Bouchard and Lefeuvre, 2000). Otherwise, detritus retention increases with the  
38 elevation gradient from low marsh (frequently inundated) to higher marsh area (Mitsch  
39 and Gosselink, 2000). The branched system of channels across that gradient also  
40 promotes the transfer of litter between marsh zones and the export to adjacent areas  
41 (Caçador et al., 2004). A number of studies have focused on *in situ* plant litter decay  
42 using nylon mesh litter bags (e.g. Valiela et al., 1985; Halupa and Howes, 2004). The  
43 decomposition of litter is strongly regulated by climate conditions, litter quality,  
44 sediment properties, bacterial and fungal populations (Valiela et al., 1985; Graça et al.,  
45 2000). Since metals in aboveground plant parts are likely to persist when tissues die and  
46 fall off, the leaching or retention of metals in decaying litter over time has been  
47 extensively investigated (Banus et al., 1974; Dorgelo et al., 1995; Zawislanski et al.,  
48 2001; Du Laing et al., 2006). Otherwise, metal-containing detritus can also be  
49 transported to adjacent marsh areas. The large majority of salt marsh ecosystems

50 exported energy-rich substances (reduced nitrogen compounds, dissolved and  
51 particulate organic matter) to deeper waters (Mitsch and Gosselink, 2000; Valiela et al.,  
52 2004). Banus et al. (1974) highlight that substantial amounts of Pb associated with *S.*  
53 *alterniflora* litter may be removed by tides from the marsh surfaces. However, where  
54 metal-containing fertilizers were supplied to the marsh a minor export of Pb, Zn and Cd  
55 associated with litter was obtained (Banus et al., 1975). Consequently, there is little  
56 agreement on the transfer of trace metals to marine environment across the detritus  
57 generated by salt marsh plants. The fate of detritus is crucial to the understanding of the  
58 metals budgets at the estuarine ecosystem level.

59 The aim of this work was to assess the budget of selected metals (Zn, Cu, Cd and Co)  
60 exported from a moderately contaminated salt marsh during a one-year growing season.  
61 While Zn and Cu are essential metals, and no metabolic functions are known for Cd,  
62 less evidence exists on the beneficial effects of Co. The partition of Zn, Cu, Cd and Co  
63 concentrations between aboveground plant parts and belowground biomass are also  
64 studied. We focused our study on the most common plants of south European salt  
65 marshes: *Sarcocornia fruticosa*, *Sarcocornia perennis*, *Halimione portulacoides* and  
66 *Spartina maritima*. The standing stock of metals was estimated for plants as a whole  
67 and also for the individual plant parts.

68

## 69 **2. Materials and methods**

70

### 71 **2.1 Study area**

72 The Tagus estuary is one of the largest on the Atlantic coast of Europe and subjected to  
73 high human pressure with ca. 4 million people living around it. Around 40% of the  
74 estuary is composed of intertidal mudflats and its southern and eastern shores contain  
75 extensive areas of salt marshes colonised mainly by *Sarcocornia fruticosa*  
76 (Caryophyllales, Chenopodiaceae), *Sarcocornia perennis* (Caryophyllales,  
77 Chenopodiaceae), *Halimione portulacoides* (Caryophyllales, Chenopodiaceae) and  
78 *Spartina maritima* (Poales, Poaceae). One of the marshes (Rosário) located in the  
79 southern shoreline of the Tagus estuary (Figure 1) covers an area of 200 ha (Crespo,  
80 1993) and is characterized by a typical zonation with homogeneous stands of *S.*  
81 *maritima* as a pioneer species, colonizing bare mud in the lower marsh area. Across the  
82 elevation (20-50 cm) transect pure stands of *H. portulacoides* follow *S. maritima*, while  
83 *S. fruticosa* and *S. perennis* are found in the upper salt marsh. This marsh is fully

84 inundated twice a day by tidal action (2-4m of tidal amplitude) through a highly  
85 branched system of channels that cross the elevation transect. These channels have 0.5  
86 to 1.5 m depth promoting the inundation of the higher marsh even at low amplitude  
87 tides. During the ebb tide water is drained into the channels due to the water table  
88 difference. The Rosário marsh is located in the proximity of a heavy industrialised area  
89 that includes several chemical plants. During the past four decades these industries  
90 discharged effluents enriched in several contaminants directly into the estuary.  
91 According to Vale (1990) both the semi-diurnal tidal inundation and the proximity to  
92 these pollution sources contribute to metal inputs to the Rosário salt marsh. Pore water  
93 depth profiles obtained from intertidal sediments will give erroneous information of the  
94 metals budgets introduced into the marsh due to post-depositional early diagenetic  
95 reactions (Shaw et al., 1990) associated with tidal flushing of pore water solutes  
96 (Caetano et al., 1995). Thus, in this particular situation it is inaccurate to estimate the  
97 amount of metals flushed to the estuary and establish the pools of metals  
98 introduced/retained in the marsh. However, available data on depth profiles of Zn, Cu,  
99 Cd and Pb in non-vegetated sediments showed that concentrations in the upper 10 cm  
100 layers are up to 10 times higher than pre-industrial levels (Caçador et al., 1993; 1996;  
101 Caetano et al., 2007).

102

### 103 **2.2 Plant material and sampling**

104 Pure stands of *S. fruticosa*, *S. perennis*, *H. portulacoides* and *S. maritima* were sampled  
105 bimonthly at Rosário salt marsh at low tide across a growing season, from October 2001  
106 to June 2002. The aboveground biomass was assessed for each species by clipping out  
107 five squares of 0.3x0.3 m, and storing in plastic bags. In the laboratory plant parts were  
108 washed with Milli-Q water (18.2 M $\Omega$ .cm) to remove dust and sediment, and separated  
109 into photosynthetic and non-photosynthetic tissues. *Sarcocornia fruticosa* and *S.*  
110 *perennis* do not have a true shoot system with leaves and stems. From these plants the  
111 swollen photosynthetic stems (referred to as “leaves” hereafter) were separated from the  
112 dry perennial shoots (referred to as “stems” hereafter). The similar procedure was  
113 followed for *S. maritima* where the tillers were divided in leaves and in non-  
114 photosynthetic tissues (referred to as “stems” hereafter). Otherwise, the shoot system of  
115 *H. portulacoides* allows separating leaves from stems. For all species, no flowering  
116 plants were sampled. In each 0.3x0.3 m square all the shedding parts of salt marsh  
117 plants (hereafter referred as detritus) found on sediment surface were collected with

118 tweezers and transported to the laboratory in plastic bags. This detritus material  
119 corresponds to the dead plant portions retained within the salt marsh. Samples were  
120 washed with Milli-Q water and oven dried at 60 °C until constant weight. Five sediment  
121 cores were taken at each study site using a core tube, 7 cm in diameter and 100 cm long.  
122 Only the upper layer in each core (0-25 cm) was used since it contains the majority of  
123 belowground components (Gross et al., 1991). The belowground biomass of this layer  
124 was sorted out from the cores and carefully separated from the sediment under a flux of  
125 Milli-Q water using a sieve with 212 µm mesh size to remove any adhering particulate  
126 matter. Plant parts were oven dried at 60 °C and powdered in a grinding ball mill (Glen  
127 CrestomMM2000) (Gross et al., 1991). Sediment samples were oven dried at 60 °C until  
128 constant weight, cleaned of roots with tweezers, passed through a 0.25 mm mesh,  
129 homogenised and ground with an agate mortar (Caçador et al., 1999).

130

### 131 **2.3 Analytical Methods**

132 Redox potential (Eh) and pH of the sediment between the roots were measured *in situ* in  
133 all sampling periods except October 2001. Five replicate measurements were obtained  
134 between 10 and 15-cm depth. The pH was measured using a combined glass electrode  
135 with one Ag/AgCl reference electrode while for Eh measurements a platinum electrode  
136 with a calomel reference electrode was used. Values of redox potential were corrected  
137 for the reference potential of H<sub>2</sub>. Pore water was extracted by centrifugation (3000 rpm,  
138 10 min) to measure salinity using a refractometer (Atago, S/Mill-E). Particle size (sand,  
139 silt, or clay) in each sediment sample was determined by the pipette method (Gee and  
140 Bauder, 1986). The organic matter content of the sediment was determined in dried  
141 samples by loss of ignition (LOI) at 600 °C for 2 hours. Sediment samples (≈100 mg)  
142 were digested with 10 mL of HNO<sub>3</sub>/HCl (3:1 v/v) at 130 °C. This procedure was  
143 repeated twice, as described by Otte (1991). Concentrations of Zn were determined by  
144 flame atomic absorption spectrometry (FAAS) with an air-acetylene flame while levels  
145 of Cu, Co and Cd were determined by graphite furnace atomic absorption spectrometry  
146 (GFAAS). Plant samples and detritus (≈200 mg) were digested with 10 mL of  
147 HNO<sub>3</sub>/HClO<sub>4</sub> (7:1 v/v) at 130 °C according to the method described by Otte (1991).  
148 Levels of Zn, Cu, Co and Cd were determined by GFAAS and detection limits were  
149 0.01, 0.10, 0.05 and 0.05 µg g<sup>-1</sup>, respectively, for all the analytical procedures used.  
150 International certified reference materials of sewage sludge (CRM 145 and CRM 146)  
151 and plant material (BCR 62- *Olea europaea*) were used to ensure accuracy of our



152 procedure. For all metals investigated, obtained values were consistently within the  
153 ranges of certified values ( $p < 0.05$ ).

154 The area occupied by each plant species in Rosário salt marsh was estimated on the  
155 basis of patches from aerial photographs scaling 1:2000. *Halimione portulacoides*  
156 colonized 132 ha, the equivalent to 66 % of the total salt marsh area (200 ha), *S.*  
157 *maritima* and *S. fruticosa* occupied 37.6 and 28.2 ha, respectively and *S. perennis* was  
158 found only in small patches within the marsh.

159

#### 160 **2.4 Data analysis**

161 Statistical analysis was performed using a one-way analysis of variance to detect  
162 differences between metal concentrations, plant type and dates. The same test was used  
163 to look for differences in the sediment characteristics. One-way ANOVA and Newman-  
164 Keuls multiple comparison tests were used to detect differences between standing  
165 stocks of metals. To obtain homogeneity of variance, data were log-transformed (Sokal  
166 and Rohlf, 1981).

167

#### 168 **2.5 Estimation of aboveground plant detritus**

169 The difference between the maximum and the minimum metal standing stocks for  
170 leaves and stems over the annual period of study, allows the estimation of aboveground  
171 net metal production of detritus (NMePD; mol m<sup>-2</sup>) for each plant species (Caçador et  
172 al., 2004):

173

$$174 \quad \text{NMePD}_i = (\max \text{Me}_{A\text{-stk}} - \min \text{Me}_{A\text{-stk}})_i \quad (1)$$

175

176 where  $i$  is the plant species and  $\text{Me}_{A\text{-stk}}$  (mol m<sup>-2</sup>) is the aboveground metal standing  
177 stock for that species. By using the surface area colonized by each plant species in the  
178 Rosário marsh, the amount of metal in *aboveground plant detritus* produced in the total  
179 marsh area ( $Q_{\text{Me}}$ ; kg) may be estimated by:

180

$$181 \quad Q_{\text{Me}} = \sum \text{NMePD}_i A_i \quad (2)$$

182

183 where  $i$  is the plant species and  $A$  is the area colonized by each plant species (m<sup>2</sup>).

184

### 185 3. Results and Discussion

186

#### 187 3.1 Sediment characteristics

188 Sediments from the upper layer (0-25cm) were formed mainly of silt ( $60\pm 0.4\%$ ) and  
189 clays ( $38\pm 0.4\%$ ) in all plots studied and at all sampling times. This suggests that these  
190 plants grow in sediment with similar grain size and retained similar suspended matter  
191 (Salgueiro and Caçador, 2007). Pore water salinity varied within the salt marsh  
192 depending on vegetation species. Pore waters from sediments colonised by high marsh  
193 plants (*S. fruticosa* and *S. perennis*) contained more salts (salinity =  $42 \pm 1.0$  and  $41 \pm$   
194  $1.7$ ) than those from the lower marsh (*H. portulacoides*: salinity =  $36 \pm 0.5$  and *S.*  
195 *maritima*: salinity =  $31 \pm 0.2$ ). This difference was observed all over the seasonal  
196 survey, although salinity was lower in spring. A seasonal variation of pH was also  
197 observed, with lower values in the winter (Table 1). In a previous study, Sundby et al.  
198 (2003) showed that the release of oxygen by roots was lower in winter, changing the  
199 state of sediment to reduced conditions. Therefore, the reactions involving the  $\text{Fe}^{2+}/\text{Fe}^{3+}$ ,  
200  $\text{Mn}^{2+}/\text{Mn}^{4+}$  and  $\text{S}^{2-}/\text{SO}_4^{2-}$  pairs prevail in the rhizosphere during the winter. As oxygen  
201 reduces in the rizosphere the decrease of pH appears to be related to the oxidation of  
202 iron sulphide compounds by Fe(III) organic complexes (Luther et al., 1996). Since no  
203 statistically significant differences of pH were found between plant species, early  
204 diagenetic reactions had similar consequences on sediment acidity and metal  
205 availability. The redox potential (Table 1) varied irregularly between plants and  
206 throughout the year with no seasonal pattern. These results are consistent with previous  
207 work done by Caçador et al. (1996) at several salt marshes in the Tagus estuary,  
208 showing that pH and Eh variations have no statistical consistency. No seasonal  
209 differences were found also for organic matter content. However, sediment colonized by  
210 *S. maritima* contained less organic matter ( $13\pm 1.0\%$ ) than the sediments from areas  
211 colonised by the other plant species (*S. fruticosa* =  $21\pm 1.4\%$ ; *S. perennis* =  $20\pm 1.6\%$ ;  
212 and *H. portulacoides* =  $20\pm 1.5\%$ ).

213 Mean concentrations of Zn, Cu, Co and Cd found in sediment between roots of the four  
214 plant species included in this study are shown in Table 2. Levels of Cu, Co and Cd were  
215 relatively constant during the sampling period as can be inferred by the low standard  
216 deviations. Although an enhanced variability was found for Zn concentrations, the  
217 standard deviation never exceed 10 % of the mean value. Moreover, no statistically  
218 significant differences were found among the metal (Zn, Cu, Co and Cd) concentrations

219 in sediments coming from areas colonised by the four plant species included in our  
220 study. Lack of seasonal variation in the metal concentrations may be attributed to higher  
221 non-reactive fraction of these metals in the solids (Caçador et al., 1996). However, it is  
222 possible that variations within the sediment layer may have occurred, but this must have  
223 been on a scale of a few centimetres only and could not be resolved by our sampling  
224 methods.

225

### 226 **3.2 Plant biomass**

227 From October 2001 to February 2002, the plant biomass overall was larger in plots of *S.*  
228 *fruticosa*, while areas with *S. maritima* had the lowest biomass values recorded from all  
229 the plant species included in this study (Table 3). In April and June total biomass was  
230 not statistically different for all the studied species. Root biomass may account for up to  
231 80 % (d.w.) of the total biomass. An exception was recorded in October for *S. fruticosa*,  
232 when the aboveground biomass accounted for 50 % of the total biomass. Presumably  
233 because dead and live roots are not easily discerned, rather small seasonal variation  
234 were observed in the biomass of roots, although higher values were found in the  
235 summer for *S. maritima* and in spring for the other species. Results from aboveground  
236 biomass showed statistically significant differences between plant species ( $p < 0.05$ ) and  
237 also between leaf and stem biomass ( $p < 0.05$ ). Although both *Sarcocornia* species had  
238 similar morphology, leaf and stem biomasses of *S. fruticosa* were up to one order of  
239 magnitude higher than those calculated for *S. perennis*. The same difference was also  
240 found by comparing aboveground plant parts biomass of *S. fruticosa* with those of *S.*  
241 *maritima* and *H. portulacoides* (Table 3). Furthermore, a clear seasonal variation was  
242 observed for leaf and stem biomass in all four species. Higher biomass values were  
243 observed in October, followed by a logarithmic decrease. However, the decline of  
244 biomass was more sharp for *S. fruticosa* (leaf: from  $3516 \pm 341$  to  $50 \pm 19$  g m<sup>-2</sup> and stem:  
245 from  $1919 \pm 195$  to  $110 \pm 29$  g m<sup>-2</sup>) than for *S. perennis* (leaf: from  $167 \pm 39$  to  $37 \pm 5$  g m<sup>-2</sup>  
246 and stem: from  $425 \pm 40$  to  $43 \pm 17$  g m<sup>-2</sup>) and *H. portulacoides* (leaf: from  $212 \pm 57$  to  
247  $46 \pm 3$  g m<sup>-2</sup> and stem: from  $733 \pm 122$  to  $180 \pm 49$  g m<sup>-2</sup>). Leaf biomass in the latter species  
248 was most reduced in December after some senescent leaves had fallen from the standing  
249 stems. A different pattern was observed for *S. maritima* since aboveground biomass was  
250 lower in December but increased afterwards. Differences in the phenology of plant  
251 species attest to specific patterns of carbon allocation to the aboveground tissues, with

252 *S. perennis*, *S. fruticosa*, and *H. portulacoides* promoting early leaf production when  
253 compared to *S. maritima* (Windham et al., 2003).

254

### 255 **3.3 Metal concentrations in plant parts**

256 Concentrations of Zn, Cu, Co and Cd in plant tissues of *S. fruticosa*, *S. perennis*, *H.*  
257 *portulacoides* and *S. maritima* are presented in Table 4. Seasonal and tissue allocation  
258 patterns of Zn, Cu, and Cd differed from Co in the four plant species studied under field  
259 conditions. For all metals except Co, the concentrations in roots were, in general, up to  
260 one order of magnitude higher than levels found in the aboveground tissues. The  
261 partitioning indicates that for *S. fruticosa*, *S. perennis*, *H. portulacoides* and *S. maritima*  
262 the dominant uptake of metals was via the root system with only small amounts being  
263 translocated to the aerial plant parts (Fitzgerald et al., 2003; MacFarlane et al., 2003;  
264 Weis and Weis, 2004). Metal concentrations in leaves showed no statistically significant  
265 differences when compared to equivalent levels in stems within the same plant or  
266 between plant species. The similarity of metal accumulation in aboveground plant parts  
267 of *S. maritima* and of both *Sarcocornia* species may result from the undistinguished  
268 shoot system of these plants. Moreover, we may suggest that photosynthetic tissues play  
269 a minor role on the storage of metals in aboveground plant parts. No seasonal pattern  
270 was observed for the concentration of essential elements, Zn and Cu in the aboveground  
271 parts of the four species. However, concentrations of Cd in leaf and stem biomass of the  
272 same plant species showed a distinct seasonal variation, with increased accumulation in  
273 February. The accumulation of Co in the tissues of the studied plants showed a  
274 distinctive pattern, quite different from that observed for the remaining elements. Up to  
275 50 % of the total metal pool was retained in the aboveground parts. While for Zn, Cu  
276 and Cd the roots act as a barrier for upward translocation this retention was not  
277 observed for Co. Detoxification of those metals by plants are associated with the  
278 phytochelatins in plant cells (Zenk, 1996). However, Co does not activate the  
279 phytochelatase synthase (Oven et al., 2002) suggesting that this metal may induce other  
280 detoxification mechanisms or play a physiological role similar to that described for  
281 algae. The coenzyme cobalamin has cobalt as component and is essential for *Euglena*  
282 *gracilis* growth, being located in several subcellular compartments, including the  
283 tilakoids of the chloroplasts (Marschner, 1995).

284

### 285 **3.4 Metal stocks in plants**

286 Our data of metal concentrations in plant parts combined with biomass allow us to  
287 compare the standing stocks of given elements in whole plant tissues and subsets, or to  
288 compare between plant species and for different seasons. Considering the standing  
289 stocks in the whole plant structure for all species in our study (Figure 2), metal pools  
290 were generally lower in *S. maritima* as a consequence of comparatively smaller biomass  
291 values. Standing stocks of Cu and Cd were higher for all plants in spring/summer  
292 ( $p<0.05$ ) indicating a net uptake of these metals in the growing season. When standing  
293 stocks of metals were analysed in leaves and stems two different patterns were observed  
294 (Figure 3). In the cases of *S. perennis*, *H. portulacoides* and *S. maritima*, stems  
295 contained comparatively higher aboveground standing stocks of Zn, Cu, Co and Cd  
296 ( $p<0.05$ ) while for *S. fruticosa* leaves emerge as the major contributor ( $p<0.05$ ). These  
297 different patterns observed for standing stocks are explained by the higher aboveground  
298 biomass of *S. fruticosa* (Table 3). There were also differences in the seasonality of  
299 aboveground metal standing stocks. While *S. perennis*, *S. fruticosa* and *H.*  
300 *portulacoides* showed a strong seasonal variation with higher values occurring in the  
301 autumn ( $p<0.05$ ; Figure 3), the standing stocks of metals in *S. maritima* were higher in  
302 February ( $p<0.05$ ; Figure 3). The early leaf production of *S. maritima* seems to be  
303 coupled to an increased transfer of some metals into the aboveground tissues.  
304 Belowground standing stocks in all plant species showed small irregular variability  
305 throughout the seasonal survey (not shown), but values were considerably higher than  
306 aboveground. This result is consistent with other investigations elsewhere showing  
307 difficulty in observing well-defined seasonal patterns for metal concentrations in roots  
308 (Gleason et al., 1979; Heller and Weber, 1998; Windham et al., 2003).

309

### 310 **3.5 Metal containing detritus**

311 Metals in aboveground tissues are likely to remain when these tissues die and turn into  
312 detritus. The seasonal variation of the metal standing stocks in *S. perennis*, *S. fruticosa*  
313 and *H. portulacoides* was mainly associated with aboveground biomass production.  
314 Therefore the *aboveground plant detritus* – *QMe* may be estimated for a growing  
315 season using equations 1 and 2. Results show that plants in our study area (200 ha)  
316 generate organic detritus containing 96 kg of Zn, 13 kg of Cu, 18 kg of Co and 0.51 kg  
317 of Cd during one growing season. However, the pool of organic material derived from  
318 aerial plant parts greatly exceeds the litter pool found in the sediment surface. The  
319 detritus on the sediment surface follows a seasonal pattern with higher values at the end

320 of the growing season (*S. fruticosa* – 1020 g m<sup>-2</sup>; *S. perennis* – 212 g m<sup>-2</sup>; *H.*  
321 *portulacoides* – 134 g m<sup>-2</sup>; *S. maritima* – 45 g m<sup>-2</sup>) and a decrease in winter (*S. fruticosa*  
322 – 146 g m<sup>-2</sup>; *S. perennis* – 25 g m<sup>-2</sup>; *H. portulacoides* – 62 g m<sup>-2</sup>; *S. maritima* – 21 g m<sup>-2</sup>). This is in line with the findings of Caçador et al. (2004) where more than 86% of the  
323 produced carbon from aboveground plant parts was exported from two different salt  
324 marshes in the Tagus estuary. The hydrological conditions of Rosário salt marsh  
325 (branched system of channels and semidiurnal tidal cycle) greatly favour the transport  
326 of organic detritus to adjoining marsh areas. The litter production may be estimated by  
327 different methods (Bouchard and Lefeuvre, 2000), although all have limitations  
328 (Hopksion et al., 1978). Thus, we chose the simple method, viz. the difference between  
329 maximum and minimum litter pools (Caçador et al., 2004). By comparing the  
330 aboveground biomass production (Table 3) with the litter pool we calculate that only  
331 5% (*S. maritima*), 21 % (*H. portulacoides*) 17 % (*S. perennis*) and 46 % (*S. fruticosa*) of  
332 the detritus produced by aboveground plant parts remain at the site. By apply these  
333 proportions to the pool of the metal-containing detritus ( $Q_{Me}$ ) we found that most of the  
334 metal containing detritus was flushed out from the marsh. During one growing season  
335 68 kg of Zn, 8.2 kg of Cu, 13 kg of Co and 0.35 kg of Cd was exported to areas  
336 adjoining the Rosário salt marsh. This metal containing detritus may act as a source for  
337 these metals before being buried in the sediments since they are easily degraded by  
338 decomposers and weathering (Weis and Weis, 2004; Du Laing et al., 2006).

340

#### 341 **4. Conclusions**

342 There is an important export of trace metals associated with marsh detritus to the  
343 estuary, even considering that all plant species included in this study have much higher  
344 belowground than aboveground biomass. The export route involves uptake of metals  
345 from sediment by roots, translocation to the aerial parts of plants, and return to the soil  
346 after senescence and fall of aerial plant parts. The major fraction of the metal-containing  
347 detritus is not retained within the marsh. Therefore, when assessing remediation  
348 techniques in a contaminated marsh we must take into account the balance between  
349 retention and export of metals from the marsh, the biology of the plant species involved  
350 and the marsh ecology.

351

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356

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495 **Figure Captions**

496

497 Figure 1 – Location of the Tagus estuary and Rosário salt marsh.

498

499 Figure 2 – Whole plant standing stock of each metal. Values presented as mean±SD.

500

501 Figure 3 – Standing stocks of Zn, Cu Co and Cd for aboveground tissues of *S. fruticosa*,

502 *S. perennis*, *H. portulacoides* and *S. maritima*. Values presented as mean±SD.

503 Dissimilar letters denote significant differences ( $p<0.05$ ).

504

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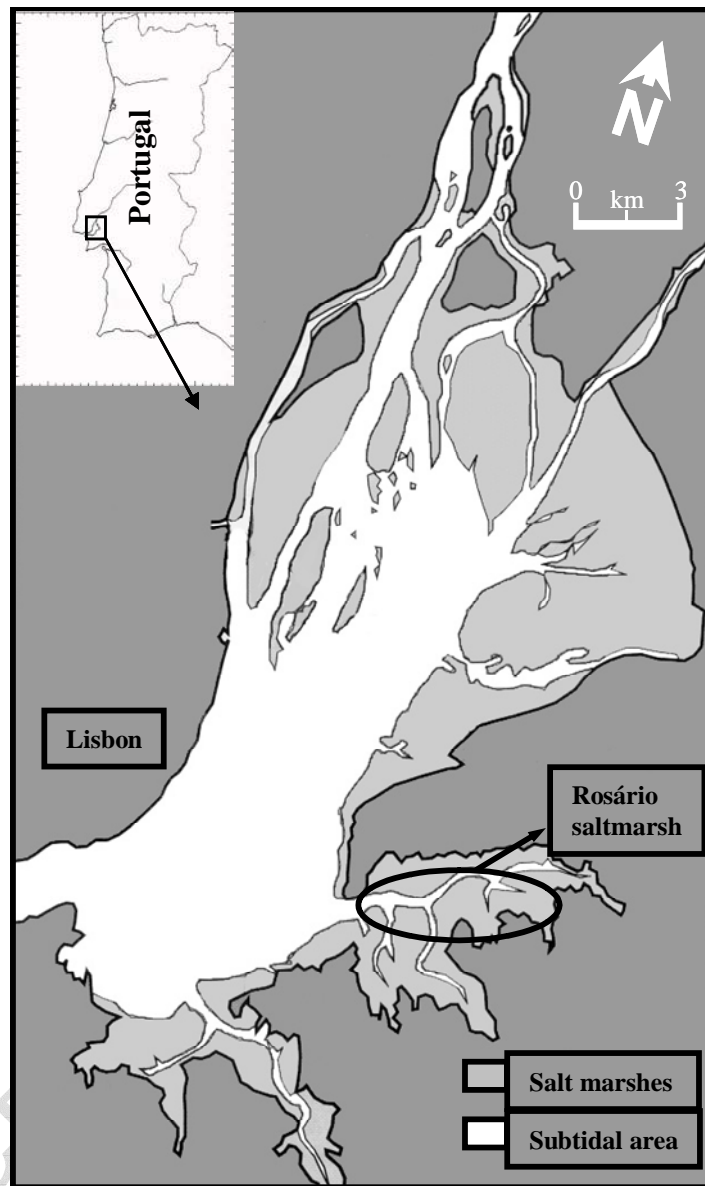


Figure 1

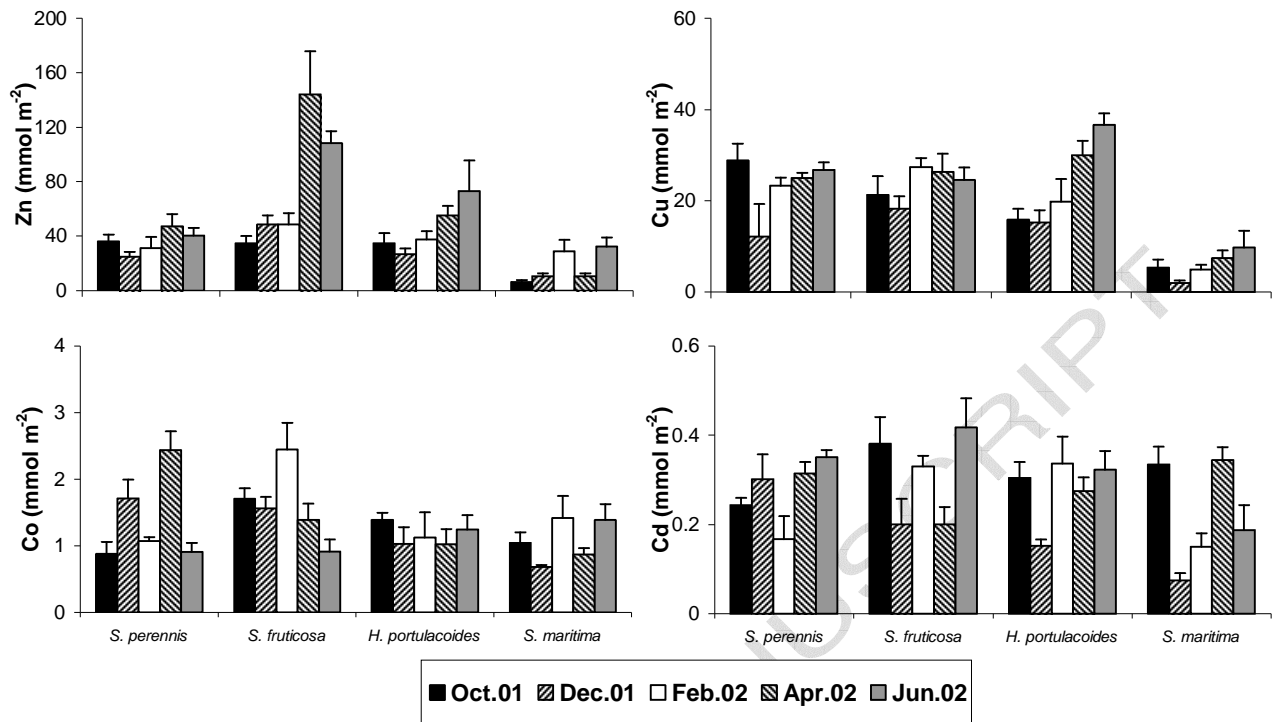


Figure 2

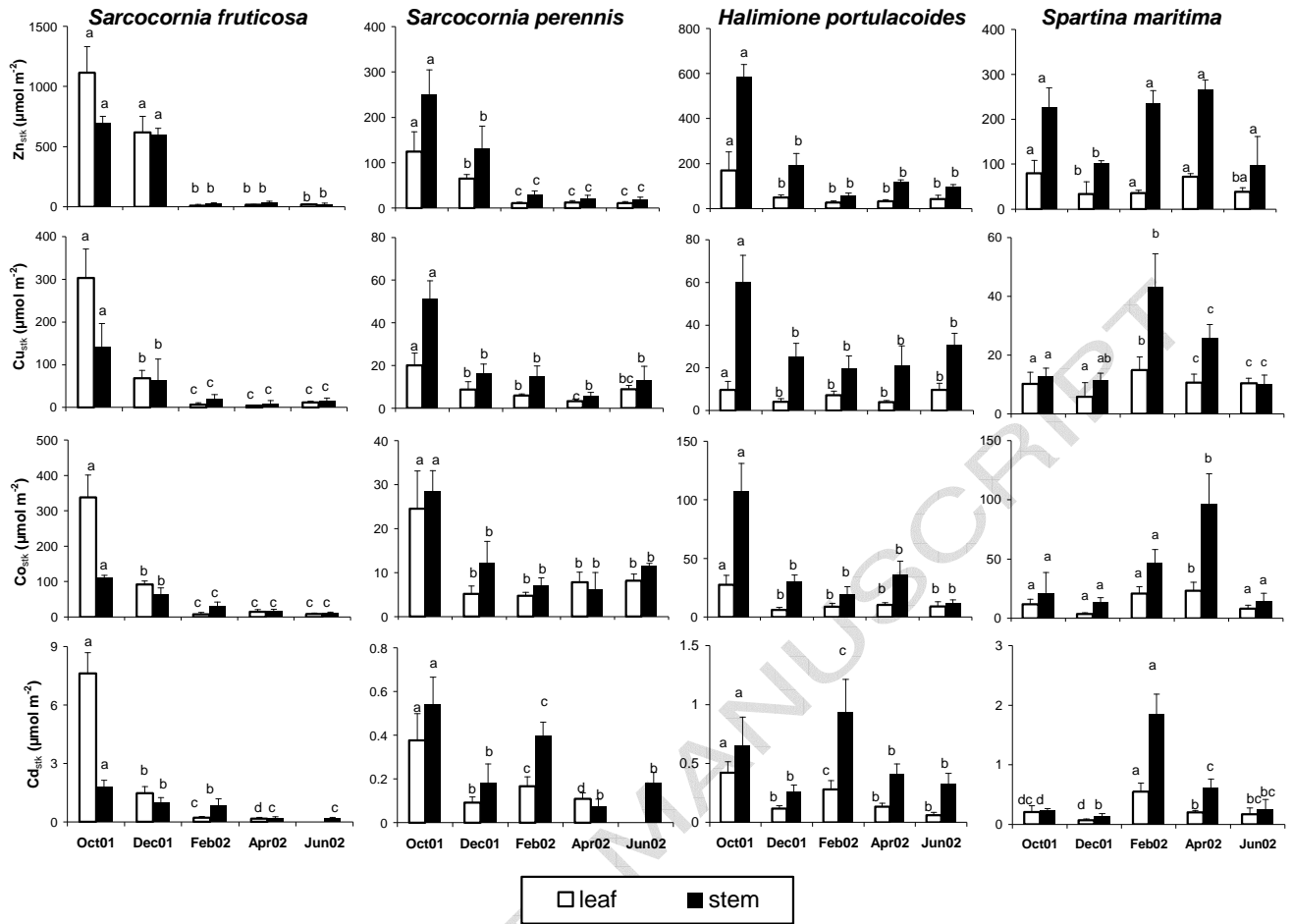


Figure 3

Table 1 - Average ( $n=5$ ) pH and redox potential (Eh, mV) in rooting sediment of *S. fruticosa*, *S. perennis*, *H. portulacoides*, and *S. maritima*.

		pH	Eh (mV)
<i>Sarcocornia fruticosa</i>	Dec.	6.9	17±11
	Feb.	7.3±0.1	-6±10
	Apr.	7.4±0.1	-25±9
	Jun.	7.9	34±13
<i>Sarcocornia perennis</i>	Dec.	6.3±0.3	43±10
	Feb.	7.7	34±14
	Apr.	7.5±0.1	73±7
	Jun.	7.9	125±19
<i>Halimione portulacoides</i>	Dec.	7.0	44±10
	Feb.	7.9	67±8
	Apr.	7.7	84±7
	Jun.	8.0	0±10
<i>Spartina maritima</i>	Dec.	6.6±0.1	91±7
	Feb.	7.8	98±7
	Apr.	7.7	93±8
	Jun.	7.8	90±12

Table 1



Table 2 – Average concentrations ( $n=5$ ) and standard deviations of Zn, Cu, Co and Cd ( $\mu\text{mol g}^{-1}$ ) in rooting sediment of *S. fruticosa*, *S. perennis*, *H. portulacoides*, and *S. marítima* all over the seasonal survey.

	<b>Species</b>	<b>Sediment</b>
Zn ( $\mu\text{mol g}^{-1}$ )	<i>S. fruticosa</i>	3.6±0.2
	<i>S. perennis</i>	5.4±0.5
	<i>H. portulacoides</i>	4.5±0.2
	<i>S. marítima</i>	4.0±0.4
Cu ( $\mu\text{mol g}^{-1}$ )	<i>S. fruticosa</i>	0.62±0.03
	<i>S. perennis</i>	0.63±0.02
	<i>H. portulacoides</i>	0.63±0.03
	<i>S. marítima</i>	0.72±0.02
Co ( $\mu\text{mol g}^{-1}$ )	<i>S. fruticosa</i>	0.48±0.01
	<i>S. perennis</i>	0.57±0.02
	<i>H. portulacoides</i>	0.57±0.02
	<i>S. marítima</i>	0.70±0.01
Cd ( $\mu\text{mol g}^{-1}$ )	<i>S. fruticosa</i>	0.029±0.001
	<i>S. perennis</i>	0.027±0.001
	<i>H. portulacoides</i>	0.026±0.0003
	<i>S. marítima</i>	0.028±0.0002

Table 2

Table 3 – Average ( $n=5$ ) dry weight biomass ( $\text{g m}^{-2}$ ) and standard deviations for total and individual plant tissues of *S. fruticosa*, *S. perennis*, *H. portulacoides*, and *S. maritima*.

		<b>Leaf biomass</b>	<b>Stem biomass</b>	<b>Root biomass</b>	<b>Total biomass</b>
		( $\text{g m}^{-2}$ )			
<i>Sarcocornia fruticosa</i>	Oct.	3516±341	1919±195	4366±225	9010±1753
	Dec.	630±151	704±215	4574±176	6023±277
	Feb.	50±19	212±67	7176±366	7287±219
	Apr.	67±20	123±50	5354±466	5172±96
	Jun.	82±16	110±29	4252±163	4444±178
<i>Sarcocornia perennis</i>	Oct.	167±39	425±40	3900±329	4594±194
	Dec.	59±18	148±56	4172±429	4179±209
	Feb.	37±5	85±2	4550±206	4725±195
	Apr.	43±6	43±17	4860±94	4946±103
	Jun.	64±12	82±17	4090 146	4287±105
<i>Halimione portulacoides</i>	Oct.	211±57	733±122	4128±111	5072±264
	Dec.	65±16	320±50	3576±283	4183±159
	Feb.	56±11	189±51	4476±276	4623±110
	Apr.	46±3	180±49	4416±75	4642±61
	Jun.	60±16	188±31	4386±227	4643±15
<i>Spartina maritima</i>	Oct.	154±56	254±62	2556±596	2998±162
	Dec.	61±16	148±29	1510±62	1751±21
	Feb.	127±30	363±77	3678±492	4360±353
	Apr.	104±18	271±53	3386±620	3730±127
	Jun.	79±20	181±80	4268±360	4379±191

Table 3

Table 4 - Metal concentrations for individual plant tissues of *S. fruticosa*, *S. perennis*, *H. portulacoides*, and *S. maritima*.

		leaf				stem				root			
		Zn	Cu	Co	Cd	Zn	Cu	Co	Cd	Zn	Cu	Co	Cd
		(μmol.g <sup>-1</sup> )				(μmol.g <sup>-1</sup> )				(μmol.g <sup>-1</sup> )			
<i>Sarcocornia fruticosa</i>	Oct.	0.36±0.05	0.071±0.011	0.051±0.008	0.00089±0.0001	0.45±0.14	0.076±0.017	0.097±0.008	0.0021±0.0001	8.1±3.9	4.8±0.87	0.26±0.06	0.087±0.024
	Dec.	0.83±0.20	0.089±0.008	0.085±0.004	0.0014±0.0001	1.0±0.3	0.11±0.01	0.14±0.01	0.0023±0.0001	10±1	4.0±0.54	0.29±0.05	0.048±0.014
	Feb.	0.12±0.03	0.095±0.013	0.15±0.01	0.0040±0.0004	0.21±0.07	0.13±0.03	0.14±0.01	0.0045±0.0007	8.7±3.0	3.8±0.38	0.31±0.06	0.049±0.008
	Apr.	0.31±0.05	0.067±0.014	0.16±0.01	0.0014±0.0002	0.24±0.04	0.057±0.011	0.23±0.05	0.0023±0.0003	26±3	4.5±0.94	0.29±0.08	0.038±0.007
	Jun.	0.22±0.05	0.14±0.02	0.11±0.01	0.0017±0.0003	0.23±0.05	0.14±0.02	0.096±0.007	--	23±6	5.7±0.64	0.20±0.05	0.091±0.020
<i>Sarcocornia perennis</i>	Oct.	0.59±0.11	0.12±0.01	0.069±0.013	0.0013±0.0002	0.74±0.16	0.12±0.01	0.14±0.02	0.0022±0.0003	8.2±2.7	6.8±1.9	0.19±0.04	0.065±0.007
	Dec.	0.90±0.20	0.11±0.03	0.095±0.003	0.0012±0.0003	1.2±0.3	0.15±0.04	0.092±0.017	0.0016±0.0001	5.6±1.3	3.6±2.0	0.37±0.09	0.069±0.012
	Feb.	0.34±0.11	0.18±0.05	0.083±0.023	0.0047±0.0008	0.29±0.04	0.16±0.01	0.14±0.01	0.0045±0.0009	5.8±2.7	5.1±0.21	0.29±0.11	0.037±0.012
	Apr.	0.47±0.05	0.13±0.01	0.13±0.06	0.0017±0.0002	0.29±0.06	0.077±0.017	0.18±0.04	0.0026±0.0006	9.8±2.9	5.1±0.27	0.44±0.12	0.059±0.011
	Jun.	0.22±0.07	0.15±0.06	0.15±0.02	0.0022±0.0003	0.17±0.04	0.14±0.02	0.13±0.02	--	9.5±1.6	6.8±0.77	0.22±0.03	0.090±0.010
<i>Halimione portulacoides</i>	Oct.	0.77±0.19	0.083±0.017	0.14±0.01	0.00087±0.0002	0.77±0.23	0.046±0.009	0.14±0.01	0.0020±0.0003	9.5±3.4	4.4±1.5	0.27±0.08	0.074±0.007
	Dec.	0.62±0.16	0.080±0.019	0.10±0.01	0.0008±0.0001	0.79±0.19	0.063±0.018	0.10±0.02	0.0018±0.0002	8.5±2.6	4.8±1.4	0.28±0.06	0.042±0.001
	Feb.	0.31±0.09	0.11±0.02	0.11±0.01	0.0049±0.0002	0.50±0.09	0.13±0.03	0.16±0.03	0.0050±0.0006	8.2±1.5	4.8±1.3	0.25±0.09	0.075±0.016
	Apr.	0.71±0.26	0.11±0.03	0.21±0.02	0.0023±0.0005	0.71±0.15	0.088±0.014	0.23±0.03	0.0029±0.0006	12±2	6.7±0.7	0.21±0.05	0.066±0.092
	Jun.	0.53±0.08	0.16±0.02	0.067±0.004	0.0017±0.0003	0.71±0.24	0.14±0.03	0.14±0.03	0.0010±0.0002	17±1	7.5±1.0	0.28±0.05	0.082±0.017
<i>Spartina maritima</i>	Oct.	0.78±0.21	0.051±0.008	0.11±0.02	0.0010±0.0002	0.48±0.07	0.066±0.008	0.079±0.019	0.0013±0.0004	2.4±0.4	2.0±0.2	0.37±0.04	0.15±0.02
	Dec.	0.71±0.15	0.075±0.008	0.091±0.006	0.00094±0.0001	0.68±0.10	0.12±0.03	0.063±0.014	0.0010±0.0002	6.5±1.6	1.3±0.3	0.41±0.06	0.045±0.017
	Feb.	0.48±0.15	0.12±0.01	0.13±0.01	0.00051±0.0005	0.27±0.02	0.12±0.02	0.16±0.01	0.0043±0.0002	7.2±1.8	1.4±0.3	0.39±0.05	0.041±0.009
	Apr.	1.00±0.19	0.091±0.009	0.35±0.03	0.0020±0.0003	0.79±0.04	0.10±0.01	0.23±0.01	0.0020±0.0001	3.4±0.6	2.2±0.3	0.26±0.06	0.11±0.03
	Jun.	0.49±0.15	0.12±0.03	0.070±0.009	0.0014±0.0004	0.51±0.22	0.22±0.13	0.11±0.01	0.0022±0.0006	6.7±1.9	2.3±0.9	0.29±0.08	0.049±0.015

Table 4