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Influence of the Sea Rush *Juncus maritimus* on Metal Concentration and Speciation in Estuarine Sediment Colonized by the Plant

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Metal accumulation by *Juncus maritimus* and the role of this plant on the physical and chemical composition of sediments, from the Douro river estuary (NW Portugal), were investigated. The contents of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were determined (by atomic absorption spectrophotometry) in sediments, rhizosediments (those among plant roots and rhizomes), and different plant tissues (roots, rhizomes, leaves, and stems). Metal fractionation in sediments through sequential extraction was carried out and used for interpretation of sediment/*J. maritimus* interactions. Two estuarine sites with different characteristics were studied: site I displayed sandy sediment with lower organic matter and metals more weakly bound to it than site II sediment, which was muddy. At both sites, higher metal contents were observed in rhizosediments than in the surrounding sediment, but metals were more weakly bound to rhizosediment. Therefore, *J. maritimus* markedly influenced the sediments among its roots and rhizomes, changing metal distribution and speciation. Different patterns of both metal uptake and metal distribution among *J. maritimus* tissues were observed at the two sites. Plant bioaccumulation was only observed for Cd, Cu, and Zn, being similar for Cd at the two sites and significantly higher for Cu and Zn (9 and 4 times higher, respectively) at site I. In conclusion, *J. maritimus* was shown to have potential for phytoextraction (or phytostabilization) of Cd, Cu, and Zn in estuarine environment. However, an eventual application of *J. maritimus* for this purpose will require a periodic removal of the plants together with their own rhizosediment.

Introduction

Estuarine sediments can be both a sink and a source of contaminants in aquatic systems. Contaminants, including trace metals, can be introduced into the aquatic environment and accumulated in sediment by several pathways, including from runoff after rain events and from human activities along coastlines and upriver (*1*).

Plants can take up chemical species from the environment, and, therefore, they are being increasingly used in phytoremediation processes, to reduce contamination (*2*). Nevertheless, the remediation and restoration of contaminated sites requires a detailed understanding of how contaminants and plants behave in a particular ecosystem, before remedial activities are carried out (*3*).

Interaction processes between plants and surrounding sediment are complex and still little understood. Recent studies indicated that the uptake, retention, and excretion of metals by plants in estuarine environments as well as metal distribution through the different plant tissues differ among plant species (e.g. refs *4*−*7*). As metal availability depends on its speciation at the sediment, it can be expected that not only plant species but also sediment characteristics will influence metal uptake by plants.

Studies in the literature (e.g. ref *8*) have pointed out that plants can alter the chemistry of the sediment in contact with its roots (rhizosediment) (for instance, causing changes in pH and redox potential), creating a different microenvironment, commonly called rhizosphere. Caçador et al. (*9*, *10*) showed that rhizosediment of the plants *Spartina maritima*, *Halimione portulacoides*, and *Arthrocnemum fruticosum* in the Tagus estuary, Portugal, were enriched in Cu, Pb, and Zn. In addition, plant roots are known to exude organic compounds capable of complexing metals (*11*), which can modify metals availability in the rhizosediment.

The aim of this work was to evaluate the capability of the sea rush *Juncus maritimus* to accumulate metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and how the sediment characteristics, including metal content and its speciation, influence this property. An additional aim was to evaluate if and how *J. maritimus* influences the sediment composition around its roots, in terms of metal concentration and availability.

J. maritimus is widely spread in salt marshes in the Atlantic coast of Europe. This plant grows in areas with different sediment characteristics in the lower Douro river estuary, Northwest of Portugal, where this study was carried out.

It was observed previously (*12*) that several locations of the Douro river estuary suffers from anthropogenic contamination by metals, namely Cr, Cu, Pb, and Zn. Therefore, it is important to understand how plants react to metal input in sediments in order to evaluate the suitability of *J. maritimus* for phytoremediation processes in the Douro river estuary or in other areas.

Experimental Section

Materials and Reagents. To prevent contamination, all sampling and labware materials were soaked in 20% (v/v) HNO_3 solution for at least 24 h, rinsed several times with bideionized water (conductivity < 0.1 $\mu S\ cm^{-1}$), and dried in a Class 100 laminar flow hood. The sample manipulation was carried out in a clean room with Class 100 filtered air. All reagents used were pro analysis grade or equivalent. Standard solutions for metal analysis were prepared daily from the stock ones (more concentrated, from BDH (Spectrosol)), in polyethylene tubes, by weighing, with filtered bideionized water or 1−10% HNO_3 solutions.

Sample Collection. *J. maritimus* (Figure 1) was collected at low tide in April 2003, at two sites in the lower mesotidal Douro river estuary (NW Portugal), which has a small area of salt marsh. In one site located upstream the salt-marsh (site I), a sandy sediment was present, while in the other site, located within the salt-marsh (site II), the sediment was much thinner and richer in organic matter content (OM) than that from site I (see Table 1; methodological details as well as more detailed information on this estuary can be found

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TABLE 1. Grain Size Distribution (% of Total Weight) and Organic Matter Content (OM) in Rhizosediments and Sediments from Sites I (Sandy Sediment) and II (Muddy Sediment)[c]

	site I		site II	
	rhizosediment	sediment	rhizosediment	sediment
grain size distribution (%)				
silt and clay ($<$0.063 mm)	2.4	2.1	76	30
very find sand (0.063–0.125 mm)	8.0	6.1	7.7	4.1
fine sand (0.125–0.25 mm)	6.1	4.6	6.4	7.8
medium sand (0.25–0.5 mm)	9.3	5.7	3.6	17
coarse sand (0.5–1 mm)	29	34	3.8	29
very coarse sand (1–2 mm)	36	39	2.1	12
gravel ($>$2 mm)	9.0	9.0	0.12	0.85
OM (%)	1.2	0.9	12	5.3
AVS (μmol g^{-1})[a]	nd[b]	nd[b]	0.13 (7)	2.7 (5)
SEM (μmol g^{-1})[a]	1.2 (4)	0.27 (1)	2.6 (4)	2.3 (4)
SEM-AVS (μmol g^{-1})[a]	1.2 (4)	0.27 (1)	2.4 (3)	−0.5 (2)

[a] Mean and standard deviation (in brackets, affecting the last digit, $n = 3$) expressed in μmol $g_{\text{dry sediment}}^{-1}$). [b] Not detected. [c] Acid volatile sulfide concentrations (AVS), sum of the concentrations of simultaneously extracted Cd, Cu, Ni, Pb, and Zn (SEM) and SEM minus AVS (SEM-AVS) are also included.

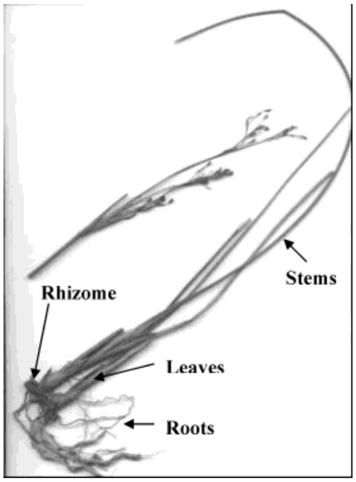

FIGURE 1. *Juncus maritimus* as collected, with the different tissues of the plant marked.

elsewhere (*12*)). Green plants without a senescent appearance and with similar size and age were collected at both sites, being carefully washed in seawater to remove debris and dead or dying root and shoot material.

Simultaneously, sediment and rhizosediment (sediment in contact with the plant roots and rhizomes, corresponding to the area of higher below ground biomass) were collected between 10 and 20 cm depth, which corresponds to the roots depth.

Each sample was put into an individual plastic bag by using plastic shovels, for rhizosediment and sediment, or plastic gloves, for plants, and was immediately (within 30 min) carried to the laboratory.

Sample Treatment. On arrival at the lab, plants were thoroughly rinsed with deionized water, placed in trays, covered with filtering paper to avoid metal contamination, and put to dry into an oven at 50 °C, up to constant weight. Rhizosediments and sediments, drained for porewater removal, were individually packed and dried as the plants but only at 30 °C. Sediments and rhizosediments were, afterward, fractionated and homogenized by sieving through nylon nets of 2 mm in order to remove large stones and dead roots. Plants were separated into stems, leaves, rhizomes, and roots (see Figure 1), which were separately crushed and homogenized. After drying, in both sites, plant roots, rhizomes, leaves, and stems represented, respectively, 3%, 18%, 21%, and 58% of the total plant weight. In a preliminary study, plant roots were washed with 0.1 mM EDTA solution (Merck) to differentiate metal outside and inside the root. As statistically significant differences were not observed between metal content in roots washed and not washed with EDTA, this procedure was not used in the present work.

For metal content determinations, aliquots of each specific tissue of the plant (ca. 0.50 g) and sediments and rhizosediments (ca. 0.25 g) were digested in closed PTFE vessels at high-pressure, with suitable amounts of suprapure concentrated HNO_3 (Merck) (and 30% H_2O_2 (Merck), only for plant tissues) using a microwave system (MLS-1200 Mega, Millestone) (methodological details are elsewhere (*12*)). The HNO_3 digestion of the sediments and rhizosediments only permits determination of total-recoverable metal contents, since it does not provide a complete dissolution of the sample, particularly silicates. Nevertheless, it has the advantage of being a strong acid digestion that dissolves all elements that could become "environmentally available".

Metal availability in the collected sediments and rhizosediments was estimated by sequential extraction (SE), using the method established by the Measuring and Testing Program of the European Community (*13*). Aliquots of dry sediment and rhizosediment (ca. 0.50 g) were treated sequentially with (a) 20.0 mL of 0.11 M CH_3COOH (Merck) solution (exchangeable fraction); (b) 20.0 mL of 0.5 M $NH_2OH{\cdot}HCl$ (Ridel) solution (fraction bound to Fe and Mn oxy-hydroxides); and (c) 25.0 mL of 1.0 M NH_4CH_3COO (Pronolab) solution (pH = 2.0), after digestion with 30% H_2O_2 solution (fraction bound to organic matter and sulfide). Each soluble fraction was obtained after 16 h of extraction on a lateral shaker (JP Selecta) at 22.0 °C, being afterward separated by centrifugation at 3000 rpm (JP Selecta, MIX TASEL). A fourth fraction, named residual fraction, was calculated as the difference between the total-recoverable elemental content (determined for a different portion of dry sediment) and the sum of elemental contents extracted in the three SE fractions. Recent studies (*14*−*16*) have pointed out that, for evaluating metal availability through SE, wet sediments were more suitable than dry ones. Therefore, a comparison of the results obtained for wet and dry sediments was carried out. Since no consistent conclusions could be drawn (results not shown), in the present study dry sediments were chosen.

Metal Determinations. Total metal content in the different plant tissues and total-recoverable metal content in sediments and rhizosediments as well as the metal content in each of the SE fractions were determined by atomic absorption spectrophotometry either with flame atomization (PU 9200X, Philips), used for Cu, Fe, Mn, and Zn, or with electrothermal atomization provided with a Zeeman background correction (4100 ZL, Perkin-Elmer coupled to an AS-

TABLE 2. Concentrations[a] of Cd, Cr, Cu, Mn, Ni, Pb, and Zn (μg per g of Dry Mass) and Fe (mg per g), Observed in the Different Types of Studied Samples[d]

	rhizosediment	sediment	EF[b]	*Juncus maritimus* stems	leaves	rhizomes	roots	T_{plant}[c]
				Site I				
Cd	0.10 (2)	0.044 (6)	2.2 (6)	0.13 (1)	0.15 (2)	0.18 (9)	0.72 (2)	0.16 (5)
Cr	13 (3)	8.9 (8)	1.5 (3)	0.429 (5)	1.9 (5)	0.45 (2)	1.5 (6)	0.8 (2)
Cu	35 (7)	7 (1)	5 (1)	12 (2)	50 (22)	66 (15)	91 (30)	32 (13)
Fe	13.8 (6)	9.0 (8)	1.5 (1)	0.199 (2)	0.9 (2)	0.13 (5)	0.5 (2)	0.35 (9)
Mn	81 (4)	59 (5)	1.4 (1)	13 (1)	45 (10)	11 (3)	33 (15)	20 (5)
Ni	8 (1)	5.7 (3)	1.5 (2)	0.43 (5)	1.5 (3)	0.99 (8)	0.6 (4)	0.8 (1)
Pb	26.6 (9)	17 (7)	1.6 (6)	1.01 (7)	5.0 (3)	1.3 (2)	9 (2)	2.1 (3)
Zn	81 (10)	46 (1)	1.8 (2)	53 (3)	67 (12)	169 (11)	175 (40)	80 (10)
				Site II				
Cd	0.19 (2)	0.094 (4)	2.0 (2)	0.18 (2)	0.22 (2)	0.26 (0.02)	1.5 (4)	0.24 (7)
Cr	23.1 (4)	18.1 (6)	1.28 (5)	0.22 (8)	1.4 (8)	1.3 (0.7)	7 (3)	0.9 (7)
Cu	21.3 (4)	14.7 (5)	1.45 (5)	6.1 (6)	6 (1)	12 (2)	17 (3)	8 (1)
Fe	20.0 (9)	15.7 (1)	1.15 (6)	0.20 (4)	1.0 (5)	0.8 (0.3)	5 (1)	0.6 (3)
Mn	138 (9)	130 (3)	1.06 (7)	48 (7)	68 (40)	14 (5)	73 (13)	47 (20)
Ni	14.5 (5)	12.0 (3)	1.21 (5)	0.31 (5)	1.0 (6)	0.8 (0.3)	3 (2)	0.6 (4)
Pb	54 (3)	32.9 (4)	1.6 (1)	0.7 (2)	4 (2)	6 (3)	22 (6)	3 (2)
Zn	141 (7)	107 (6)	1.3 (1)	27 (1)	31 (5)	84 (19)	154 (31)	42 (10)

[a] Mean and standard deviation (in brackets, affecting the last digit, $n = 3$). Results are total-recoverable metals for sediments and rhizosediments and total metal content for plant tissues. [b] Enrichment factor: ($[M]_{rhizosediment}/[M]_{sediment}$). Standard deviations were calculated attending to the propagation of errors. [c] Total metal content in the plant as a whole, calculated as a weighted average of the mass of the different plant tissues. [d] The plant grew at both site I, with sandy sediment, and site II, with muddy sediment.

70 autosampler), used for Cd, Cr, Ni, and Pb. Aqueous matched standards were used for external calibrations. The total metal content per plant (T_{plant}) was calculated considering a weighed average of the total mass of the different plant tissues and respective metal contents.

To check the accuracy of the analytical procedures used for sediments, Estuarine Sediment BCR 277 reference material, certified for total metal content, and BCR 701, certified for the extractable metal content by SE (*13*), were analyzed. For BCR 277 statistically identical results were obtained for Cd, Ni, Pb, and Zn and recovery percentages of 90% Cu, 80% for Cr and Fe, and 70% for Mn. Some lower values were expected as the total-recoverable metals instead of total contents were measured. For BCR 701 statistically identical results for Cd, Cr, Ni, Pb (only in the first and second fractions), and Zn and significantly lower results for Cu in all fractions (10−20% lower) and Pb in the third fraction (ca. 50% lower) (no certified or indicative values were available for Fe and Mn) were observed. The lower values obtained for Cu and Pb were taken into consideration for the interpretation of the results of this work and were shown not to compromise any relevant conclusion. For plant tissues, as certified reference material was unavailable, a procedure previously optimized for grapevine tissues (*17*) was used.

Blank solutions were prepared for each type of sample, following the respective sample treatment. Three independent replicates of each sample were prepared and analyzed, and, after blank subtraction, mean values and respective standard deviations were calculated. Statistically significant differences among samples were evaluated through *t*-paired tests ($P < 0.05$).

Results and Discussion

Metal Concentration and Speciation in Sediments. Table 2 shows that higher metal contents (twice as much) were registered in the muddy sediment from site II. Sediments containing relatively high percentages of fine-grained fractions, as is the case of those at site II (Table 1), have relatively high specific surface area for the adsorption of metals (*18*). Therefore, metal concentration normally increases with decreasing grain size (*19*).

Table 2 also shows that, for all analyzed elements, rhizosediment presented significantly higher metal concentrations than sediment, which indicates that *J. maritimus* influenced the distribution of metallic contaminants in the sediments of the estuary. The only exception was Mn at site II for which statistically identical results were obtained in rhizosediment and sediment. To obtain the metal enrichment in rhizosediment compared with sediment, enrichment factors (EFs) were calculated and included in Table 2. The EFs varied with both the metal nature and the sediment characteristics. Significantly higher EFs were observed at site I for Cu, Fe, Mn, and Zn, indicating a higher metal mobility in sediments from this site. For the remaining metals (Cd, Cr, Ni, and Pb) statistically identical EFs were obtained in the two sites. An enrichment of total Cu, Pb, and Zn concentrations in sediments around the roots of other marsh plants, *S. maritima*, *H. portulacoides*, and *A. fruticosum*, which have grown at the Tagus estuary, Portugal, has been also reported (*9*, *10*).

The enrichment of nutrients around plant roots has been reported for crop plant in soils (*20*), and it has been attributed to mass flow effects. That is, nutrients are carried in the flow of water that is being absorbed by the plant, and, if the plant does not absorb these nutrients, they accumulate at the surface of the roots. Nevertheless, in the present study, as *J. maritimus* grows in the intertidal zone, mass flow effects will be affected by water movements during tidal cycles, which increases the complexity of the process and the interpretation of the results.

Identification and quantification of elemental fractions associated with individual sediment components can lead to a better understanding of mobility and bioavailability of potentially toxic elements in sediments or soils.

For instance, the ratio of acid volatile sulfide (AVS) to the sum of the concentrations of Cd, Cu, Ni, Pb, and Zn simultaneously extracted (SEM) has been postulated to estimate the bioavailability of metals in anoxic sediments (*21*). Sediments with an excess of reactive sulfide to trace metals (SEM-AVS < 0) will exhibit very low dissolved metal concentrations in interstitial waters, thus reducing metal environment availability and mobility. In the estuarine area under study, oxic sediment and rhizosediment were found

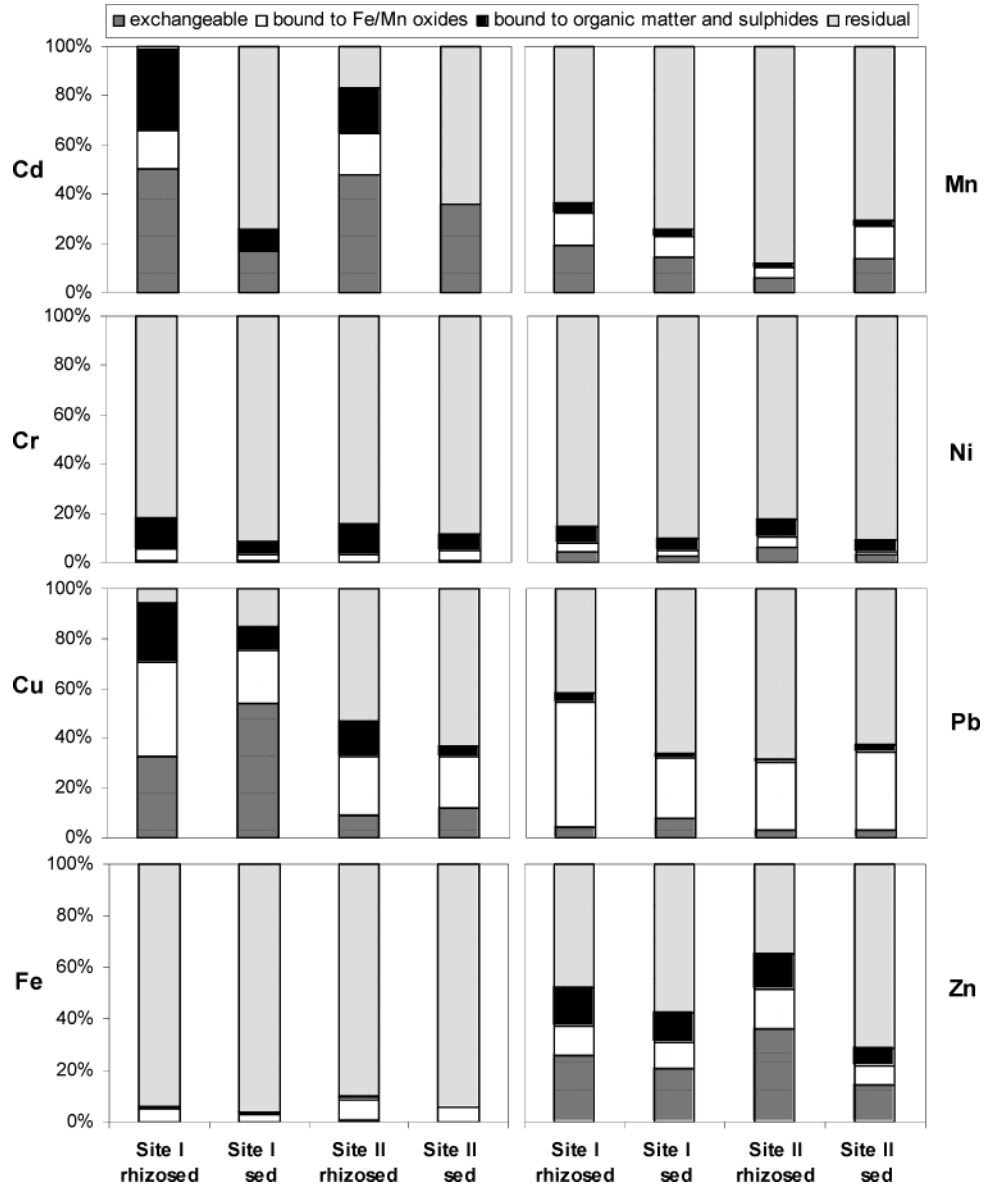

FIGURE 2. Percentages (relative to the total-recoverable concentration) of metal obtained in the different SE fractions (*13*) for rhizosediments and sediments. The residual fraction was calculated as the difference between total-recoverable elemental concentration and the sum of concentrations extracted in the three SE fractions. The site I sediment was sandy and that of site II was muddy.

at site I (AVS was not detected) probably as a result of the relatively high grain size, which promote a more efficient percolation pumped by the ebb tide. At site II sediments the above-mentioned trace metals were mainly as metal sulfides (Table 1). However, $\mathrm{AVS}_{\mathrm{rhizosediment}}$ was much lower than $\mathrm{AVS}_{\mathrm{sediment}}$, indicating that *J. maritimus* oxidized the medium among its roots. This is in agreement with other studies (e.g. ref *8*), which have showed that sediments colonized by plants displayed higher oxidizing conditions (higher redox potential) than sediments without vegetation.

A more detailed metal fractionation in the samples was obtained by application of the SE procedure (*13*): (a) exchangeable, which gives the fraction that is more weakly bound to the sediment; (b) bound to Fe and Mn oxy-hydroxides; (c) bound to organic matter and sulfide; and (d) residual, which corresponds to that more intensively bound to sediment, although still potentially available. It should be stressed that this SE scheme provides only operationally defined fractionation and cannot directly determine the chemical speciation of metals in sediments. The distribution of the metals among the different fractions (Figure 2) varied with both the metal nature and the sediment characteristics. At sediments from both sites, Cr, Fe, and Ni were mostly in the residual fraction, while the strengths of Cd, Cu, Mn, Pb, and Zn binding to the sediment were weaker. Therefore, Cr, Fe, and Ni probably will be much less available than the remaining ones. Figure 2 also shows that most Cd at rhizosediments of both sites was present in easily mobilizable forms (exchangeable and bound to Fe and Mn oxy-hydroxides), whereas in sediments without plants Cd was mostly in the residual fraction. Releasing by plant roots of exudates with capacity to change the forms of Cd in the sediment to more available ones probably contributed to the observed results. It deserves an allusion the release of oxalic and malonic acids by this plant (*22*), which showed to be able of extracting large fractions of Cd (and others) from the sediment and, therefore, of altering Cd availability. In addition, it was observed in microalgae cultures that Cd strongly promoted the liberation of exudates, particularly those identified as glutathione by cathodic stripping voltammetry (*23*). However, Cd may also be present in organic forms from dead plants (prehumic substance) as *J. maritimus* can accumulate Cd (see below). Marked differences between sediments and rhizosediments metal

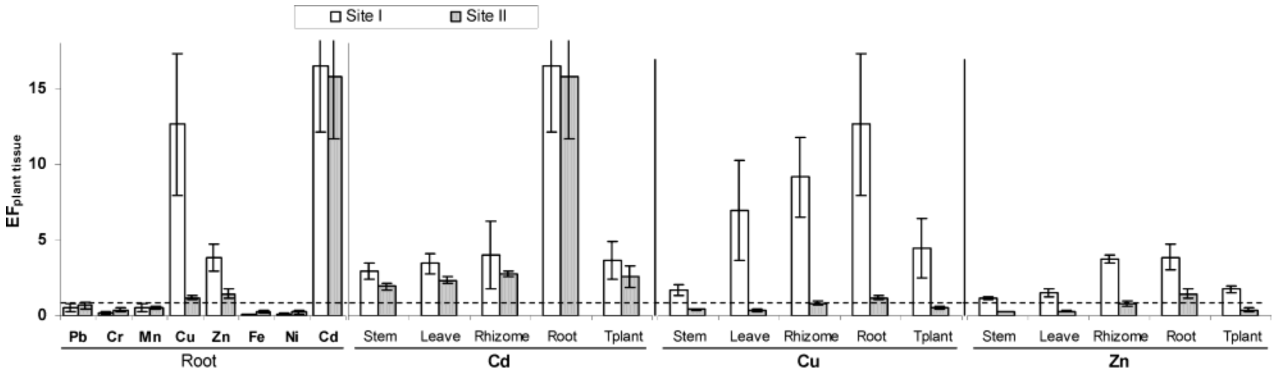

FIGURE 3. Enrichment factor ($EF_{plant\ tissue} = [M]_{plant\ tissue}/[M]_{sediment}$) of metals in plant tissues and sediments. T_{plant} was calculated as a weighted average of the mass of the different plant tissues. Standard deviations were calculated attending to the propagation of errors. The site I sediment was sandy and that of site II was muddy. The dash line indicates EF = 1.

fractionation were only observed for Cd. Nevertheless, the percentages of several other metals (Cr, Fe, Mn (only at site I), Ni, and Zn) in the respective residual fraction were significantly lower in the rhizosediments than in the respective sediments from both sites. Therefore, the presence of the plant seems to change metal fractionation in the sediment. Marked differences between metal fractionation in sediment compositions with and without vegetation have been observed before (*9*) for another estuary and other plants (*S. maritime*, *H. portulacoides*, and *A. fruticosum*). However, those authors have observed that metals in rhizosediments were mainly associated with the residual fraction, which differs from what was observed in the present study (where the residual fraction was higher in sediments than in rhizosediments). Thus, the influence a plant can have on the sediment surrounding its roots may depend on the plant species (and probably also on sediment composition).

Therefore, the present results demonstrated that *J. maritimus* was able to change the characteristics of the sediment among its roots, altering metal fractionation (metals more weakly bound to the rhizosediment than to the sediment, this effect being quite pronounced for Cd) and metal distribution (concentrating metals around its roots). Such changes can influence directly metal mobility and availability (and toxicity) of the trace metals in the surrounding environment, particularly to organisms that live and feed there.

As plants can play an important role in sediment (or soil) chemistry, in studies concerning sediment (or soil) contamination and interactions between plants and contaminants, rhizosediment should also be taken in consideration and thoroughly characterized.

Metal Content and Distribution in Plant Tissues. The concentrations of the measured metals found in the different parts of *J. maritimus* as well as in the whole plant (T_{plant}) are also presented in Table 2. *J. maritimus* roots presented, in most cases, significantly higher metal contents than the other parts of the plant, namely rhizomes, up to 7 (site I) or 6 (site II) times higher; leaves, up to 5 (site I) or 7 (site II) times higher; and stems, between 2 and 9 (site I) or 3 and 30 (site II) times higher. Higher metal contents in roots were expected, as for most salt-marsh plants the dominant uptake pathway of metals from the sediment is via the root system. Consequently, most metals tend to accumulate in the roots rather than in shoots (*7*). Nevertheless, there is a high degree of variability among metals and among various plant species (*7*).

The distribution of each metal among the different parts of *J. maritimus* varied with the element (Table 2). For instance, in the plants collected at both sites, Cd contents were similar in rhizomes and stems (ca. 0.2 μg g^{-1}), whereas Cu, Ni, and Zn contents were significantly higher in rhizomes than in stems.

It deserves to be mentioned that the metal distribution among the different tissues of *J. maritimus* varied also between the two sites. This resulted probably due the differences in the sediment characteristics, since plants of similar size, age, and apparent physiological state were selected from both sites. Table 2 shows that at site I, Cr, Mn, and Pb contents in plant rhizomes and stems were statistically identical (ca. 0.42 μg g^{-1}, 12 μg g^{-1}, and 1.1 μg g^{-1}, respectively), while at site II, Cr and Pb contents were, respectively, 7 and 8 times higher in the rhizomes than in the stems and Mn was 4 times lower. Metal contents in the leaves from site II were significantly lower (Cu and Zn) or statistically identical (remaining elements) to those in respective rhizomes, while at site I the leaves metal contents were significantly higher for several metals (Cr, Fe, Mn, Ni, and Pb), statistically identical for Cd and Cu and significantly lower only for Zn. Comparing stems and leaves, leaves from site I presented significantly higher metal contents for most elements, whereas in leaves from site II statistically identical levels to those in stems were observed. It follows therefrom that the distribution of most metals among the tissues of the plants that grew at site I was roots $\geq$ leaves $\geq$ rhizomes $\geq$ stems, whereas for those collected at site II it was roots $\geq$ rhizomes $\geq$ leaves $\geq$ stems. Therefore, for most metals the potential to migrate within the plant (through its tissues) was greater at site I than at site II, which may be the result of these metals being in different forms at the sediments of the two sites.

Metal Bioaccumulation. The capability of salt-marsh plants to accumulate trace metals is, in principle, advantageous, as it reduces the levels of those metals in the estuarine sediments and, therefore, their potential toxicity to other organisms living there. For the most favorable cases, such plants may have application in phytoremediation.

To evaluate whether *J. maritimus* can accumulate some trace metals, $EF_{plant\ tissue}$ ($EF_{plant\ tissue} = [M]_{plant\ tissue}/[M]_{sediment}$) were calculated. For this purpose, the composition of the sediment from the area around the roots but not properly the rhizosediment was considered. This choice was based on the following considerations. The objective of the study was to evaluate (i) the capability of *J. maritimus* to remove metals from the sediment, cleaning it, and (ii) the influence of the plants on the estuarine environment. As the rhizosediment is already a consequence of the presence of the plants, sediments seemed to be a more suitable parameter for estimating bioaccumulation.

Figure 3 shows that *J. maritimus* was capable of accumulating Cd, Cu, and Zn ($EF_{root} > 1$) but not Cr, Fe, Mn, Ni, or Pb in its roots (the tissue that accumulated most metal).

At site I, the elements bioaccumulated were those that presented higher mobility from the sediment to the rhizosediment (highest EF, see Table 2). At site II, Cd displayed the highest EF, followed by Pb, Cu, and Zn. From these, only Pb was not accumulated by the plant. This result indicates that Pb may not be available for the plant or that the plant may have resistance mechanisms against Pb uptake and/or accumulation. Pb has been, in generally, considered unavailable for plant uptake (*2*). This metal is difficult to remove from the soil/sediment because it forms stable complexes with both organic and inorganic (particularly phosphates, carbonates, and (hydr)oxides, all insoluble at neutral and alkaline pHs) ligands from soils/sediments (*2*).

Among the bioaccumulated metals, both Cu and Zn are micronutrients of the plant, being included in the structure of several enzymes (*2*), whereas Cd is not recognized as an essential element, being toxic to plants (*2*). Nevertheless, toxic metals are thought to enter root cells by means of the same uptake processes that move essential micronutrient metal ions (*24*). Competitive relationships between Cd and Zn have been reported, due to similarity in their chemistry (*2*), and relationships between Cd and other nutrients may exist. For instance, a competitive transport of Cd via voltage-gated cation (like Ca) channels has been pointed out as a way of Cd absorption by roots (*2*). The highest bioaccumulation occurred, in fact, for Cd, the levels in *J. maritimus* roots being about 16 times higher than at the sediments, at both sites. At site II, bioaccumulation of Cu and Zn in the roots, although significant, was very small. In contrast, roots of the plants from site I accumulated significantly more Cu (11 times more) and Zn (3 times more) (see Figure 3), despite higher metal contents (twice as much) being registered in sediments from site II (see Table 2).

Figure 3 also compares Cd, Cu, and Zn accumulation in roots, rhizomes, leaves, and stems as well as in the whole plant (T_{plant}). Cd was significantly more accumulated (about 5 times) in roots than in the other plant tissues. Further, the accumulation in stems, leaves, and rhizomes was statistically identical. No significant difference was observed between the accumulation of Cd in plants collected at site I and II. Plants from site I were also able to accumulate Cu and Zn in all plant tissues, the bioconcentration decreasing as follows: roots ≥ rhizomes ≥ leaves ≥ stems. In contrast, plants from site II bioaccumulated Cu and Zn only in the roots. Therefore, accumulation pattern through the plant tissues ($\mathrm{EF}_{\mathrm{plant\ tissue}}$) varied with both metal nature and sediment characteristics. Plants show a range of different mechanisms for protecting themselves against an uptake of toxic elements and for restricting their transport within the plant. These mechanisms include the subcellular compartmentalization of the metal, namely in vacuoles, and the sequestration of the metal by specially produced organic compounds, like phytochelatins, concentrating metal in the plant roots (*24*). Cd is known as an important inducer of the production of phytochelatins (*24*), which could explain the higher accumulation of Cd in *J. maritimus* roots compared with that in the remaining plant tissues. Despite the fact that the plant accumulated a great deal of Cd, no toxicity signs were perceptible (though physiological tests were not carried out).

Considering the plant as a whole, Figure 3 also shows that it could accumulate Cd from both sites and Cu and Zn only from site I. Therefore, *J. maritimus* would be useful for the phytoextraction, or at least for the phytostabilization, of Cd in Douro river estuarine environment (and probably in other estuaries). As concerns other trace metals, *J. maritimus* has also potential for phytoremediation of Cu and Zn, but the role of the plant seemed to be markedly dependent on the sediment composition. Nevertheless, the usefulness of *J. maritimus* for decontamination of estuarine areas requires periodic removal of plants together with rhizosediment (richer in metal than sediment). Fall maybe the best season to withdraw the plants, to avoid that metal taken up and translocated above-ground (in leaves and stems) could later return to the surface of sediment, owing to the plant death, and become bioavailable to the ecosystem.

Influence of Metal Speciation in the Sediment on Metal Uptake − a Two Way Interaction Between *J. maritimus* and Sediments. The observed differences in Cu and Zn bioaccumulation may result in these metals being in different forms at the sediments of the two sites. The fact that *J. maritimus* accumulated less Cu and Zn at site II would be compatible with the relative magnitude of SEM-AVS at the two sites, since at site II (but not at site I) these metals are predominantly stabilized as sulfides (Table 1). Nevertheless, $\mathrm{AVS}_{\mathrm{rhizosediment}} < \mathrm{AVS}_{\mathrm{sediment}}$ at site II (Table 1), indicating that *J. maritimus* oxidized the medium among its roots. Such oxidation could render metals more available increasing bioavailability. Therefore, the AVS/SEM model might not be useful to explain data in the present case. Particularly, it does not explain the behavior of Cd that was accumulated similarly by the plant at the two sites. In more general terms, the role of plants in reducing AVS content in soil/sediments is difficult to predict, because the presence of sulfate-reducing bacteria (*Desulfovibrio* bacteria) can produce sulfide in microbial degradation of biomass, thus neutralizing the oxidizing action of the plants. For instance, reduction of oxygen content in the soil between crop plant roots has been reported (*20*).

The difference observed in bioaccumulation of Cu and Zn between the two sites could also be related to the differences in the OM, much higher at site II (Table 1), since some organic compounds can form complexes with trace metals, which changes their availability, frequently reducing it (*19*). Nevertheless, OM did not affect the Cd uptake by *J. maritimus* in this study, indicating that either the organic compounds present did not have high affinities to Cd or some complexes formed were directly available to the plant. For instance, it has been reported that the addition of soil amendments, rich in OM, significantly reduced Zn bioavailability but did not affect Cd concentration present in the plant tissues, suggesting that the effect on bioavailability is metal specific (*25*).

On the other hand, metals that were accumulated by *J. maritimus*, Cd, Cu, and Zn, were those more weakly bound to the sediment, while Cr, Fe, and Ni (more intensively bound to the sediment) were not accumulated by the plant (Figure 2). However, Mn and Pb, although more weakly bound to the sediment than Cr, Fe, or Ni, were also not bioaccumulated. Regarding Pb, its concentration in the SE exchangeable fraction (Figure 2) was very low, and as previously noted, it is a complex metal to remove from the soil/sediment. Besides, Pb is also a nonessential toxic element to the plant (*2*), and the plant could have resistance mechanisms to prevent Pb accumulation. The element Mn, like Cu and Zn, is also essential for plants (*2*), but its percentage in the SE residual fraction (Figure 2) was higher than Cu and Zn, particularly at site I, which is compatible with its lower uptake.

At site I the percentages of Cu and Zn in the residual fraction were lower than those at site II, which is also compatible with the difference in Cu and Zn accumulation by the plants which grew at the two sites.

The high bioaccumulation of Cd cannot be explained through the metal fractionation observed in the sediments, but it may be related to the fractionation observed in the rhizosediments. Figure 2 shows that most Cd at rhizosediments of both sites is present in easily available forms (exchangeable and bound to Fe and Mn oxy-hydroxides), whereas in sediments Cd is mostly in the residual fraction. As Cd and Zn have similar chemical properties, compounds released by the plant for facilitating Zn uptake may promote Cd uptake too. As previously mentioned, *J. maritimus* roots can exude both oxalic and malonic acids, which were capable of extracting large fractions of Cd and Zn (and others) from the sediment (*22*).

Also for Cu and Zn, metal fractionation varied between rhizosediment and sediment (Figure 2). In general, the percentages in the residual fraction in the rhizosediments of both sites were lower than that in the respective sediments, which is compatible with higher metal availability. Therefore, the suitability of *J. maritimus* for Cu and Zn phytoremediation seems to be much more dependent on the physical-chemical composition of the sediments than that for Cd, at least in terms of OM and metal speciation. These results stress the role of metal speciation on metal availability and uptake. They argue against application of generic remediation processes and in favor of a site-specific phytoremediation approach. Therefore, sediment (and rhizosediment) characteristics should be considered in studies aimed for evaluating the suitability of a plant for phytoremediation purposes.

Taking into consideration all the discussed results, one must be aware that *J. maritimus* has potential for phytoremediation (at least, of Cd, Cu, and Zn). For this purpose, a periodic removal of the plant together with the sediment surrounding its roots (which is richer in metal than the sediment not colonized by the plants) is required. Otherwise, these plants may act as a secondary source of metal contamination in estuarine ecosystems, principally for organisms living and/or feeding on the rhizosediment.

Finally, it must be noted that the present results were obtained in spring (sampling in April), and both plants and estuary display seasonal variations. Studies are in progress in order to investigate seasonal variability of the *J. maritimus*/estuarine sediment system.

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