Hydrobiologia (2011) 671:95–103
DOI 10.1007/s10750-011-0706-4

PRIMARY RESEARCH PAPER

The role of two *Spartina* species in phytostabilization and bioaccumulation of Co, Cr, and Ni in the Tinto–Odiel estuary (SW Spain)

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Received: 17 December 2010 / Revised: 29 March 2011 / Accepted: 3 April 2011 / Published online: 13 April 2011

Abstract Vascular plants in salt marshes strongly influence processes of heavy metal accumulation. Many studies have focused on this issue; however, there is a lack of information regarding the effects of plants on the distribution of certain poorly studied metals, such as Co, Cr, and Ni. The aim of this study was to comparatively evaluate the capability of *Spartina densiflora* Brongn. and *Spartina maritima* (Curtis) Fernald, to accumulate Co, Cr, and Ni and influence the sediment composition around their roots, investigating whether the observed behavior can change with different levels of sediment pollution. Concentrations of Co, Cr, and Ni were determined in tissues of *S. densiflora* and *S. maritima* and in sediments and rhizosediments from the Odiel and Tinto marshes (SW Spain), one of the estuaries most polluted by heavy metals in the world. Concentrations of Co, Cr, and Ni in the belowground tissues of both *Spartina* species were higher than those in aboveground tissues in all sites sampled. Both species showed potential for phytostabilization of Co, possibly by promoting the formation of high amounts of Fe-oxides in the rhizosphere, which can act to retain the metal within the sediment around the roots. In addition, both *Spartina* species were found to accumulate Co in their roots, thereby avoiding the translocation of this metal to photosynthetic tissues. At the Tinto marsh, there were no differences recorded in metal levels between sediments and rhizosediments of both species, a fact that could be explained by the extremely high background levels of metals at this site, which may impair the ability of the plant to alter the chemistry of the sediment in contact with the roots. The potential for the immobilization of a large amount of Co in the soil, exhibited by *S. densiflora* and *S. maritima*, indicates that both species could be highly useful in the phytostabilization of Co contaminated environments.

Keywords Coastal marshes · Heavy metals · Phytostabilization · Sediments · *Spartina*

Handling editor: Karen McKee

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Introduction

Owing to their toxicity and their ability to accumulate in the biota, pollution caused by heavy metals is a serious problem. Although extensive studies have been made of contamination by heavy metals, most of these have focused on elements such as As, Cd, Cu, Hg, Pb, and Zn, which are considered to be the most harmful to human health (Hu, 2002; Weiss et al., 2006). Other metals have not been so well studied, and there is a lack of information regarding aspects such as the effects of plants on the distribution of these metals. Chromium (Cr) in trivalent form, cobalt (Co), and nickel (Ni) are considered as essential elements when present at trace levels; however, when present at excessive levels, these metals are toxic to living organisms, and are considered as contaminants to soil, surface water, and groundwater (Denny et al., 1995; Kabata-Pendias & Pendias, 2001). Co and Ni are released into the atmosphere from the combustion of coal and oil and from metal-processing operations, whereas Cr is released by various sources, of which the main ones are industrial waste and municipal sewage sludge (Kabata-Pendias & Pendias, 2001).

The joint estuary of the Tinto and Odiel rivers (SW Spain) is one of the areas most polluted by heavy metals in the world, with high concentrations of Co, Cr, and Ni (Ruiz, 2001; Sáinz & Ruiz, 2006; Morillo et al., 2008). This is partially due to the fact that the Tinto and Odiel rivers drain the Iberian Pyrite Belt, one of the most important mining areas of south-western Europe, with extremely high concentrations of heavy metals (Davis et al., 2000). Sewage sludge from coastal towns and industrial dumps are important additional sources of pollution of this area. Within this estuary, the austral cordgrass *Spartina densiflora* Brongn. (Poaceae) has colonized a wide range of habitats: the species has proven to be a vigorous invader and ecosystem engineer that spreads by clonal growth and prolific seed production (Bortolus, 2006), and has come into contact with the indigenous *Spartina maritima* (Curtis) Fernald. Nieva et al. (2001) described high tolerance of *S. densiflora* populations to various environmental factors (flooding, low soil redox potential, high soil conductivity, and soil texture) and a dense occupation of the available below- and aboveground spaces. *S. maritima* has a potentially wide elevational tolerance and an absolute lower limit substantially below that of *S. densiflora*. This native species has an important role as a primary colonist of intertidal mudflats since it is able to trap and stabilize sediment efficiently, thus facilitating successional development (Castillo et al., 2000).

Vascular plants in salt marshes are crucial to the dynamics of the estuarine ecosystem and strongly influence processes of heavy metal accumulation (Alberts et al., 1990; Weis et al., 2002; Windham et al., 2003). Several studies have pointed out that plants can alter the chemistry of the sediment in contact with their roots (rhizosediment) (Madureira et al., 1997; Almeida et al., 2006). In this way, plant activity can influence metal mobility, depending on the existing physicochemical properties in the salt marsh sediment (Jacob & Otte, 2003). Despite the large presence of these two species of the *Spartina* genus in the joint estuary of the Tinto and Odiel rivers, both the distribution of Co, Cr, and Ni in plant tissues, and the influence of these species on the metal composition of the sediment have barely been studied previously. Luque et al. (1999) recorded high levels of Cr and Ni in leaves of both species at the Odiel marshes, but Co levels were not assessed in their study.

This study was undertaken to investigate the capability of the invasive *S. densiflora* and the native *S. maritima* to influence processes of accumulation of Co, Cr, and Ni in two marshes with different levels of pollution, and to characterize the distribution of these metals in the tissues of both species.

Materials and methods

Study site and plant material

Plants of *S. maritima* and *S. densiflora* were collected in February 2007 at low tide from three sites in the joint estuary of the Odiel and Tinto rivers in Huelva, on the Atlantic coast of SW Spain (37°15′N, 6°58′W; Fig. 1). Two of these sites are located in the Odiel marshes: the first is a well-drained lagoon with only *S. densiflora* present, while the second is a poorly drained lagoon (“Laguna de Ludovico” of Figueroa et al., 2003) where only *S. maritima* is present. Finally, the third site, located within the Tinto marshes, is a low and well-drained marsh, where both species coexist. Measurements of sediment conductivity ($n = 5$), pH ($n = 5$) and redox potential ($n = 15$) were obtained at low tide, on bare sediments (0–10 cm depth) at each sampling site in February 2007. Conductivity of the sediment was determined in the laboratory with a conductivity

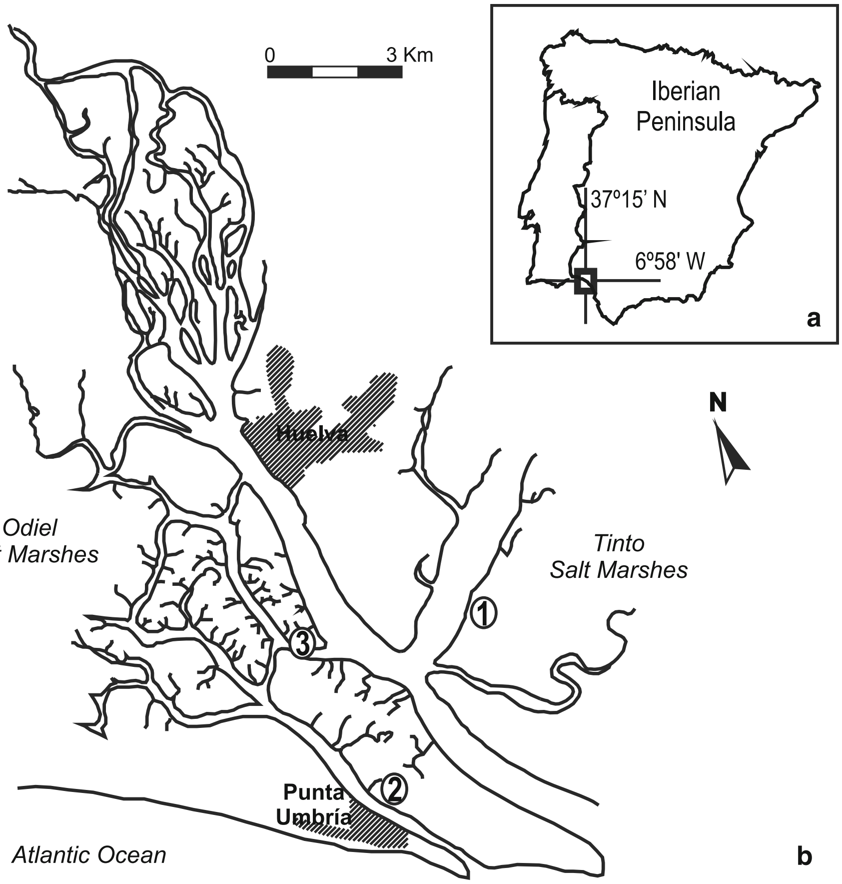

Fig. 1 Map showing **a** the location of the Odiel and Tinto salt marshes (Huelva, SW Spain) and **b** the sampling sites

meter (Crison-522, Spain) after diluting the sediments with distilled water (1:1). The redox potential and pH of the sediment were determined in the field with a portable meter and calibrated electrode system (Crison pH/mV p-506, Spain). Percentages of sand, silt, and clay were determined using the Bouyoucos hydrometer method (Bouyoucos, 1936). The physicochemical properties of the soil are given in Table 1.

From all sites, five clumps of the *Spartina* species present (site 1, five clumps of *S. densiflora*; site 2, five clumps of *S. maritima*; and site 3, five clumps of both species) with approximately 30 tillers were taken and transported to the laboratory in plastic bags. The plants were carefully washed with distilled water and separated into belowground tissues (rhizomes and roots), stems, and old and young leaves (upper and lower leaves on the stem, respectively). All samples were dried at 80°C for 48 h and ground. In addition, samples of the first 20 cm of sediment (corresponding to rooting depth) were taken at each site ($n = 5$) by taking sediment cores (10-cm diameter) in areas where vegetation was absent (>0.5 m distance from the nearest plant). In addition, when clumps of *Spartina* species were collected, the sediments removed with the roots and rhizomes of each plant (rhizosediment) was collected separately for analysis. All samples of sediment and rhizosediment were put into individual plastic bags and transported to the laboratory. Samples were dried at 80°C for 48 h, and ground and homogenized by sieving to <2 mm to remove large stones and dead plant material.

Metal analysis

All samples were dried at 80°C for 48 h and ground (Redondo-Gómez et al., 2007). Samples were acid-digested using concentrated HNO_3 for 15 min at room temperature. These were then placed on a hot plate, until dry. The cooled residue was extracted with HF and H_2O_2. Plant samples (0.5 g; $n = 5$) were digested with 6 ml HNO_3, 0.5 ml HF, and 1 ml

Table 1 Physicochemical properties of soils from the Tinto and Odiel marshes in SW Spain

Parameter	Soil		
	Odiel (site 1)	Odiel (site 2)	Tinto
Clay (%)	8	10	39
Silt (%)	23	17	41
Sand (%)	69	73	20
pH	6.9 ± 0.02	7.2 ± 0.03	6.2 ± 0.06
Conductivity (mS cm^{-1})	12.2 ± 0.1	12.6 ± 0.4	11.9 ± 0.5
Redox potential (mV)	+131 ± 4.2	−125 ± 6.9	+121 ± 7.1

The values given are the mean ± SE of five replicates (except for the clay, silt and sand percentages), and of 15 replicates for redox potential

H_2O_2, while sediment (sediment and rhizosediment) samples were digested with 2 ml HNO_3, 1 ml HF, and 5 ml H_2O_2. The concentrations of elements were then measured by inductively coupled plasma (ICP-AES) spectroscopy (ARL-Fisons 3410, USA). Reference materials from Fisons®, certified for Co, Cr, and Ni, were used to check the accuracy and precision of the analysis of total metals. In all cases, the average uncertainty of metal ion determination was <2%.

Statistical analysis

Statistical analysis was carried out using Statistica v. 6.0 (Statsoft Inc.). Data were analyzed using Student test (*t*-test) and a one-way analysis of variance (*F*-test). Data were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Brown–Forsythe test. Data were transformed using $\sqrt{x}$ function to improve normality and homogeneity of variance. Significant test results were followed by Tukey tests for identification of important contrasts.

Results

Metal content and distribution in plant tissues

Both species exhibited higher levels of Co, Cr, and Ni in below- than in aboveground tissues in all the sites sampled (ANOVA, $P < 0.005$; Table 2). Co and Ni levels were significantly higher in stems than in leaves (ANOVA, $P < 0.05$), while stem and leaf Cr contents were not significantly different in both the species studied (ANOVA, $P > 0.05$). There were no significant differences in metal levels recorded between older and young leaves (*t*-test, $P > 0.05$; Table 2).

The content of Co in tissues ranged from ≤0.1 to 35.8 and from ≤0.1 to 43.4 μg g^{-1} for *S. densiflora* and *S. maritima*, respectively. Co concentrations measured in tissues of both species were significantly

Table 2 Ranges of cobalt, chromium, and nickel (μg g^{-1} of dry mass) concentrations, measured in roots and rhizomes, stems, and old and young leaves of both *Spartina densiflora* and *Spartina maritima* in the two marshes of the joint estuary of the Odiel and Tinto rivers

	S. densiflora		*S. maritima*	
	Odiel	Tinto	Odiel	Tinto
Co (μg g^{-1})				
Root and rhizome	1.8–12.6	12.5–35.8	7.7–16.1	24.3–43.4
Stem	≤0.1–2.7	≤0.1–4.2	1.8–3.1	3.1–5.9
Old leaf	≤0.1	≤0.1	≤0.1	≤0.1
Young leaf	≤0.1	≤0.1	≤0.1	≤0.1
Cr (μg g^{-1})				
Root and rhizome	2.7–18.8	11.1–17.9	9.5–25.2	2.3–23.1
Stem	4.5–7.1	4.8–10.3	5.2–8.7	5.8–10.6
Old leaf	6.2–14.8	8.4–10.5	3.8–6.7	5.3–8.7
Young leaf	2.0–14.2	2.3–13.4	1.4–4	≤0.1–3.2
Ni (μg g^{-1})				
Root and rhizome	7–10.1	6.5–11.1	4.3–12.6	≤0.5–15.6
Stem	2.2–3.4	2.5–4.8	2.7–4.2	2.6–6
Old leaf	≤0.5–5	2.4–5	≤0.5–2.4	≤0.5–3
Young leaf	≤0.5	≤0.5	≤0.5	≤0.5

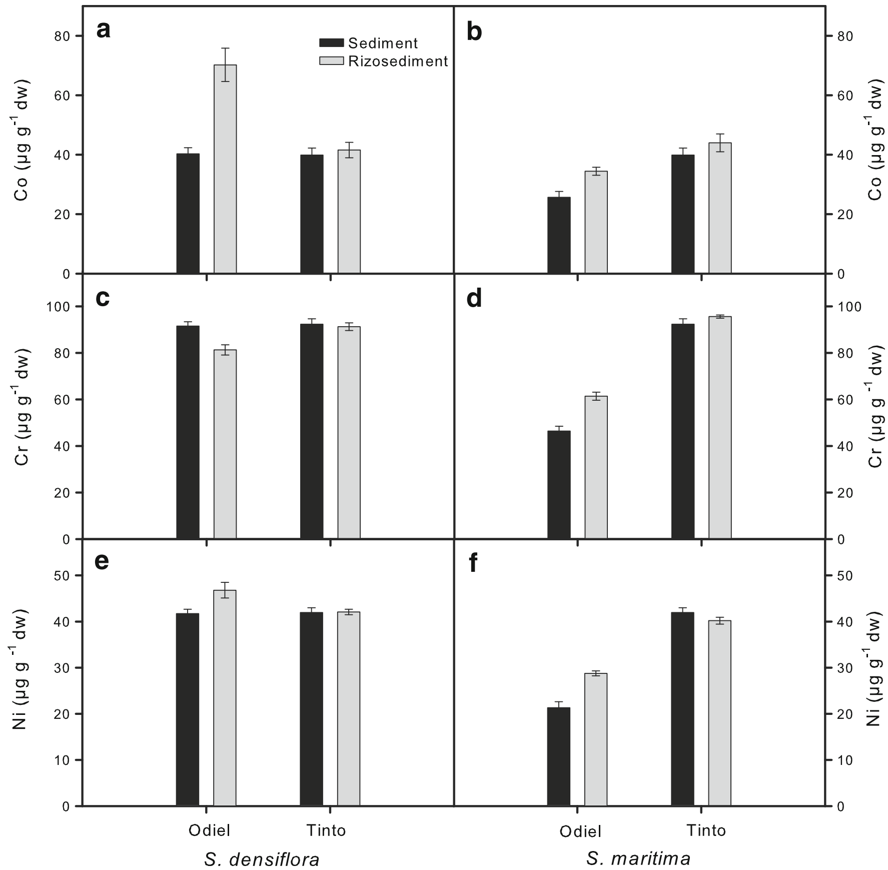

Fig. 2 Concentrations of cobalt (**a**, **b**), chromium (**c**, **d**) and nickel (**e**, **f**) (μg g^{-1} of dry mass), measured in sediments and rhizosediments of both *Spartina densiflora* (**a**, **c**, **e**) and *Spartina maritima* (**b**, **d**, **f**) in the two marshes of the joint estuary of the Odiel and Tinto rivers. The values given are the means ± SE of five replicates

higher for samples from the Tinto marshes than those from the Odiel marshes (*t*-test, $P < 0.05$). Moreover, Co levels in belowground tissues of both species were significantly higher in the Tinto marshes (*t*-test, $P < 0.005$, Table 2).

The content of Cr in tissues ranged from 2 to 18.8 and from ≤0.1 to 25.2 μg g^{-1} for *S. densiflora* and *S. maritima*. Tissue Ni concentrations ranged from ≤0.5 to 11.1 and from ≤0.5 to 15.6 μg g^{-1} for *S. densiflora* and *S. maritima*, respectively. There were no significant differences between the concentrations of Cr and Ni in the plant tissues from both sites (*t*-test, $P > 0.05$, in both cases; Table 2).

Metal distribution in the sediments

At the Odiel marsh site, metal concentrations in the rhizosediments of *S. maritima* were significantly higher than those in the sediments (*t*-test, $P < 0.005$; Fig. 2b, d and f). Similar results were obtained in *S. densiflora* for Co and Ni in this site (*t*-test, $P < 0.005$; Fig. 2a, e). The cited differences between Co concentrations of sediments and rhizosediments were higher for *S. densiflora* (*t*-test, $P < 0.001$). Focusing on the Tinto marshes, no differences were recorded for Co, Cr, and Ni between sediments and rhizosediments in both species (*t*-test, $P > 0.05$; Fig. 2).

Discussion

Metal content and distribution in plant tissues

It is well known that most salt marsh plants accumulate large amounts of metals in their above and belowground organs (Caçador et al., 1996; Weis & Weis, 2004; Cambrollé et al., 2008). Although a large quantity of studies exist on this theme, very few have focused on the Co, Cr, and Ni content in the tissues of salt marsh plants. Concentrations of Co, Cr, and Ni in tissues of *S. densiflora* and *S. maritima* detected at the Odiel and Tinto marshes were similar to the average contents reported by different authors in *S. maritima* in the polluted estuary of Tagus (Portugal) (Caetano et al., 2008; Caçador et al., 2009). The tissue metal distribution detected in our study indicates a restriction on upward transport. Caetano et al. (2008) found higher levels of Cr and Ni in belowground tissues, than in aboveground tissues, of *S. maritima* in two Portuguese estuaries with different anthropogenic pressures. Luque et al. (1999) also detected this pattern in both species in the Odiel salt marshes. Published data on *Spartina* metal distributions imply that they function as excluders (Breteler & Teal, 1981; Alberts et al., 1990). The fact that most metals tend to accumulate within plant roots rather than in the aerial portions suggests that the plant adopts either external or internal exclusion mechanisms to hinder upward translocation of heavy metals (Hansel et al., 2001). In this way, Burke et al. (2000) showed that *Spartina alterniflora* can release large quantities of metals into the marsh environment, through both excretion and leaf deposition.

It could be expected that stems accumulate metals to a lesser extent than leaves, since stems (which consist of vascular tissue) exhibit lower metabolic activity (Sawidis et al., 1995). However, the higher concentrations of Co and Ni detected in stems compared to those in leaves indicate a restriction on the aboveground allocation of these metals within the photosynthetic tissues. Our results suggest that the potential for migration through plant tissues depended on the individual properties of the element concerned. On the other hand, the metal distribution in the aerial portions of the plants indicates that translocation to old leaves cannot be considered as a tolerance mechanism.

Data concerning Co pollution in plants are scarce: Sillanpää & Jansson (1992) analyzed wheat and corn from 30 countries, and concluded that soil texture is the most significant parameter influencing the Co level in plants. In addition to the influence of soil factors on Co levels in plants, the ability of plant species to absorb Co shows considerable variation (Kabata-Pendias & Pendias, 2001). In our study, both *Spartina* species showed only slight translocation from roots to the aerial tissues. Indeed, the content of Co in photosynthetic tissues of both species was below the minimum detection limit (MDL $\leq$0.1). In contrast to our results, various studies realized in the Tagus estuary (Portugal) report poor retention of Co in the belowground tissues of *S. maritima* (Reboreda et al., 2008; Caçador et al., 2009).

Plant Cr content is controlled mainly by the soluble Cr content of soils. Concentrations of Cr in plants vary widely according to tissue type and growth stage, and the pattern of Cr variation appears to be irregular (Mertz et al., 1974; Kabata-Pendias & Pendias, 2001). We found a high translocation of Cr from roots to shoots in both *Spartina* species, which concurs with various studies demonstrating that roots retain small quantities of Cr (Windham et al., 2003; Almeida et al., 2004). Luque et al. (1999) found similar concentrations of Cr in *S. densiflora* and *S. maritima* at Odiel marshes.

Echevarria et al. (1997) demonstrated the high phytoavailability of Ni to plants with an experiment in which the isotopic composition of the element in the growth medium was closely controlled. However, our results suggest poor mobility of this element into the plant tissues. Cataldo et al. (1978) found that both the absorption of Ni by soybean roots and Ni^{2+} translocation from roots to shoots were inhibited by the presence of Cu^{2+}, Zn^{2+}, and Fe^{2+}. In the joint estuary of the Tinto and Odiel rivers, there are extremely high concentrations of Cu, Fe, and Zn (Ruiz, 2001; Morillo et al., 2008). Moreover, Cambrollé et al. (2008) found extremely high levels of these elements in leaves of both species in the Odiel and Tinto marshes. These high levels of Cu, Fe, and Zn could therefore be inhibiting the absorption and translocation of Ni in both *Spartina* species.

Metal distribution in the sediments

At the Odiel marsh site, both species influenced the distribution of Co, Cr, and Ni in the sediment. Various studies have pointed out that plants can favor the accumulation of metals in the sediment in contact with its roots through several mechanisms (Madureira et al., 1997; Almeida et al., 2006). Moreover, root activity is able to modify characteristics such as Eh, pH and microbial activity, which eventually modifies metal retention capacity (Williams et al., 1994; Almeida et al., 2004). Reboreda et al. (2008) found that *S. maritima* was able to induce a higher concentration of metals between its roots in three salt marshes within the Tagus estuary (Portugal). At the Odiel marsh, the differences between Co concentrations of rhizosediments and adjacent sediments were higher for *S. densiflora*. The higher growth of the invasive *S. densiflora* (Figueroa et al., 2003; Bortolus, 2006) must be the consequence of the creation of a large flow of nutrients to the rhizosphere of the species, which could be probably mediated by a mass-flow effect (Comeford, 2005). This large flow of nutrients may be responsible for the higher Co levels in rhizosediments of *S. densiflora* than those of *S. maritima*.

Metal concentrations were similar in sediments and rhizosediments at the Tinto marsh site. Bowen (1979) concluded that when biological and structural properties of root cells are altered, the roots lose the capability to modify the biochemistry of the elements in soil. In this way, the extremely high levels of metals in Tinto marshes (Ruiz, 2001; Cambrollé et al., 2008) could be altering the properties of root cells of the species and reducing the ability of the plant to alter the chemistry of the rhizosediment. In addition, since metals tend to adhere very strongly to the silt and clay particles, the high percentage of these fractions detected in the Tinto marshes (Table 1) may reduce the mobility of metals, thereby reducing their accumulation in the rhizosediment.

Relation between rhizosediment concentration and metal content in tissues

The enrichment of Co found in the rhizosediments for both species at the Odiel marsh was reflected in lower concentrations in the plant tissues, as significant differences were found in concentrations of this metal between plants growing in different marshes. In fact, both species showed the ability to influence the concentration of Co in the rhizosediment at Odiel marshes, preventing the translocation of high levels of this metal into the roots. Oxidation of the root zone and movement into the rhizosphere could be considered responsible for this finding. Both *Spartina* species seem to have the ability to oxygenate their rhizosphere, because of a well-developed aerenchyma in roots and rhizomes, thus generating an oxidizing soil environment around their belowground tissues (Castillo et al., 2000). Since Fe oxides are known to have a great affinity for selective adsorption of Co (Kabata-Pendias & Pendias, 2001), the high concentrations of Fe in the joint estuary of the Odiel and Tinto rivers (Egal et al., 2008), coupled with the oxidation of the root zone by both *Spartina* species, could be promoting the formation of high amounts of Fe-oxides in the rhizosphere, leading to the immobilization of Co through sorption processes. Accordingly, our results showed that Co levels in roots of both species were higher in the Tinto marshes than those in the Odiel marshes. In spite of this fact, Co concentrations in leaves of both species in Tinto marshes were below metal detection limits (≤ 0.1). Our results suggest that both species accumulate Co in their roots avoiding the translocation of this metal to photosynthetic tissues. Restriction of upward movement is one strategy of metal tolerance in plants, since the movement of metal ions into photosynthetic tissues can induce severe stress (Carbonell et al., 1998; Sneller et al., 1999).

It is well known that naturally occurring Cr compounds have a principal valence of +3 (chromic) and +6 (chromate), and that highly oxidized forms of Cr are much less stable than Cr(III) (Kabata-Pendias & Pendias, 2001). The dominant effect of organic matter is to stimulate the reduction of readily soluble Cr(VI) to the more stable Cr(III) (Bolt & Bruggenwert, 1976). Comparing the high concentrations of Cr detected in sediments with those recorded in tissues of both species, our results suggest the slight occurrence of transport of this metal from soil to plant tissues at all sites sampled (see Fig. 2 and Table 2), thus indicating that Cr(III) predominates in the joint estuary of the Tinto and Odiel rivers. This estuary is a highly anthropized area, with large inputs of organic matter from nearby urban areas and the paper pulp industry (González-Pérez et al., 2007). In this context, the high input of organic matter and the high accumulation of dead belowground biomass (high amounts of organic matter) in the case of *S. densiflora* (see Figueroa & Castellanos, 1988), may favor reduction of Cr(VI) to Cr(III), leading to the slight absorption and translocation of this metal from soil to plant tissues. The enrichment of Cr found in rhizosediments of *S. maritima* at the Odiel marsh was not reflected in lower concentrations in plant tissues. Taking into account the fact that the mobility of trace metals (especially of Cd, Cu, Cr, and Zn) increases in poorly aerated soils (Kabata-Pendias and Pendias, 2001), these results could be explained by the lower redox potential recorded in the Odiel marsh sediments (Table 1), which may increase the solubility of Cr at this site. On the other side, our results showed that tissues of both *Spartina* species showed similar concentrations of Ni in both marshes, and the slightly lower levels of Ni recorded in tissues of *S. maritima* from the Odiel marsh compared to those from the Tinto marsh could be attributed to the lower concentrations of the metal detected in sediments and rhizosediments from this site.

In conclusion, both *S. densiflora* and *S. maritima* show potential for the immobilization of a large amount of Co in the soil, thereby reducing translocation from rhizosediment to root. In this way, these two *Spartina* species could be highly useful in the phytostabilization of Co contaminated environments. The capability of both species to immobilize metals is limited by environmental factors that should be taken into account for phytoremediation purposes.

Acknowledgments We are grateful to Mr. K. MacMillan for the revision of the English version of the manuscript. We also thank the Spanish Ministry of Education (FPU programme, AP2007-04420), the Junta de Andalucía for financial support (RMN-224), and the Directorate of the Odiel Marshes Natural Park for their collaboration.

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