

***Sarcocornia fruticosa* AND *Spartina maritima* AS HEAVY METALS REMEDIATORS IN SOUTHWESTERN EUROPEAN SALT MARSH (RIA FORMOSA, PORTUGAL)**

M. MOREIRA DA SILVA^{a,b,c*}, J. ANÍBAL^{a,c}, D. DUARTE^{a,d},
L. CHÍCHARO^{a,b,d}

^a*CIMA – Centro de Investigação Marinha e Ambiental. Universidade do Algarve, Faro, Portugal*
E-mail: msanti@ualg.pt

^b*International Centre for Coastal Ecohydrology (ICCE) – UNESCO*

^c*Instituto Superior de Engenharia da Universidade do Algarve, Campus Penha, 8005-139 Faro, Portuga*

^d*Faculdade de Ciências e Tecnologia da Universidade do Algarve, Campus Gambelas, 8005-139 Faro, Portugal*

Abstract. Salt marshes are transition zones between land and sea, exposed to several sources of different pollutants, including heavy metals, which tend to be accumulated in sediments. Halophyte vegetation which colonises sediments affects retention and bioavailability of the pollutants that reach salt marsh areas. The accumulation capacity and the pattern of metal distribution in salt marsh plant tissues vary among plant species, and with sediment characteristics. The aim of this study was to survey the behaviour of *Spartina maritima* and *Sarcocornia fruticosa* on heavy metals contents and distribution amongst sediment and plant tissues in an European Southwestern lagoon (Ria Formosa, Portugal). Both species could fix metals from the surrounding belowground environment and accumulate them mainly in roots (and in rhizomes for *S. maritima*). Metal translocation to aerial organs was residual. *S. maritima* acted as a more effective metal stabiliser than *S. fruticosa*.

Keywords: salt marshes, heavy metals, halophyte vegetation, phytoremediation.

AIMS AND BACKGROUND

Salt marshes are wetlands positioned at the land–sea interface and though they occupy only 4% of the Earth total land, these areas are exposed to a large number of anthropogenic impacts as urbanisation, tourism activities, industrial activities and agricultural uses¹. Salt marshes have an important ecological role and provide several eco-services, including: sediment accumulation; nutrients cycling; filtering of contaminants; wildlife habitat; flood regulation and storm protection^{2–6}. When pollutants, including heavy metals resulting from human activities, enter saltmarsh areas they disperse with the tides, interacting with soil and biotic communities^{7,8}.

* For correspondence.

Heavy metals can have serious effects on sediment and water quality, biota and human health⁹⁻¹⁴. Aquatic organisms that produce epibenthic structures such as salt marsh plants (halophytes) play key roles in habitat functioning¹⁵. Halophytes that colonise salt marshes have ability to accumulate heavy metals or tolerate high levels of toxic ions in the environment¹¹. These capabilities make halophytes excellent candidates for phytoextraction and phytostabilisation of heavy metals in contaminated sediments¹¹. Salt marsh plants accumulate large amounts of metals in their aerial and belowground tissues¹⁶⁻¹⁹. The solubility and availability of metals in marshes, in general, and for vegetation in particular, may be affected by several factors such as concentration and speciation of metals and characteristics of the sediment, such as grain size, organic matter content, biotic aspects, concentrations of inorganic and organic ligands including plants exudates, cation exchange capacity, etc. Therefore, mutual interactions between plants and surrounding chemical environment, which determine the role played by plants on trace metal distribution and uptake, may vary among plant species and, for a single plant, among locations with different characteristics. Metal translocation and storage capacity is rather heterogeneous and controlled by genetic, environmental and toxicological factors^{17,20,21}. Previous studies showed that roots are the main pool of metals within salt marsh plants^{18,22-29}. The dominant producer of the lower salt marshes in Ria Formosa is the small cordgrass *Spartina maritima* (Poales: Poaceae), which is a pioneer specie in the lower marsh areas with a typical zonation forming clear homogeneous stands. *Sarcocornia fruticosa* (Caryophyllales: Chenopodiaceae) appears frequently in the upper saltmarsh in pure stands³⁰. The aim of this study was to evaluate the assess the role of *S. maritima* and *S. fruticosa* as heavy metals remediators in Ria Formosa saltmarsh.

EXPERIMENTAL

Ria Formosa is a coastal lagoon located in SW Coast of the Iberian Peninsula, which includes a large intertidal zone, about 55 km long (E-W), five sandy barrier islands and six inlets form a roughly triangular chain, with a maximum of 6 km (N-S) at its widest point. Ria Formosa has been recognised as an important natural and permanent wetland with marine water, being National Park since 1987, and European and International level by its acceptance as a Natura 2000 and a Ramsar site³¹. Sampling was carried out in March 2007, at Ria Formosa salt marsh (37°1'54.83" N; 7° 48'44.52" W). Two pure stands of *S. fruticosa* and of *S. maritima* and a non-vegetated local were surveyed. Aboveground biomass was determined by clipping the vegetation at ground level in three circles with 45.36 cm² area. Three sediment cores with 30 cm depth of each site were collected at low tide and sliced in 5 cm thickness layers. The belowground biomass was collected with the rhizosediments and separated at the laboratory. Redox potential and pH

were determined using a HANNA pH/mV meter (HI 931401), with a combined platinum electrode (HI 3131B) for redox potential and a combined glass electrode (HI 1131) for pH. Values of redox potential were accepted after a 5 min period of stabilisation. At the laboratory the sediments were oven dried at 40°C and then weighed), each sample was divided in two portions, one part was used for grain size determinations and the other was homogenised in an agate mortar for further analysis. The organic matter contents of the sediments were determined by loss on ignition at 600°C for 2 h (LOI). The belowground plant material of each depth was separated from sediment, under a flux of water, using a 212 µm mesh size and sieved to remove any adhering particles. *Spartina maritima* roots were separated from rhizomes, and in *S. fruticosa* the amount of rhizomes was negligible when compared to roots. In both species no separation between dead and live parts of the plants was done. The aerial tissues were washed with deionised water to remove dust and sediment. In *S. fruticosa* aerial organs were separated into chlorophylline and non-chlorophylline and in *S. maritima* were separated into leaves and stems. All vegetal material was oven dried at 40°C, weighed and grounded with an agate mortar for further analysis. Three replicates, about approximately 300 mg of each sediment and vegetal material sample, were digested by microwave in high-pressure Teflon vessels. A procedural blank was prepared and included in each digestion batch of 10 samples. Metals and Si were analysed by atomic absorption spectrometry-AAS provided with a deuterium background correction and determined with the standard additions method. Aluminium and Si were analysed by flame AAS with a nitrous oxide-acetylene flame and Fe, Mn and Zn with an air-acetylene flame. Silver, Cd, Cu, Cr, Mo, Ni and Pb were analysed with electrothermal atomisation and an auto-sampler. The accuracy of the analytical procedure was assessed by the analysis of certified references materials (SRM1646a, MESS3, BCSS1, CRM281, BCR61). Procedure blanks always accounted for less than 1% of the metal concentrations in samples. All statistical analyses were carried out using SPSS 12.0 for Windows. Data normality and homocedasticity were tested prior to analysis. To compare the concentration of metals in different organs of both plants was used Student *t*-test. Principal components analysis (PCA) was used to establish relations between means of metal concentrations and organic matter content in depth, from the sediments. To evaluate whether *S. maritima* and *S. fruticosa* can accumulate trace metals in belowground biomass, enrichment factors were calculated:

$$EF = [\text{Me}]_{\text{belowground tissues}} / [\text{Me}]_{\text{rhizosediment}}$$

RESULTS AND DISCUSSION

Visual inspection during sampling showed that main active belowground biomass of *S. maritima* was present in the top 10 cm. Below this depth, roots and rhizomes were inactive (belowground litter). Rhizosediments of *S. fruticosa* displayed visible

inorganic precipitates forming rhizoconcretions below 15 cm, which is compatible with the presence of active biomass capable of oxidising the rhizosediment. Very distinct redox potential depth profiles were observed in the three sites. Non-vegetated sediment was anoxic (or reductive: from -192 to -6 mV). *S. fruticosa* rhizosediment presented positive and relatively high redox potential (oxidative sediment: from 62 to 316 mV) and rhizosediment of *S. maritima* displayed redox potential values between the other two sites (from -9 to 144 mV). Similar reddish brown deposits in which Fe-oxides have been identified on plant roots have been observed in several flood-tolerant plants and attributed to the oxidative capacity of roots³². The release of oxygen by roots can cause a decrease of pH in the rooting zone, possibly due to sulphide oxidation (S^{2-}/SO_4^{2-}) and iron oxidation (Fe^{2+}/Fe^{3+}) followed by Fe^{3+} hydrolysis. In the present case, rhizosediments displayed more acidic conditions than non-vegetated sediment, showing lower pH values (*S. maritima*: 6.62 ± 0.05 ; *S. fruticosa*: 7.05 ± 0.07 ; non-vegetated: 7.68 ± 0.12). Rhizosediment of *S. maritima* was the poorest in silt and clay particles and, only small differences in terms of grain size depth distribution, were observed between rhizosediment of *S. fruticosa* and non-vegetated sediment. Rhizosediment of *S. maritima* was the richest in organic matter, particularly in the first 10 cm depth. The highest organic matter value occurred at surface sediment, 14%, and decreased down to 17.5 cm, remaining practically constant in deeper layers, the global mean value being of $9.9 \pm 2.6\%$. In contrast, *S. fruticosa* displayed organic matter depth profile and magnitude closest to non-vegetated sediments. Organic matter global mean value (between 0 and 30 cm) was slightly higher for the sediment colonised by *S. fruticosa* than for non-vegetated one ($6.5 \pm 1.5\%$ versus $5.8 \pm 1.1\%$).

Metals are distributed differently at the three sites, not only non-vegetated sediment differed from vegetated ones, but also marked differences between sediments colonised by *S. maritima* and *S. fruticosa*. Performed PCA based on contents of those parameters and organic matter at different depth confirmed that each plant gives rise to a very specific environment (Fig. 1).

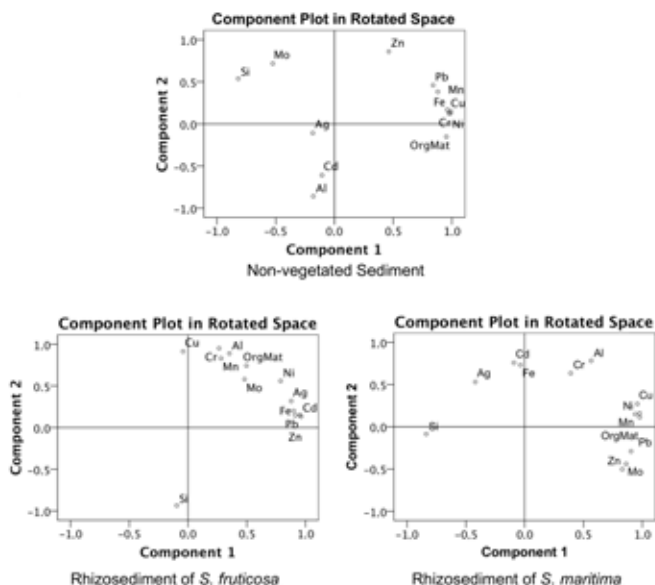


Fig. 1. PCA plots of metals and organic matter measured from 0–30 cm in the three sampling sites

The presence of plants conditioned metal concentration in rhizosediments in different ways. For instance, Al concentration was higher in non-vegetated sediment, the levels decreasing as follows: non-vegetated sediment > (2.5 times higher than) *S. maritima* sediment > (2 times higher than) *S. fruticosa* sediment. In the case of Cd, much higher levels occurred in non-vegetated sediment: non-vegetated sediment > (5 times higher than) *S. maritima* sediment \approx *S. fruticosa* sediment (except for depths > 15 cm, where the levels were slightly higher, but still lower than in non-vegetated sediment). Lower levels in rhizosediments of both plants were also found for Mn (only for depth > 10 cm). For other metals, like Cr and Pb, significant and marked differences between rhizosediments of the two plants were observed: metal levels in sediment colonised by *S. fruticosa* were lower than those founded in both non-vegetated and *S. maritima* sediments. Iron occurred in much higher concentrations in rhizosediment of *S. maritima*, whereas *S. fruticosa* rhizosediment displayed levels identical to those of non-vegetated sediment. Significant influence of the plants was not observed for Ag and Mo levels, whereas Zn occurred in much higher concentration in rhizosediments of both plants than in non-vegetated one. Significant differences were found for metal concentrations ($\mu\text{g g}^{-1}$) in depth between non-vegetated sediment, rhizosediment of *S. maritima* and rhizosediment of *S. fruticosa*, except for Cu and Mo. Therefore, the studied halophytes could influence the composition of sediment among roots and that influence was very specific (depending on the plant species).

The profile of EF in function of depth varied markedly with both the metal nature and plant species (Fig. 2). Enrichment factors higher than 1 have supported the idea that salt marshes can act as metal phytoestabilisers^{27,33}, thus contributing for decreasing ecosystem metals availability.

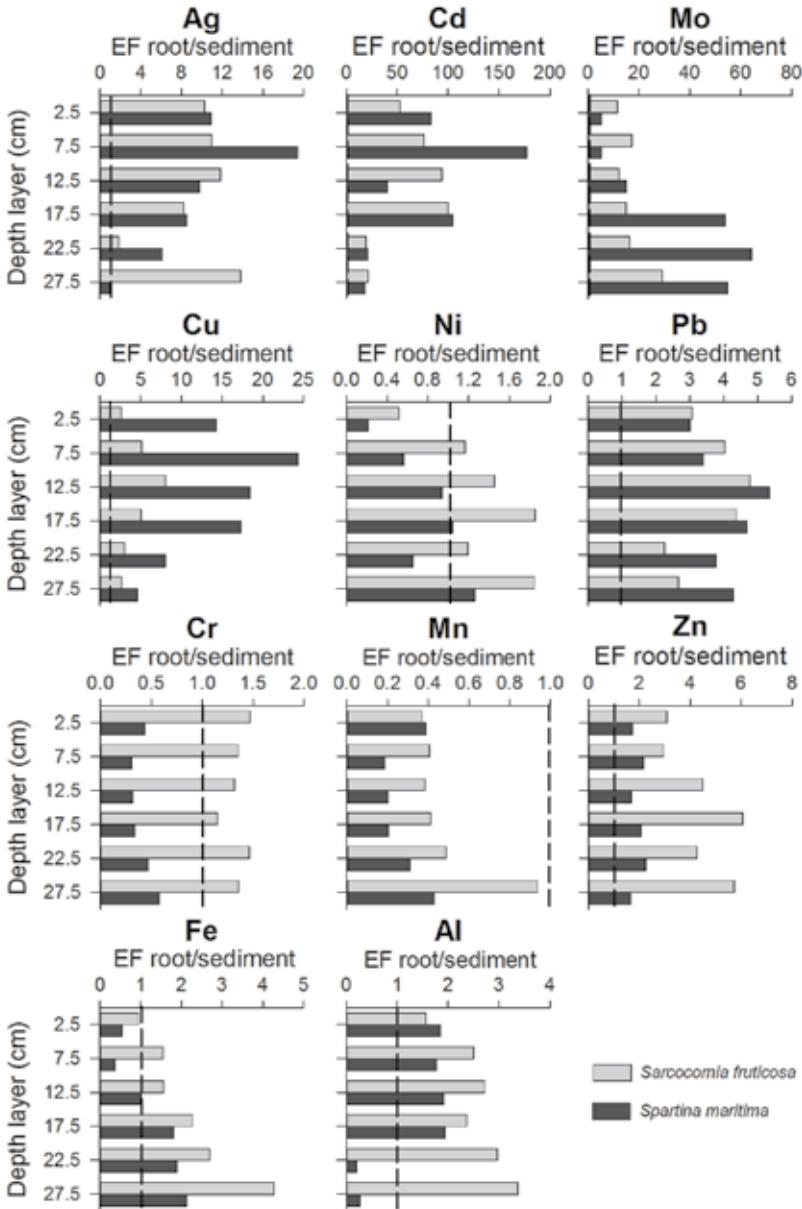


Fig. 2. Heavy metals enrichment factors (EF) per depth layer (the dash line indicates EF = 1)

Influence of plant species resulted of the differences in the plant characteristics, as *S. maritima* is a monocotyledonous and *S. fruticosa* is a dicotyledonous, having unlikeness in density and structure of belowground biomass and biological response.

Enrichment factors were higher than 1 for Ag, Cd, Mo, Cu, Pb, and Zn, for both species and at all depth layers. *S. maritima* displayed lower EF than *S. fruticosa* for Cr, Ni, Zn, Al and Fe and higher for Cu and Pb (below 10 cm depth). Differences in chemical speciation of the different elements, physicochemical characteristics of the rhizosphere and biomass characteristics, all together will condition the observed results. For instance, *Sarcocornia fruticosa* removed Cr from sediment to roots at all depths, while *S. maritima* did not showed the same behaviour.

Once inside the plants, metals can be translocated from belowground vegetal tissues to the aerial organs, leaves and stems for *S. maritima* and clorophyline and non-clorophyline organs for *S. fruticosa* (Table 1).

Table 1. Metal distribution (%) among the plants organs

	Ag	Cd	Cr	Cu	Ni	Pb	Zn	Mn	Mo	Fe	Al
<i>Spartina maritima</i>											
Leaves	0.63	0.13	0.10	0.07	0.00	0.03	1.70	11.32	0.00	8.06	0.41
Stems	0.51	0.21	0.09	0.05	0.00	0.07	1.58	8.60	0.02	5.55	0.34
Rhizomes	22.47	21.67	10.05	4.07	20.67	11.73	23.16	20.96	69.23	20.42	34.15
Roots	76.39	78.00	89.76	95.81	79.33	88.17	73.56	59.12	30.75	65.97	65.10
<i>Sarcocornia fruticosa</i>											
Aerial clorophyline organs	7.56	2.16	0.64	0.74	2.57	0.29	2.28	16.18	1.00	36.70	4.63
Aerial non-clorophyline organs	8.00	7.33	0.98	1.32	2.37	1.46	5.55	34.58	2.24	43.79	7.57
Roots	84.44	90.52	98.39	97.94	95.06	98.26	92.16	49.24	96.75	19.51	87.80

Metal translocation to both kinds of aboveground organs was significantly ($p < 0.05$) higher in *S. fruticosa* than in *S. maritima*, for all metals. The magnitude of translocation for Fe and Mn was very much distinct of that for the remaining studied metals. Iron in aerial organs is closely related to chlorophyll formation and all plants have iron-containing enzymes³⁴. Manganese is an activator of a number of enzymes involving in the tricarboxylic acid cycle, previous studies reported accumulation in shoots of *Phytolacca americana* of about 90% of the whole Mn that plant absorbed³⁵.

Capacity of these saltmarsh species to accumulate and translocated Ag to aboveground tissues was observed and described for the first time, leading to further studies of Ag uptake by other plants, having in mind the possibility of using

phytoremediation for cleaning Ag contaminated soils. Silver residues may occur as a result of some industrial activities, such as photographic films and paper, batteries, mirrors, photosensitive glass, etc. Metal translocation to aboveground tissues may be very valuable for phytoremediation of areas where the plants can be cultivated and harvested, thus removing the pollutant from the soil or sediment. Thus being the case, metal translocation to aerial tissues may result in a drawback of salt marshes as possible sites for phytostabilisation of metals. Metal in aboveground tissues may be accumulated in leaf and stem litter, returning to the marsh system and thus acting as a potential source of metals. In a previous study¹⁹ including Cu, Cd, and Pb, was concluded that areas colonised by *H. portulacoides* are potential sources of metals to the marsh ecosystem, *S. maritima* seeming to contribute more effectively to the metal stabilisation in saltmarsh sediment.

During the last decades, wetland plants have been shown to play important roles in constructed wetlands to remove metals from wastewater. Therefore, phytoremediation on wetlands can be considered an important type of eco-services to society, based on 'green' technologies and low energy consumption³⁶⁻³⁸.

CONCLUSIONS

Spartina maritima and *S. fruticosa* acted as heavy metals remediators, changing the sediment metal distribution in depth and accumulating them, mainly in roots. In both plants, metals translocation to aerial organs was less than 10% of the total accumulated content, excepted for Fe and Mn. Moreover, these results demonstrated that the use of EF, by itself, can not ensure that a marsh colonised by an halophyte species can act as a metal stabiliser. The biomass density and oxidative power of the plant roots are also relevant factors to the balance between metal accumulation and release into the bulk of sediments, conditioning final metal stabilisation.

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