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Halophytes as sources of metals in estuarine systems with low levels of contamination

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Abstract. Heavy metal concentrations present in the above- and beowground tissues of *Scirpus maritimus* L., *Spartina maritima* (Curtis) Fernald and *Zostera noltii* Hornem were analysed seasonally in the Mondego Estuary, Portugal. The sediments of the estuary were confirmed to contain only low concentrations of heavy metals. The belowground tissues of all three species showed higher heavy metal concentrations than the aboveground tissues. Although the sediments only contained low levels of contamination, because the area occupied by *S. maritimus* and *Z. noltii* was large, significant quantities of heavy metals were accumulated and exported to the surrounding water bodies. In contrast with observations of highly contaminated estuaries, it was found that in spite of the low level of contaminants in the sediments of the Mondego Estuary, aquatic vegetation functioned as a source of metals for nearby systems.

Additional keywords: estuary, halophytes, heavy metal, Portugal.

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Introduction

The approval of the Water Framework Directive (WFD) requires the member states of the European Union (EU) to monitor the biological quality of all of their water bodies and elaborate a management plan for their river basins to prevent potential problems (EC 2000). The WFD CIS (2003) advises that, in transitional waters (TW), the intertidal areas from the highest tide limit to the lowest tide limit should be included in the monitoring program, which encompasses salt marshes.

Salt marshes are natural deposits of heavy metals in an estuarine system (Caçador *et al*. 1996; Doyle and Otte 1997). When located near polluted areas, these ecosystems receive large amounts of pollutants from industrial and urban wastes, which either drift downstream with the river flow or are dumped directly from nearby industrial and urban areas (Reboreda and Caçador 2007). When metals enter salt marshes, they spread through the system with the tides and periodic floods and interact with the sediment and the biotic community (Suntornvongsagul *et al*. 2007; Caçador *et al*. 2012). Most salt marsh plants accumulate large amounts of metals in both their aerial and belowground organs (Caçador *et al*. 2000). Salt marsh species greatly influence the inputs and outputs of metals and nutrients in the marsh (Caçador *et al*. 2004; Reboreda and Caçador 2007; Reboreda *et al*. 2008; Sousa *et al*. 2008; Caçador *et al*. 2009) owing to the different uptake rates of these elements. As the highest levels of metal accumulation are found in the root tissues (Caçador *et al*. 2000; Reboreda and Caçador 2007), the decomposition of root litter may also be a source of metals released to the surrounding sediment through leaching (Weis and Weis 2004; Pereira *et al*. 2007). Metals are not well studied in the Mondego Estuary and so it is important to know if the system has some kind of metal contamination. *Scirpus maritimus* L., *Spartina maritima* (Curtis) Fernald and *Zostera noltii* Hornem together occupy ~50% of the area (J Neto, unpubl. data), covering a large part of the salt marsh, making these three species very important in the system.

The present study considers the dynamics of *S. maritimus*, *S. maritima* and *Z. noltii* in order to evaluate their primary production and senescence mechanisms and their implications for metal cycling at the estuarine level. In this work we report the metal concentrations in the salt marsh sediments of the Mondego Estuary and the role of plants in metal cycling in the estuarine system.

Materials and methods

Study area

The Mondego Estuary (Fig. 1) is a temperate system located in the central Atlantic coast of Portugal (40°08′N, 8°50′W) (Marques and Nogueira 1991). The terminal portion of the estuary, 7 km long and 2–3 km across at its widest, consists of two arms (North and South) with highly divergent hydrological characteristics

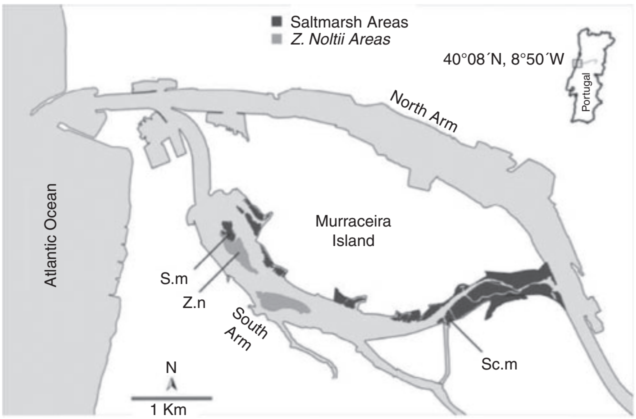

Fig. 1. Terminal part of the Mondego Estuary, Portugal, showing *Zostera noltii* (Z.n), *Spartina maritima* (S.m) and *Scirpus maritimus* (Sc.m) sampling location indicated by the arrows.

separated by the Murraceira Island (Marques *et al*. 2003). The South arm is shallower than the North arm (2–4 m during high tide), is characterised by large areas of intertidal mudflats (almost 75% of the area) exposed during low tide (Neto *et al*. 2008), contains sediments with high percentages of silt and clay and is considered a rich estuarine habitat in terms of productivity and biodiversity (Marques *et al*. 1993).

Sampling and laboratory procedures

For each sampling, three sediment cores (50 cm depth) were taken in pure stands of *Spartina maritima* (Curtis) Fernald, *Zostera noltii* Hornem and *Scirpus maritimus* L. The stands were located along the marsh, with a minimum distance of 10 m between each stand. All samples were collected during low tide. Pure stands of *Z. noltii*, *S. maritima* and *S. maritimus* were sampled in the spring, summer and autumn of 2010 and the winter of 2011. The aboveground biomass was assessed for each species by clipping out three 0.3×0.3 m squares. Three additional sediment cores were also taken to sample belowground biomass at each species site using a tubular probe with an 8 cm diameter for sampling the first 30 cm, which contains the majority of belowground components (Caçador *et al*. 2004). The belowground plant material was carefully separated from the sediment under a stream of water, using a sieve with a 212 µm mesh size to remove any adhering particulate matter, and then was washed with Milli-Q water (18.2 ΩU cm). Plant organs were oven-dried at 60°C and powdered in a grinding ball mill (Glen Crestom MM2000, London, UK) (Gross *et al*. 1991). Sediment samples were oven-dried at 60°C until constant weight (~ 3 days). After the oven, the sediments were cleaned of roots with tweezers, passed through a 0.25 mm mesh, homogenised and ground with an agate mortar.

Pore water was extracted by centrifugation at 6704*g* for 10 min at 4°C and the salinity was estimated using a refractometer (Atago, S/Mill-E; Borivali, Mumbai, India). The sediment organic matter content was determined in dried samples by loss on ignition (LOI) at 600°C for 2 h, following the method described by Caçador *et al*. (2000). Sediment grain size was determined by mechanical sequential sediment-sieving, using analytical sieves housed in a shaker, to evaluate the relative abundance (Folk 1954) and were classified as coarse sand ($\geq$0.5 mm), fine + medium sand (>0.063 and <0.5 mm) and silt + clay (<0.063 mm). Sediment samples (~100 mg) were mineralised with 2 mL of HNO_3/HCl (3 : 1 v/v) at 110°C. Plant samples were digested with 2 mL of HNO_3/HClO4 (7 : 1 v/v) at 110°C (Duarte *et al*. 2011). Metal concentrations (copper, zinc, lead, cobalt, cadmium and chromium) were determined by flame atomic absorption spectrometry (FAAS, Duisburg, Germany) with an air-acetylene flame. International certified reference materials (CRM145, CRM 146 and BCR 62) were used to ensure accuracy, and precision was determined by analysing replicate samples. Trace metal concentrations in the reference materials determined by FAAS were not statistically different from the certified values (Student's *t*-test; $\alpha = 0.05$).

Data analysis

Above and belowground net primary production (NPP, g) for each species were calculated according to Caçador *et al*. (2007), using Eqn 1, where the minimum biomass value found in the work period was subtracted of the maximum biomass value found in the same period:

$$\text{NPP} = \text{maximum biomass} - \text{minimum biomass}. \quad (1)$$

The root decomposition was calculated as percentage of NPP (grams) using the following formula:

$$\text{Root decomposition} = \left(1 - \frac{\text{Minimum root biomass}}{\text{Maximum root biomass}}\right) \times \text{Root NPP}. \quad (2)$$

Aboveground biomass losses (g) were assessed for the biomass lost during senescence, using the biomasses recorded as described above for root decomposition (Eqn 2). Metal pools (mg) were determined by multiplying the biomass of a sample at time t by the metal content per mg at time t to assess the effective amount of metal retained in the plants:

$$\text{metal pool} = [\text{metal}]_t \times \text{biomass}_t. \quad (3)$$

Metal primary accumulation (MPA, mg) was calculated according to the following equation using the maximum and minimum metal pool values verified during the sampling season:

$$\text{MPA} = \text{maximum metal pool} - \text{minimum metal pool}. \quad (4)$$

Metal exports (mg) were calculated as a percentage of MPA (Eqn 4) as described above, taking into account the percentage of mass losses due to decomposition of the belowground (metal export_{dec}) or senescence of the aboveground organs (metal export_{sen}):

$$\text{metal export}_{dec} = \text{root decomposition} \times \text{root MPA}, \quad (5)$$

$$\text{metal export}_{sen} = \text{aboveground senescence} \times \text{aboveground MPA}. \quad (6)$$

To calculate an annual balance between the vegetation and the sediment, an average metal concentration was derived for the sediment. Metal returns to the sediment due to biomass losses (root decomposition and aboveground organs decomposition) were determined as by Wen-jiao *et al*. (1997):

$$\text{Metal return} = \left(\frac{\text{Export rate} \times \text{MPA}}{\text{Metal concentration in sediment}}\right) \quad (7)$$

The cycling coefficient and turnover period were also calculated according to Wen-jiao *et al*. (1997) and Válega *et al*. (2008).

$$\text{Cycling coefficient} = \frac{\text{Metal returned due to litter fall}}{\text{MPA}} \quad (8)$$

The biomass parameters (NPP and maximum biomass) were used to determine the turnover rate of each plant part, while the metal accumulation parameters (MPA and metal pool) were used to assess the turnover rate of each metal pool:

$$\text{Turnover rate} = \frac{\text{NPP or MPA}}{\text{Maximum biomass or metal pool}} \quad (9)$$

Statistical analysis

To check for differences between the biomasses and the metal contents, one-way ANOVA tests were employed using the software Statplus ver. 5.8 (AnalystSoft Inc., Vancouver, Canada). A non-metric multidimensional scaling (nMDS) was analysed together with the ANOVA for a better view of the metal concentration between species results. The nMDS analyses were performed using the PRIMER ver. 6 (Primer-E Ltd., Lutton, Ivybridge, UK).

Results

Sediment characteristics

Grain size composition in the *S. maritimus* zone was composed of ~32% of coarse sand, 55% of fine + medium sand and 13% by silt + clay. In contrast, *Z. noltii* showed low values of coarse sand (5%) but high values of fine + medium sand (73%) and silt + clay particles composed 22% of the sediment in the *Z. noltii* area. Similar to that in *Z. noltii* area, in the rhizosediments colonised by *S. maritima* a low proportion for coarse sand (9%) was found, but the results for fine + medium sand (53%) were similar to those under *S. maritimus*; the percentage found for silt + clay was 38%, and higher than for the other two species.

Biomass production

Regarding plant biomass (Fig. 2; Table 1), both *S. maritimus* and *S. maritima* had significantly higher biomass values in their belowground rather than in their aboveground organs ($P < 0.05$). *S. maritimus* always presented the highest belowground biomass, with a maximum during the spring season. When comparing the belowground NPP values of the three species, significant differences were only found between *S. maritimus* and *Z. noltii* ($P < 0.05$). The NPP mean value of *S. maritimus* (1010 g m^{-2}) was more than three times higher than that of *Z. noltii* (270 g m^{-2}). The highest value of NPP (Table 1) for the aboveground organs was found in *S. maritimus*, followed by *S. maritima* and *Z. noltii*. All differences between the NPP of the aboveground organs were significant ($P < 0.05$). Turnover rates for aboveground organs were significantly different ($P < 0.05$) between all species pairs except *S. maritimus* and *S. maritima*. There were no significant differences in turnover rates for belowground organs.

Heavy metal concentrations in plants

S. maritima showed higher ($P < 0.05$) metal concentrations in the belowground organs than in the aboveground organs (Fig. 3), with the exception of Cu, for which the difference was not significant. This species had seasonal differences ($P < 0.05$) for Co and Cr in above and belowground organs, but not for the others metals. The highest average concentration for the majority of

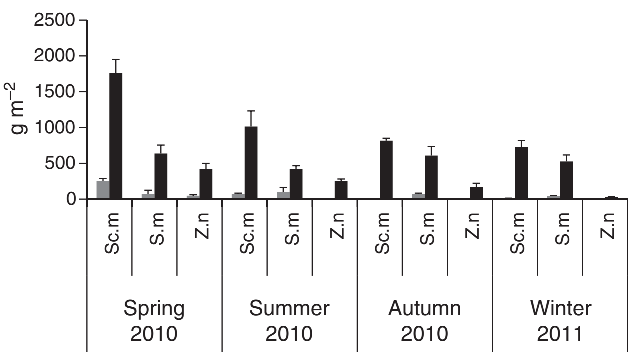

Fig. 2. Above- and belowground seasonal biomass average (grey, aboveground; black, belowground) of the three studied species over 1 year period and s.d. Sc.m, *Scirpus maritimus*; S.m, *Spartina maritima*; Z.n, *Zostera noltii*.

Table 1. Biomass (g m^{-2}) for each season, NPP (g m^{-2}) and turnover (g m^{-2}) ± standard deviation

Statistical seasonal differences (*, $P < 0.05$). Above- and belowground organs statistically different from each other in all seasons (**, $P < 0.05$)

	Spring	Summer	Autumn	Winter	NPP	Turnover ($year^{-1}$)
Aboveground organs						
*S. maritimus**,**	252 ± 35	70 ± 13			190 ± 90**	0.76 ± 0.09
*S. maritima***	72 ± 51	102 ± 61	71 ± 11	45 ± 2	80 ± 30**	0.77 ± 0.28
*Z. noltii**,**	47 ± 12	2 ± 0.55	5 ± 1.8	8 ± 1	70 ± 52**	1.53 ± 0.35**
Belowground organs						
*S. maritimus***	1761 ± 191	1014 ± 216	815 ± 34	887 ± 278	1010 ± 330**	0.66 ± 0.15
*S. maritima***	637 ± 116	420 ± 45	607 ± 127	526 ± 90	600 ± 340**	0.73 ± 0.19
*Z. noltii**,**	419 ± 81	249 ± 31	167 ± 54	31 ± 12	270 ± 200**	0.66 ± 0.13**

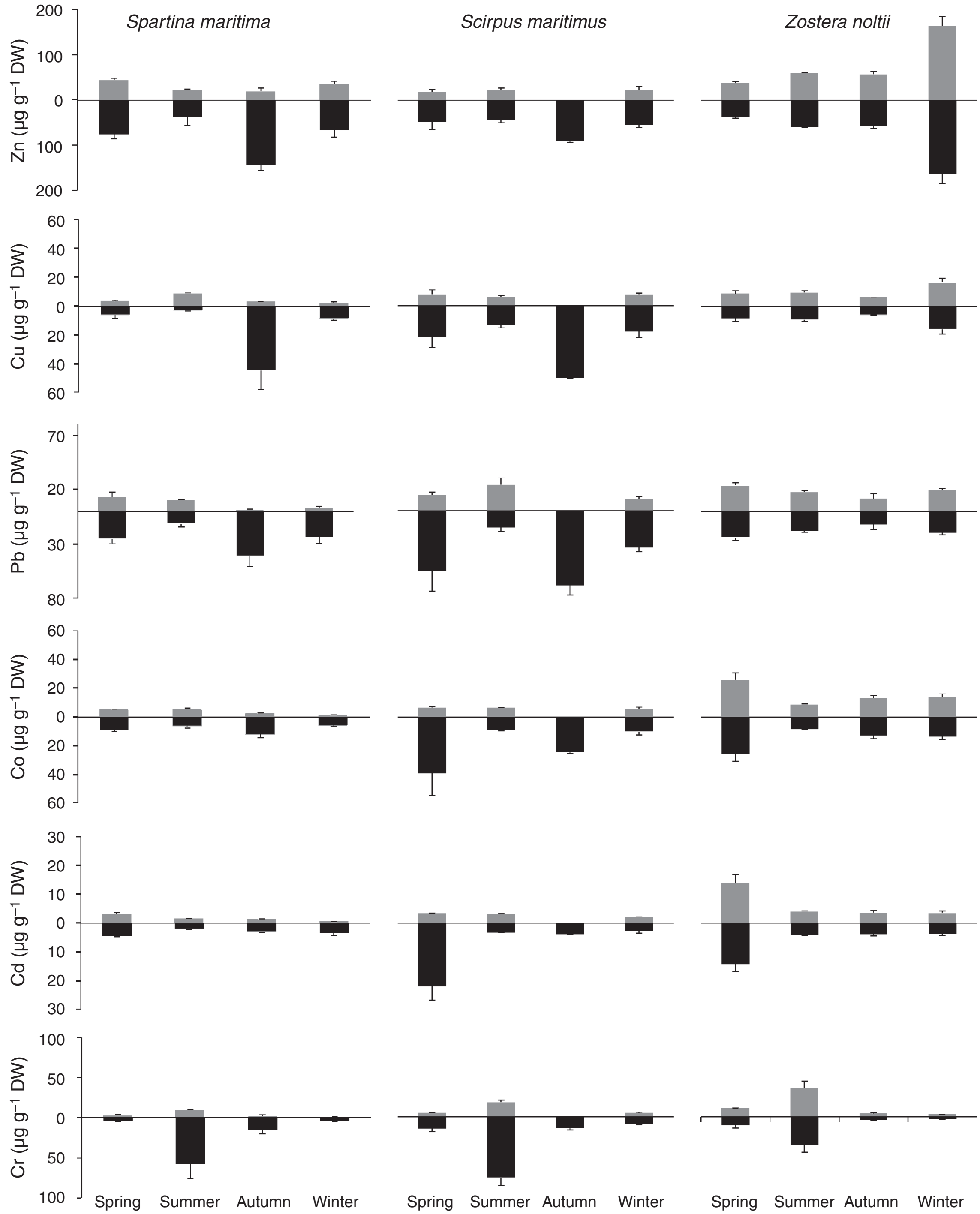

Fig. 3. Above- and belowground seasonal metal concentration average for *Scirpus maritimus*, *Zoostera noltii* and *Spartina maritima* and s.d. over 1 year period. (grey, aboveground organs; black, belowground organs).

analysed metals (Zn, Cu, Pb and Co) occurred in autumn and the lowest in summer. *S. maritimus* exhibited similar behaviour to *S. maritima*, with higher ($P < 0.05$) metal concentration in the belowground organs (Fig. 3). Except for Cu, where the difference was not significant, *S. maritimus*, presented seasonal differences ($P < 0.05$) for all metals, and had half of the analysed metals (Zn, Cu and Pb) with their highest average concentration in autumn. Like the other two species, *Z. noltii* showed significant seasonal variation of metal concentrations ($P < 0.05$) for the majority of studied metals. The above- and belowground organs, in this species had similar concentrations ($P > 0.05$) (Fig. 3). Figure 4 shows the differences that occured between species, showing that

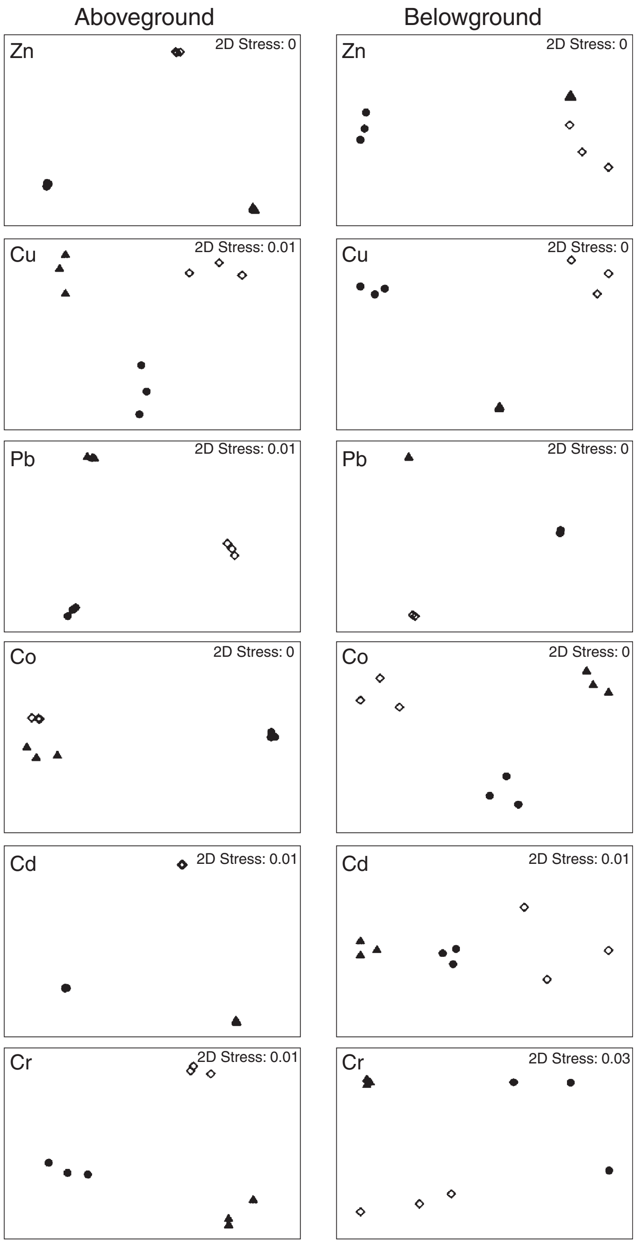

Fig. 4. nMDS of the metal concentrations for the above and belowground organs in the three studied species for one year period. ▲, *Scirpus maritimus*; ◇, *Spartina maritima*; ●, *Zoostera noltii.*

pratically for all metals the species were separated from each other. Half of analysed metals (Pb, Co and Cd) had their highest average values in spring.

Heavy metal concentration in sediments

Significant differences ($P < 0.05$) were found between the sediment heavy metal concentrations of the three studied species for all metals except Zn and Cd. These two elements were found to be the most and least abundant elements (Zn and Cd respectively) in the sediments, whereas the other metals had similar concentrations. Sediments colonised by *S. maritimus* presented the highest values for all metals, with the exception of Cd (Fig. 5). Cu had a higher concentration than Cr ($P < 0.05$) between the sediments under *S. maritimus* and *S. maritima*. All metal levels, with the exception of zinc and cadmium, were significantly different ($P < 0.05$) between the sediments under *S. maritimus* and *Z. noltii*. In comparison with the elemental composition of the Earth's crust (Turekian and Wedepohl 1961), only Pb and Cd were enriched in the sediments (twice for Pb and 30 times for Cd).

Heavy metal cycling

The metal primary accumulation (MPA) and exports for the aboveground and belowground organs of each species studied are shown in Fig. 6. In *S. maritimus* aboveground organs, all of the accumulated metals were exported to the sediment or water column. A similar behaviour was observed in *Z. noltii*, which accumulated only small amounts of metals in its aboveground and belowground organs, whereas *S. maritima* showed a lower exportation rate. High accumulation values occurred in the belowground organs of both *S. maritima* and *S. maritimus*. With the exception of Cr, *S. maritimus* had a higher aboveground turnover rate for all metals than *S. maritima*. When comparing *S. maritimus* and *Z. noltii*, only Co and Cd aboveground turnover rates showed significant differences ($P < 0.05$). Only Zn and Pb rates showed statistically significant differences ($P < 0.05$) between *S. maritimus* and *S. maritima*, whereas only Cr rates showed significant differences ($P < 0.05$) between *Z. noltii* and *S. maritima*. For the metal cycling coefficient (Table 2) of the aboveground organs, *S. maritimus* had higher values for Cu, Pb and Cr, whereas *Z. noltii* had higher values for Zn, Co and Cd. For the belowground organs, *S. maritima* showed higher values for Zn and Cu, and *S. maritimus* had higher values for Pb, Co, Cd and Cr. With the exception of the comparison between *S. maritimus* and *S. maritima* aboveground organs for zinc, all other cycling coefficient comparisons did not show significant differences ($P > 0.05$). Table 3 shows the amounts of metals retained and exported by each species. With an estuarine coverage area of ~16.4 ha, *S. maritimus* accumulated more than 2 kg of each of the analysed metals in one year, with Pb being the most retained metal. *S. maritima*, with an area of 2.8 ha, retained more Zn, with a total of 1.8 kg in 1 year, and *Z. noltii*, with an area of 10.4 ha, accumulated more zinc and cobalt.

Discussion

Although the absolute heavy metal concentrations in the sediments of the Mondego Estuary are very low, if the extent of the plant coverage is considered, the heavy metal concentrations in plant biomass acquire new ecological importance.

As previously noted for other halophytes in other coastal systems (Caçador *et al*. 2009; Duarte *et al*. 2010), higher amounts of plant biomass were found in the belowground than the aboveground organs. When plants accumulate metals, the roots and rhizomes generally show higher concentrations than the aboveground parts (Sinicrope *et al*. 1992). This behaviour could be seen in other studies like Reboreda *et al.* (2008) and Duarte *et al*. (2010). It was also confirmed that heavy metal accumulation

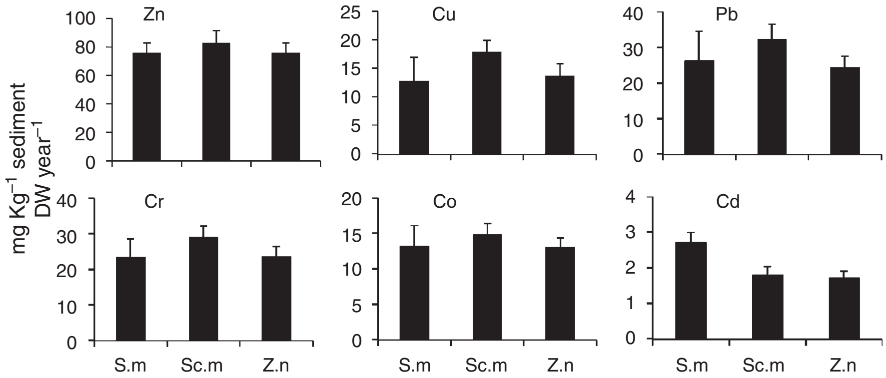

Fig. 5. Average metal concentrations in sediment for 1 year period and s.d. (S.m, *Spartina maritima*; Sc.m, *Scirpus maritimus*; Z.n, *Zostera noltii*).

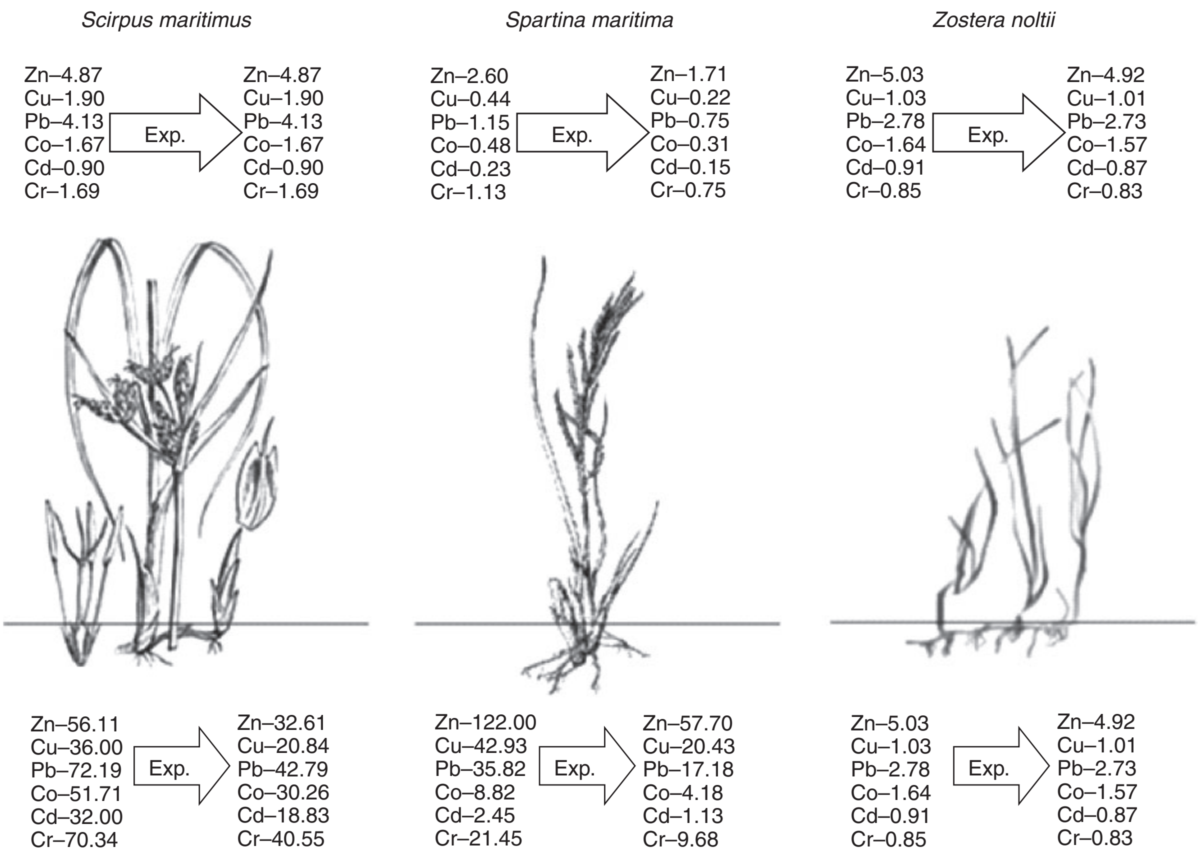

Fig. 6. Metal primary accumulation (MPA, mg m^{-2}) and exports of each studied species, for above- (values at top) and belowground (values at bottom) organs. Exp., export.

is not strictly independent of biomass production (Caçador *et al.* 2009; Duarte *et al.* 2010). Although all species presented similar patterns of biomass production, with higher values during spring and lower values towards the winter, *S. maritimus* is peculiar in that it completely loses all of its aboveground biomass during the autumn. This life history trait likely has implications on the ecosystem metal budget, as all of the aboveground biomass in areas colonised by *S. maritimus* is converted into necromass and exported to the adjacent water column.

Because of the very different areas colonised by each of the investigated halophytes, these endogenous processes are of great importance when addressing the metal budgets between sediments, halophytes and the estuary. Heavy metals absorption in seagrasses appears to be influenced by seasons (Larkum *et al.* 2006). What was corroborated by the results in this study, was that seasonal differences occurred in *Z. noltii* metal concentrations. This species also presented high biomass exportation percentages. *Zostera* individuals can become

Table 2. Mean cycling coefficient values ± s.d. for all the studied species for each metal in 1 year period
Significant differences between above- and belowground organs are indicated: *, $P < 0.05$

	Zn	Cu	Pb	Co	Cd	Cr
Aboveground organs						
Scirpus maritimus	0.058 ± 0.005*	0.106 ± 0.015*	0.128 ± 0.005*	0.113 ± 0.007*	0.505 ± 0.006*	0.058 ± 0.005*
Zostera noltii	0.063 ± 0.004	0.073 ± 0.002	0.112 ± 0.003	0.121 ± 0.009	0.512 ± 0.061	0.035 ± 0.007
Spartina maritima	0.007 ± 0.003*	0.016 ± 0.006*	0.028 ± 0.003*	0.023 ± 0.009*	0.785 ± 0.057	0.032 ± 0.019*
Belowground organs						
Scirpus maritimus	0.381 ± 0.082*	1.137 ± 0.015*	1.297 ± 0.085*	2.051 ± 0.097*	10.470 ± 0.689*	1.395 ± 0.275*
Zostera noltii	0.063 ± 0.004	0.073 ± 0.002	0.112 ± 0.003	0.121 ± 0.009	0.512 ± 0.061	0.035 ± 0.007
Spartina maritima	0.744 ± 0.140*	1.595 ± 0.006*	0.629 ± 0.244*	0.317 ± 0.079*	0.825 ± 0.023	0.414 ± 0.082*

Table 3. MPA, export and accumulation for all the studied species in their total area for each metal (g)

Species	MPA/export	Organ	Zn	Cu	Pb	Co	Cd	Cr
Scirpus maritimus	MPA	Aboveground	799	311	677	273	147	277
		Belowground	9205	5906	11843	8483	5250	11540
	Export	Aboveground	799	311	677	273	147	277
		Belowground	5350	3419	7020	4964	3089	6652
	Accumulated	Total	3855	2487	4823	3519	2160	4887
Spartina maritima	MPA	Aboveground	751	127	332	138	6	32
		Belowground	3526	1241	1035	254	70	620
	Export	Aboveground	494	635	21	896	4	21
		Belowground	1667	590	496	120	32	279
	Accumulated	Total	1884	656	550	139	40	351
Zostera noltii	MPA	Aboveground	527	108	291	171	95	89
		Belowground	527	108	291	171	95	89
	Export	Aboveground	515	105	286	164	91	87
		Belowground	515	105	286	164	91	87
	Accumulated	Total	24	6	10	14	8	4

detached from sediments due to extreme events (Den Hartog 1987). This process has enormous implications due to the vast areas covered by *Zostera* meadows along the Mondego Estuary and the biomass exported during these events. Additionally, it is important to consider that the entire plant is exported during these *Zostera* release events, not only the aboveground organs, suggesting that the amount of heavy metal-contaminated biomass exported at these events is comparable to that assessed for *S. maritimus.*

Solís *et al.* (2007) found similar metal concentration for above and belowground organs in the seagrass *Thalassia testudinum*. Similar behaviour was noted in *Z. noltii* in the present work, but for the other two studied species, the aboveground biomass presented lower metal values in comparison with the belowground biomass, which is in agreement with previous studies (Duarte *et al*. 2010). Our results showed that the heavy metal concentrations were higher in the belowground organs (in agreement with Cambrollé *et al*. 2008; Reboreda *et al*. 2008; Duarte *et al*. 2010) of both *S. maritimus* and *S. maritima*, which suggests the ability to accumulate heavy metals; in contrast, *Z. noltii* showed similar concentration levels in both aboveground and belowground organs but still accumulated metal. The three studied species presented higher belowground biomass values in the spring than the other seasons, and many of the heavy metal concentrations were higher in autumn and winter. The absorption and exportation by the three species did not show significant differences, demonstrating that all species absorb and export metals at similar quantity.

With the exception of the contents of the aboveground organs of *S. maritimus* (which are washed out due to senescence), the remaining two species showed low turnover, indicating that metal cycling between roots and sediments occurs at a rather slow pace. The annual mean of all of the sediment heavy metal concentration values found in this work was low in comparison with other estuaries around the world, such as Kyeongi Bay in Korea (Ahn *et al*. 1995), Sheldt in the Netherlands (Van Alsenoy *et al*. 1993), Thames in the UK (Attrill and Thomes 1995), Port Philip Bay (Talbot *et al*. 1976), the Bilbao Estuary (Cearreta *et al*. 2000) and Jurujuba (Baptista Neto *et al*. 2000). Indeed, all of the heavy metal values in the salt marsh of the Mondego Estuary are below the limits prescribed in the Guidance on the assessment and redevelopment of contaminated land (ICRLC 1987). Zhang *et al.* (2001), in the intertidal sediment of *Scirpus mariqueter* and *Scirpus trigueter*, also had higher values for Cu and Zn than the values found in this work for *S. maritimus.* Another example can be found in the work of Izquierdo *et al.* (1997), where higher values of Cu, Zn, Pb, Cd and Cr in the Odiel (Spain) salt marsh sediment were found. Mondego salt marsh has very low values of these six heavy metals (Zn, Cu, Pb, Cd, Co and Cr) and can therefore be used as a reference for non-contaminated salt marshes. Although rather low concentrations of heavy metals were verified in the salt marsh sediments, the plants of the ecosystem function not only as a sink for heavy metal retention, but as a source, exporting metals to nearby systems.

Conclusion

The role of halophytic plants as metal sinks, mostly through their root systems, is well known in contaminated salt marshes. The Mondego Estuary salt marshes have relatively low heavy metal concentrations in their sediments. This system is also largely colonised by two species of aquatic vegetation with high biomass exportation rates, either due to senescence mechanisms (*S. maritimus*) or physical factors (*Z. noltii*). Although the amount of metals taken up by the aquatic vegetation is relatively low, the exportation of biomass has enormous implications on system contamination at the estuarine level. From analysis of the concentration of metals in the above and belowground organs and in the sediment it was possible to conclude that the Mondego Estuary, although low in contaminants, could act as a source of metals for nearby systems.

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References

Ahn IY, Kang YC, Choi JW (1995) The influence of industrial effluents on intertidal benthic communities in Panweol, Kyeonggi Bay (Yellow Sea) on the west coast of Korea. *Marine Pollution Bulletin* **30**, 200–206. doi:10.1016/0025-326X(94)00125-S

Attrill MJ, Thomes RM (1995) Heavy metal concentrations in sediment from the Thames Estuary, UK. *Marine Pollution Bulletin* **30**, 742–744. doi:10.1016/0025-326X(95)98339-X

Baptista Neto JA, Smith BJ, McAllister JJ (2000) Heavy metal concentrations in surface sediments in a nearshore environment of Jurujuba Sound, Brazil. *Environmental Pollution* **109**, 1–9. doi:10.1016/S0269-7491(99)00233-X

Caçador I, Vale C, Catarino F (1996) The influence of plants on concentration and fractionation of Zn, Pb, and Cu in salt marsh sediments (Tagus Estuary, Portugal). *Journal of Aquatic Ecosystem Health* **5**, 193–198. doi:10.1007/BF00124106

Caçador I, Vale C, Catarino F (2000) Seasonal variation of Zn, Pb, Cu and Cd concentrations in the root-sediment system of *Spartina maritima* and *Halimione portulacoides* from Tagus Estuary salt marshes. *Marine Environmental Research* **49**, 279–290. doi:10.1016/S0141-1136(99)00077-X

Caçador I, Costa A, Vale C (2004) Carbon storage in Tagus saltmarsh sediments. *Water Air and Soil Pollution Focus* **4**, 701–714. doi:10.1023/B:WAFO.0000028388.84544.ce

Caçador I, Costa A, Vale C (2007) Nitrogen sequestration capacity of two salt marshes from the Tagus estuary. *Hydrobiologia* **587**, 137–145. doi:10.1007/BF00124106

Caçador I, Caetano M, Duarte B, Vale C (2009) Stock and losses of trace metals from salt marsh plants. *Marine Environmental Research* **67**, 75–82. doi:10.1016/j.marenvres.2008.11.004

Caçador I, Costa JL, Duarte B, Silva G, Medeiros JP, Azeda C, Castro N, Freitas J, Pedro S, Almeida PR, Cabral H, Costa MJ (2012) Macroinvertebrates and fishes as biomonitors of heavy metal concentration in the Seixal Bay (Tagus Estuary): which species perform better? *Ecological Indicators* **19**, 184–190. doi:10.1016/j.ecolind.2011.09.007

Cambrollé J, Redondo-Gómez S, Mateos-Naranjo E, Figueroa ME (2008) Comparison of the role of two *Spartina* species in terms of phytostabilization and bioaccumulation of metals in the estuarine sediment. *Marine Pollution Bulletin* **56**, 2037–2042. doi:10.1016/j.marpolbul.2008.08.008

Cearreta A, Irabien MJ, Leorri E, Yusta I, Croudace IW, Candy AB (2000) Recent anthropogenic impacts on the Bilbao Estuary, Northern Spain: geochemical and microfaunal evidence. *Estuarine, Coastal and Shelf Science* **50**, 571–592. doi:10.1006/ecss.1999.0582

Den Hartog C (1987) 'Wasting disease' and other dynamic phenomenain *Zostera* beds. *Aquatic Botany* **27**, 3–14. doi:10.1016/0304-3770(87)90082-9

Doyle M, Otte M (1997) Organism-induced accumulation of Fe, Zn and As in wetland soils. *Environmental Pollution* **96**, 1–11. doi:10.1016/S0269-7491(97)00014-6

Duarte B, Caetano M, Almeida PR, Vale C, Caçador I (2010) Accumulation and biological cycling of heavy metal in four salt marsh species, from Tagus Estuary (Portugal). *Environmental Pollution* **158**, 1661–1668. doi:10.1016/j.envpol.2009.12.004

Duarte B, Freitas J, Caçador I (2011) The role of organic acids in assisted phytoremediation processes of salt marsh sediments. *Hydrobiologia* **674**, 169–177. doi:10.1007/s10750-011-0731-3

EC (2000) Directive 2000/60/EC of the European Parliament and of the Council Establishing a Framework for Community Action in the Field of Water Policy. PE-CONS3639/1/00 REV 1 EN.

Folk RL (1954) The distinction between grain size and mineral composition in sedimentary rock nomenclature. *Journal of Geology* **62**(4), 344–359. doi:10.1086/626171

Gross M, Hardisky M, Wolf P, Klemas V (1991) Relationship between aboveground and belowground biomass of *Spartina alterniflora* (smooth cordgrass). *Estuaries* **14**, 180–191. doi:10.2307/1351692

ICRLC (1987) Guidance on the Assessment and Redevelopment of Contaminated Land. Guidance Note 59/83. Department of Environment, London.

Izquierdo C, Usero J, Gracia I (1997) Speciation of heavy metals in sediments from salt marshes on the Southern Atlantic Coast of Spain. *Marine Pollution Bulletin* **34**, 123–128. doi:10.1016/S0025-326X(96)00059-8

Larkum AWD, Orth RJ, Duarte CM (2006) 'Seagrasses: biology, ecology and conservation.' (Springer: Dordrecht, The Netherlands)

Marques JC, Nogueira A (1991) Life cycle, population dynamics, and production of *Echinogammarus marinus* (Leach) (Amphipoda) in the Mondego Estuary (Portugal). *Acta Oceanologica* **11**, 213–223.

Marques JC, Maranhão P, Pardal MA (1993) Human impact assessment on the subtidal macrobenthic community structure in the Mondego Estuary (Western Portugal). *Estuarine, Coastal and Shelf Science* **37**, 403–419. doi:10.1006/ecss.1993.1064

Marques JC, Nielsen SN, Pardal MA, Jørgensen SE (2003) Impact of eutrophication and river management within a framework of ecosystem theories. *Ecological Modelling* **166**, 147–168. doi:10.1016/S0304-3800(03)00134-0

Neto JM, Flindt MR, Marques JC, Pardal MA (2008) Modelling nutrient mass balance in a temperate meso-tidal estuary: implications for management. *Estuarine, Coastal and Shelf Science* **76**, 175–185. doi:10.1016/j.ecss.2007.06.013

Pereira P, Caçador I, Vale C, Caetano M, Costa AL (2007) Decomposition of belowground litter and metal dynamics in salt marshes (Tagus Estuary, Portugal). *The Science of the Total Environment* **380**, 93–101. doi:10.1016/j.scitotenv.2007.01.056

Reboreda R, Caçador I (2007) Halophyte vegetation influences in salt marsh retention capacity for heavy metals. *Environmental Pollution* **146**, 147–154. doi:10.1016/j.envpol.2006.05.035

Reboreda R, Caçador I, Pedro S, Almeida PR (2008) Mobility of metals in salt marsh sediments colonised by *Spartina maritima* (Tagus Estuary, Portugal). *Hydrobiologia* **606**, 129–137. doi:10.1007/s10750-008-9340-1

Sinicrope TL, Langis R, Gersberg RM, Busnardo MJ, Zedler JB (1992) Metal removal by wetland mesocosms subjected to different hydroperiods. *Ecological Engineering* **1**, 309–322. doi:10.1016/0925-8574(92)90013-R

Solís C, Martínez A, Lavoisier E, Martínez MA, Isaac-Olivé K (2007) Trace metal analysis in sea grasses from Mexican Caribbean Coast by particle induced X-ray emission (PIXE). *Revista Mexicana de Física* **54**(1), 50–53.

Sousa AI, Lillebø AI, Caçador I, Pardal MA (2008) Contribution of *Spartina marítima* to the reduction of eutrophication in estuarine system. *Environmental Pollution* **156**, 628–635. doi:10.1016/j.envpol.2008.06.022

Suntornvongsagul K, Burke DJ, Hamerlynck AP, Hahn D (2007) Fate and effects of heavy metals in salt marsh sediments. *Environmental Pollution* **149**, 79–91. doi:10.1016/j.envpol.2006.12.010

Talbot V, Magee RJ, Hussain M (1976) Distribution of heavy metals in Port Philip Bay. *Marine Pollution Bulletin* **7**, 53–55. doi:10.1016/0025-326X(76)90093-X

Turekian KK, Wedepohl KH (1961) Distribution of the elements in some major units of the Earth's crust. *Geological Society of America Bulletin* **72**, 175–191. doi:10.1130/0016-7606(1961)72[175:DOTEIS]2.0.CO;2

Válega M, Lillebø A, Pereira M, Caçador I, Duarte A, Pardal M (2008) Mercury mobility in a salt marsh colonized by Halimione portulacoides. *Chemosphere* **72**, 1607–1613. doi:10.1016/j.chemosphere.2008.04.007

Van Alsenoy V, Bernard P, Van Grieken R (1993) Elementary concentrations and heavy metal pollution in sediments and suspended matter from the Belgian North Sea and Scheldt Estuary. *The Science of the Total Environment* **133**, 153–181. doi:10.1016/0048-9697(93)90119-Q

Weis JS, Weis P (2004) Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. *Environment International* **30**, 685–700. doi:10.1016/j.envint.2003.11.002

Wen-jiao Z, Xiao-yong C, Peng L (1997) Accumulation and biological cycling of heavy metal elements in *Rhizophora stylosa* mangroves in Yingluo Bay, China. *Marine Ecology Progress Series* **159**, 293–301. doi:10.3354/meps159293

WFD CIS (2003) Overall approach to the classification of ecological status and ecological potential. Guidance Document No. 13. Directorate General Environment of the European Commission, Brussels.

Zhang W, Yu L, Hutchinson SM, Xu S, Chen Z, Gao X (2001) China's Yangtze Estuary: I. Geomorphic influence on heavy metal accumulation in intertidal sediments. *Geomorphology* **41**, 195–205. doi:10.1016/S0169-555X(01)00116-7