BIJP #821451, VOL 00, ISS 00

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TABLE OF CONTENTS LISTING

The table of contents for the journal will list your paper exactly as it appears below:

Potential of *Spartina Maritima* in Restored Salt Marshes for Phytoremediation of Metals in a Highly Polluted Estuary
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International Journal of Phytoremediation, 00:1–12, 2013
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ISSN: 1522-6514 print / 1549-7879 online
DOI: 10.1080/15226514.2013.821451

POTENTIAL OF *SPARTINA MARITIMA* IN RESTORED SALT MARSHES FOR PHYTOREMEDIATION OF METALS IN A HIGHLY POLLUTED ESTUARY

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Sedimentary abiotic environment, and concentration and stock of nine metals were analyzed in vegetation and sediments to evaluate the phytoremediation capacity of restored* Spartina maritima *prairies in the highly polluted Odiel Marshes (SW Iberian Peninsula). Samples were collected in two 10 –m long rows parallel to the tidal line at two sediments depths (0–2 cm and 2–20 cm). Metal concentrations were measured by inductively coupled plasma spectroscopy. Iron, aluminum, copper, and zinc were the most concentrated metals. Every metal, except nickel, showed higher concentration in the root zone than at the sediment surface, with values as high as ca. 70 g Fe kg^{-1}. The highest metal concentrations in* S. maritima *tissues were recorded in its roots (maximum for iron in* Spartina *roots: 4160.2 ± 945.3 mg kg $^{-1}$). Concentrations of aluminum and iron in leaves and roots were higher than in superficial sediments. Rhizosediments showed higher concentrations of every metal than plant tissues, except for nickel. Sediment metal stock in the first 20 cm deep was ca. 170.89 t ha^{-1}. Restored* S. maritima *prairies, with relative cover of 62 ± 6%, accumulated ca. 22 kg metals ha^{-1}. Our results show* S. maritima *to be an useful biotool for phytoremediation projects in European salt marshes.

KEY WORDS biomass, halophytes, Odiel Marshes, phytoremediation, pollution, roots

INTRODUCTION

Coastal marshes are very vulnerable to metal contamination since they are located at river mouths (Beeftink 1977; Williams *et al*. 1994a), especially in the vicinity of mining and industrial areas (Curado *et al*. 2010). Potentially halophytes are ideal candidates for phytoextraction or phytostabilization of metal polluted soils and moreover of metal polluted soils affected by salinity (Manousaki and Kalogerakis 2011; De Lange *et al*. 2013). Constructed wetlands are commonly used to treat contaminated freshwater effluent. However, experience with saline systems is more limited (De Lange *et al*. 2013). In this context, some marsh plants such as *Spartina alterniflora* Loisel., *Phragmites australis* (Cav.) Trin. ex Steud., *Sarcocornia perennis* (Miller) A.J. Scott and *Juncus maritimus* Lam. can be used in restoration projects for phytoremediation in polluted estuaries since they concentrate

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contaminants in colonized sediments and in their tissues (Weis and Weis 2004; Czako *et al.* 2006; Gomes and Costa 2009; Duarte *et al.* 2010; Marques *et al.* 2011).

Once a restoration project has been implemented, good monitoring is essential to improving the restoration methodology for future applications, and to solving of unexpected problems during its evolution (England *et al.* 2008). Nevertheless, although a great deal of laboratory, microcosm and greenhouse studies of metal phytoremediation by wetland macrophytes have been carried out (e.g., Tang 1993; Weiss *et al.* 2006; Yadav *et al.* 2012; Anning *et al.* 2013), only a handful of studies have monitored the results of phytoremediation efforts in constructed wetlands in field settings and long-term field-based studies are rare (Williams 2002; Bert *et al.* 2009). However, Imfeld *et al.* (2009) discussed some of the key characteristics of constructed wetlands for removal of organic chemicals, and Vymazal *et al.* (2010) showed that concentrations of metals in the sediments of constructed wetlands used to treat municipal wastewater were low and comparable with those found in unpolluted natural wetlands. Teuchies *et al.* (2012) described how removal of metals and burial of contaminated sediments in restored salt marshes emphasize the potential of restoration projects to decrease contamination risks.

In the Odiel Marshes, globally one of the most metal-polluted salt marshes (Pérez *et al.* 1991; Ruiz 2001), an innovative restoration project was carried out from November 2006 to January 2007 using plantations of the Small Cordgrass, *Spartina maritima* (Curtis) Fernald. This project included phytostabilization of metal-polluted sediments as a specific restoration goal (Castillo and Figueroa 2009), since natural *S. maritima* prairies contribute effectively to the stabilization of metals in the sediments (Reboreda and Caçador 2007; Cambrollé *et al.* 2008; Reboreda *et al.* 2008; Caçador *et al.* 2009; Castillo and Figueroa 2009; Duarte *et al.* 2010).

The aim of this study was to analyze the sedimentary abiotic environment and to quantify the concentration and stock of nine metals (Al, As, Cd, Cr, Cu, Fe, Ni, Pb, and Zn) in the colonized sediment and tissue of *Spartina maritima* 28 months after planting, as a component of the integral monitoring and evaluation of the restoration project carried out in the Odiel Marshes (Castillo and Figueroa 2009). We hypothesized that expanding plantations of *S. maritima* growing on very polluted sediments would accumulate high metal loads in their tissues, as well as in colonized sediments. This study increases our knowledge about the phytoremediation capacity of salt marsh restoration projects based on halophytes plantations, specifically those carried out with cordgrasses.

MATERIAL AND METHODS

Study Site

Our work was carried out in a restored salt marsh area that borders the main channel of the joint estuary of the Odiel and Tinto rivers (south-west Iberian Peninsula; 37°08′–37°20′N, 6°45′–7°02′W). This area was restored from November 2006 to January 2007 using mainly *S. maritima* plantations (8.37 ha). *S. maritima* clumps coming from natural populations were planted manually at a density of 1 clump m^{-2} (ca. 20 shoots $clump^{-1}$) after invasive *Spartina densiflora* Brongn. was eliminated manually from 2.00 ha around the site (Castillo and Figueroa 2009). During the study period plant community composition in restored marshes was mainly continuous prairies of *S. maritima* with a relative cover of ca. 62% and a tiller height of ca. 34 cm. Isolated clumps of *Zostera noltii* Hornem. grew at lower elevations and *S. perennis*, with ca. 15% of relative cover,

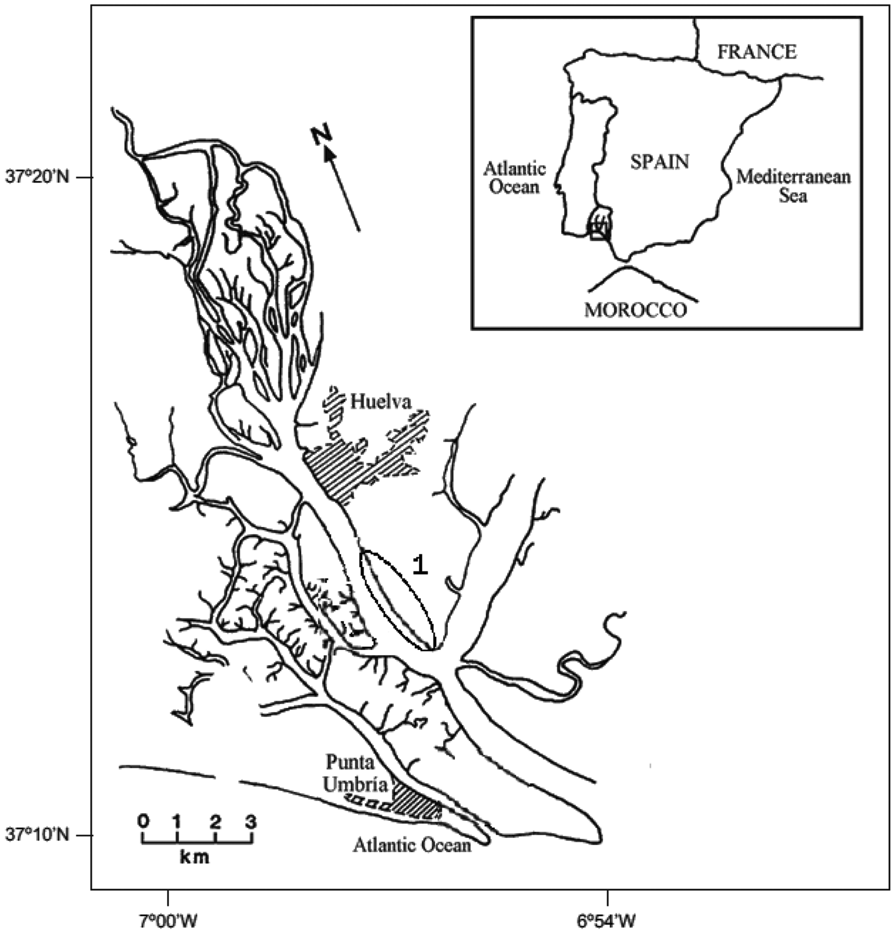

Figure 1 Location of Odiel Marshes on the Atlantic coast of Southwest Iberian Peninsula (37°08′–37°20′N, 6°45′–7°02′W), and the restored area where our work was carried out (1).

was the most abundant halophyte besides *S. maritima* at higher elevations. The hybrid *S. perennis* x *fruticosa*, *Atriplex portulacoides* L., *Suaeda maritima* (L.) Dumort., *Arthrocnemum macrostachyum* (Moric.) Moris., *Salicornia ramosissima* J. Woods and *Suaeda vera* Forsskal ex J.F. Gmelin. were also present at higher elevations (Curado *et al*. 2012, 2013). The area is very polluted with metals coming from two sources: industrial activities developed in the estuary and long-term mining activities carried out landward at the Iberian Pyrite Belt (van Geen *et al*. 1997; Leblanc *et al*. 2000) (Figure 1).

Abiotic Environment

Every abiotic characteristic described below was recorded from sampling points along two 10 –m long rows in sediments colonized by *Spartina maritima* that we established parallel to the tidal line (10 equidistant sampling points per row) between +2.16 and +2.67 m SHZ in May–July 2009 (n = 20) (Fig. 2).

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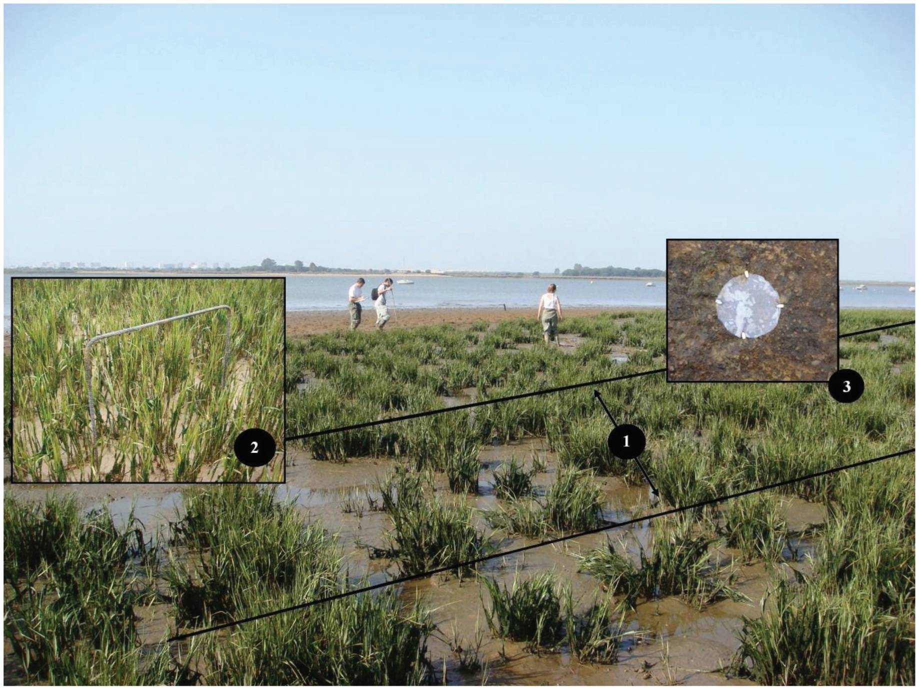

Figure 2 Restored marshes planted with *S. maritima* in Odiel Marshes showing sampling points along rows parallel to the tidal line (1), accretion / erosion marker (2), and nylon horizon to collect deposited sediments (3) (Color figure available online).

Elevation relative to Spanish Hydrographic Zero (SHZ) was surveyed to a resolution of 2 cm with a Leica NA 820 theodolite (Singapore); reference points were determined in relation to measurements of tidal extremes (Ranwell *et al*. 1964). Every sediment characteristic was recorded between 0 and 10 cm deep, except for the redox potential, which was sampled at the surface (0–2 cm) and at depth (2–20 cm). pH (pH / redox Crison with the electrode *M-506*) and electrical conductivity (conductivity meter, Crison-522) were recorded in the laboratory after adding distilled water to the sediment with 1:1, v/v and 1:2, v/v, respectively. Redox potential of the sediment was determined in the field with a portable meter and electrode system (Crison pH/mV p-506). Sediment bulk dry density was recorded by weighing (DW) the volume of sediments in a cylindrical core of 5 cm diameter × 5 cm height. Sedimentation rate was determined by markers consisting in an iron structure with two vertical posts (ca. 1.5 m tall and 1 cm diameter) inserted in the sediment to a depth of approximately 1 m in *S. maritima* areas and supporting a horizontal crossbar (ca. 0.5 m long). The distance from the middle point of the crossbar to the sediment surface was measured quarterly from March 2009 to March 2010 ($n = 9$) (Curado *et al*. 2012) (Fig. 2).

Metal Analysis

Samples were collected for determination of the concentrations of Al, As, Cd, Cr, Cu, Fe, Ni, Pb, and Zn from sampling points along the same two 10 –m long rows established parallel to the tidal line where the abiotic parameters were measured. These samples included: (1) superficial sediments (0–2 cm deep) collected in *S. maritima* areas on nylon horizons between April and October 2008 (Salgueiro and Caçador 2007); (2) sediments between 2–20 cm deep colonized by *S. maritima* roots (n = 10) in July 2009; and (3) leaves, non-photosynthetic stems, rhizomes and roots of *S. maritima* in July 2009 (n = 10) (Fig. 2).

Samples were dried to constant weight at 80 °C for 48 h, pulverized using a grinder (Cyclotec, Foss Tecator AB, Höganäs, Sweden), and then sieved through an 80 μm screen. Samples were digested in 6 ml HNO_3 and 25 ml ultrapure water using microwaves (Anton Paar, multiwave 3000, Austria). The product was measured by inductively coupled plasma (ICP) spectroscopy (Horiba Jobin Yvon, Última 2, France).

The lowest detection hold showed when the concentration was below the detection limit. For the sediment samples that showed metal concentrations under the detection limit, mean concentration was calculated considering these samples with a value of 0.1 mg kg^{-1} DW for Zn, Cu, Cd, Cr, Ni, Al, Fe; 0.3 mg kg^{-1} DW for As and 0.6 mg kg^{-1} DW for Pb in soil. For *S. maritima*, these values were 0.05 mg kg^{-1} DW for Zn, Cu, Cd, Cr, Ni, Al, Fe; 0.15 mg kg^{-1} DW for As and 0.3 mg kg^{-1} DW for Pb.

Metal Stocks

Metal stocks in *S. maritima* sediments were calculated as the product of each metal concentration (in mg kg^{-1} dry weight (DW)) and the mass of sediment at the surface (0–2 cm deep) and at depth (2–20 cm deep). The mass of sediment was calculated as the product of the volume (restored area * depth) and its bulk dry density.

Metal stocks in *S. maritima* tissues were calculated both for areas totally colonized by the cordgrass (monospecific cover of 100%) and for the entire restored area. Firstly, each metal concentration (in mg g^{-1} DW) was multiplied by the biomass of every plant organ (in g DW m^{-2}) to calculate metal stocks for areas totally colonized by *S. maritima*. Then, the metal stocks for the entire restored area was calculated by multiplying the metal stocks for the totally colonized areas by the total restored area (8.37 ha) and by *S. maritima* relative cover (relative cover was 0.62 in *Spartina* prairies; Curado *et al*. 2012). *S. maritima* biomass was recorded in October 2009 in totally colonized 10-cm quadrant plots (n = 10). In the laboratory, biomass was washed carefully, plant structures were separated and dried to constant weight at 80 °C for 48 h. In addition, net annual standing above- and below-ground productivity (NAPP and NBPP) for *S. maritima* prairies were calculated as the total AGB or BGB, respectively, divided by years since transplantation. Sampling plots for biomass were located in areas with bare sediments adjacent to clumps just after transplanting, to ensure that all the standing biomass was effectively produced in situ after restoration plantings (Castillo *et al*. 2008a). No evidence of herbivory by *cattle, rabbits or crabs* was observed during the study.

Statistical Analysis

Analyses were carried out using SPSS release 14.0 (SPSS Inc., Chicago, IL). Deviations were calculated as the standard error of the mean (SEM). Data were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Levene test ($P > 0.05$). When no homogeneity of variance between groups was found, data were transformed using the following functions: ln(x), 1/x and $\sqrt{x}$. Student's *t*-test for independent samples was applied to compare two means. If homogeneity of variance was not achieved by data transformation, then means were compared using Mann–Whitney *U*-test. Variations in metal loads between organs were compared by one-way Anova (analysis of variance). Tukey's test between means was calculated only if the *F*-test was significant ($P < 0.05$). If homogeneity of variance was not achieved by data transformation, then the means were compared by a Kruskal–Wallis non parametric Anova.

RESULTS AND DISCUSSION

Abiotic Environment

In the restored *S. maritima* marshes, sediment surface was at a mean elevation of +2.28 ± 0.06 m SHZ, sediment pH was close to neutrality (7.1 ± 0.1) and sediment electrical conductivity was 15.2 ± 1.5 mS cm^{-1}. Redox potential was similar at sediment surface (–5 ± 18 mV) and at depth (–44 ± 20 mV) (*t*-test, $P > 0.05$). Sediment bulk dry density was 0.80 ± 0.06 g cm^{-3} and sedimentation rate was +2.6 ± 0.3 cm yr^{-1}.

Metal Concentrations

Iron, aluminum, copper, and zinc were the most concentrated metals in both superficial sediments and rhizosediments (sediment surrounding *Spartina* roots). This same trend was described in North America (Hudson River estuary) where the most abundant metals in superficial sediments also were iron, aluminum, copper, and zinc as well as lead. Fe concentration was lower in the Odiel Marshes while Cu and Zn concentration were higher in our study than those recorded in the contaminated Hudson River estuary (Feng *et al.* 1998). Every metal, except nickel, showed higher concentration in the root zone than at the surface (Al, Cu and Fe: Mann–Whitney *U*-test, $P < 0.001$; Cd, Cr, Pb, Zn, and As: *t*-test, $P < 0.01$, df = 8), with values as high as ca. 70 g Fe kg DW^{-1} (Table 1). The high concentration of metals in rhizosediments could be related with transport and precipitation of metals in the rhizosphere (Caçador *et al.* 1996a). Previous studies have recorded higher metal concentrations in sediments colonized by roots of *S. maritima* than in sediments without roots (Caçador *et al.* 1996a, 1996b; Reboreda and Caçador 2007; Cambrollé *et al.* 2008; Reboreda *et al.* 2008). Reported metal concentrations were in accordance with those

Table 1 Metal concentrations (mg kg DW^{-1}) in superficial sediments (0–2 cm deep) and rhizosediments (2–20 cm deep) 28 months after transplanting *Spartina maritima* in the Odiel Marshes (south-west Iberian Peninsula) (n = 10). Different coefficients indicate significant differences between depths (*t*-test or *U*-test, $P < 0.01$)

Metal	*Surface (0–2 cm)*	*Depth (2–20 cm)*	TOTAL
Al	568.4 ± 102.5^{a}	43375.6 ± 4065.2^{b}	43944.0 ± 4087.1
As	138.3 ± 34.4^{a}	340.4 ± 51.8^{b}	478.7 ± 78.6
Cd	0.4 ± 0.1^{a}	19.5 ± 1.8^{b}	19.9 ± 1.8
Cr	18.0 ± 4.9^{a}	68.0 ± 2.9^{b}	86.0 ± 4.7
Cu	405.2 ± 114.3^{a}	3085.5 ± 293.0^{b}	3490.7 ± 294.4
Fe	808.0 ± 179.7^{a}	69138.7 ± 6509.0^{b}	69946.7 ± 6572.0
Ni	10.5 ± 4.2^{a}	21.4 ± 2.18^{a}	31.9 ± 4.6
Pb	120.3 ± 30.9^{a}	512.6 ± 61.3^{b}	632.9 ± 81.2
Zn	467.8 ± 105.4^{a}	1831.4 ± 179.8^{b}	2299.2 ± 232.7
TOTAL	2536.7 ± 565.8^{a}	118393.0 ± 10816.7^{b}	120929.7 ± 10996.0

Table 2 Metal concentrations (mg kg DW^{-1}) in leaves, stems, rhizomes and roots 28 months after transplanting *Spartina maritima* in the Odiel Marshes (n = 10). Different coefficients indicate significant differences between organs (analysis of variance, $P < 0.05$). (* measurements under the detection threshold)

Metal	Leaves	Stems	Rhizomes	Roots
Al	1356.4 ± 130.7^{a}	236.5 ± 33.7^{b}	297.8 ± 38.4^{b}	1334.9 ± 109.6^{a}
As	5.7 ± 0.4^{a}	1.2 ± 0.1^{b}	3.2 ± 0.3^{c}	29.0 ± 7.1^{d}
Cd	0.4 ± 0.0^{a}	0.7 ± 0.2^{ab}	1.0 ± 0.2^{b}	2.4 ± 0.5^{c}
Cr	3.7 ± 0.3^{a}	1.1 ± 0.1^{b}	1.3 ± 0.1^{b}	3.9 ± 0.6^{a}
Cu	83.0 ± 5.0^{a}	35.9 ± 4.3^{b}	74.1 ± 9.9^{a}	348.3 ± 58.2^{c}
Fe	1513.2 ± 136.7^{a}	270.0 ± 22.3^{b}	635.9 ± 83.1^{c}	4160.2 ± 945.3^{d}
Ni	99.6 ± 9.1^{a}	118.6 ± 11.5^{ab}	199.4 ± 0.6^{b}	245.4 ± 48.9^{c}
Pb	4.5 ± 0.7^{a}	*	0.4 ± 0.1^{c}	6.0 ± 2.4^{b}
Zn	102.1 ± 9.4^{a}	32.5 ± 10.9^{b}	48.2 ± 13.9^{b}	193.1 ± 54.1^{c}
TOTAL	3168.5 ± 274.2^{a}	696.5 ± 57.7^{b}	1261.0 ± 103.5^{ab}	6323.1 ± 967.7^{c}

recorded previously in sediments from the Odiel Marshes (e.g. Luque *et al*. 1998; Santos Bermejo *et al*. 2002[]; Cambrollé *et al*. 2008, 2011; Sánchez-Moyano *et al*. 2010). **Q1**

The highest metal concentrations for *S. maritima* tissues were recorded in the roots (Table 2), denoting a high capacity for metal immobilization in the subterranean biomass to protect photosynthetic tissues (Fitzgerald *et al*. 2003; Duarte *et al*. 2010). Species of *Spartina*, such as *Spartina patens* (Aiton) Muhl and *S. densiflora* also accumulated metals in their roots without significant translocation into their shoots (Suntornvongsagul *et al*. 2007; Cambrollé *et al*. 2008). *S. densiflora* has also been described as accumulating high concentrations of organochlorine compounds in its BGB in South America salt marshes (Menone *et al*. 2000).

Aluminum and chrome in *S. maritima* showed similar concentrations in roots and leaves (Al: *t*-test, $P > 0.05$; Cr: *U*-test, $P > 0.05$) (Table 2). Reported metal concentrations were in accordance with those recorded previously for *S. maritima* in the Odiel Marshes (Cambrollé *et al*. 2008) and Tagus estuary, except for lead and copper (Caçador *et al*. 1996a; Reboreda *et al*. 2008; Duarte *et al*. 2010). In Portuguese marshes in the same season, lead concentration was higher and copper concentration was lower in *S. maritima* roots than in our study.

S. maritima roots had a metal load three times higher than superficial sediments (however Zn, Pb, and As were less concentrated in *Spartina* roots than in the sediments). Nevertheless, rhizosediments showed higher concentrations of every metal than in plant tissues, except for nickel, which was more concentrated in plant tissues (*t*-test or *U*-test, $P < 0.001$) (Tables 1 and 2). Previous works with halophytes have described that the metal concentration in sediments was not reflected in their tissues; only zinc concentrations in plant material reflected levels within the sediment (Williams *et al*. 1994b). In contrast, we recorded lower zinc concentration in plant tissues than in sediments (Tables 1 and 2). Nickel was more accumulated in all plant tissues than in the sediments (Tables 1 and 2), but not hyperaccumulated, according to Brooks *et al*. (1977). Hyperaccumulation thresholds in the aerial plant tissues have been established as 1000 mg kg^{-1} for copper, chrome, nickel, lead, arsenic and aluminum, 10000 mg kg^{-1} for zinc, and 100 mg kg^{-1} for cadmium (Brooks *et al*. 1977; Baker and Brooks 1989; Jansen *et al*. 2002; Robinson *et al*. 2006). For iron, it was not possible to find any general threshold of hyperaccumulation (Branquinho *et al*. 2007). Following this, *S. maritima* only hyperaccumulated aluminum in aerial tissues and iron was accumulated above 1000 mg kg^{-1} DW, reaching a value of ca. 0.42% DW in *Spartina* roots. Thus, aluminum and iron in *S. maritima* were accumulated at higher concentrations than in superficial sediments both in leaves (*U*-test, $P < 0.001$) and roots (*U*-test, $P < 0.001$) (Tables 1 and 2). In anoxic (low redox potential) and neutral sediments with salinities as high as those of the studied restored marshes, zinc, chrome and cadmium would be the most bioavailable metals (Guo *et al.* 1997; López-González *et al.* 2005). In fact, cadmium was more concentrated in rhizomes and roots of *S. maritima* than in superficial sediments (rhizomes: *t*-test = –2.733, $P < 0.05$, df = 18; roots: *U*-test = 14.000, $P < 0.01$). However, cadmium and chrome did not reach high concentrations in plant tissues, probably because their total sediment concentrations were low (Tables 1 and 2).

Metals Stocks

Sediment metal stock in the first 20 cm deep was ca. 1430.3 t (170.89 t ha^{-1}). Iron was the most abundant metal (ca. 834 t, 99.69 t Fe ha^{-1}), followed by aluminum (ca. 524 t, 62.55 t Al ha^{-1}), copper (ca. 38 t, 4.51 t Cu ha^{-1}), and zinc (ca. 23 t, 2.71 t Zn ha^{-1}) (Table 3). 2176 m^{3} of sediments were deposited annually in *Spartina* areas (8.37 ha), which represented 1.3 times the pool of metals in the first 2 cm (Table 3). Previous work in *S. maritima* natural and restored marshes recorded also high sedimentation rates in accordance with our results (Salgueiro and Caçador 2007; Curado *et al.* 2012).

Biomass of *S. maritima* in leaves (356 ± 53 g DW m^{-2}) and in roots (192 ± 44 g DW m^{-2}) showed higher total metal stocks than stems (935 ± 145 g DW m^{-2}) and rhizomes (424 ± 60 g DW m^{-2}) (Kruskal–Wallis, $\chi^2 = 22.515$, $P < 0.001$, df = 3) (Table 4). Iron and aluminum showed the highest metal stocks in *S. maritima* tissues and cadmium, lead and chrome the least (Tables 2, 3, and 4). About 2.5 yr after transplanting, *S. maritima* prairies, with relative cover of 62 ± 6% in 8.37 ha of restored marshes, accumulated 182 ± 12 kg of metals (ca. 22 kg ha^{-1}), corresponding to 152 kg of iron and aluminum (ca. 18 kg ha^{-1}) (Table 3). The recorded values of BGB for *S. maritima* (ca. 0.63 kg DW m^{-2}) were lower than those reported previously for natural populations in the Tajo estuary (3.60 ± 0.15 kg DW m^{-2} by Reboreda and Caçador 2007) and in the Odiel Marshes (from 4.82 ± 0.59 to

Table 3 Metal stock (kg ha^{-1}) in the first twenty centimeter of sediment and in *Spartina maritima* biomass for restored salt marshes 28 months after transplanting (8.37 ha with a *S. maritima* relative cover of 62%) in the Odiel Marshes (n = 10). Different coefficients indicate significant differences between surface and depth (*t*-test or *U*-test, $P < 0.05$)

	Sediments colonized by *S. maritima*		TOTAL in sediments	*S. maritima* biomass
Metal	(0–2 cm)	(2–20 cm)	(0–20 cm)	
Al	90.9 ± 16.4^{a}	62460.8 ± 5853.8^{b}	62551.8 ± 5857.2	6.7 ± 0.6
As	22.1 ± 5.5^{a}	490.1 ± 74.5^{b}	512.2 ± 78.2.6	0.1 ± 0.0
Cd	0.1 ± 0.0^{a}	28.1 ± 2.6^{b}	28.2 ± 2.6	0.0 ± 0.0
Cr	2.9 ± 0.8^{a}	97.9 ± 4.2^{b}	100.7 ± 4.0	0.0 ± 0.0
Cu	64.8 ± 18.3^{a}	4443.1 ± 422.0^{b}	4507.9 ± 419.0	1.0 ± 0.1
Fe	129.3 ± 28.8^{a}	99559.8 ± 9373.0^{b}	99689.1 ± 9382.7	11.5 ± 1.3
Ni	1.7 ± 0.7^{a}	30.8. ± 3.1^{b}	32.5 ± 3.2	1.7 ± 0.1
Pb	19.2 ± 4.9^{a}	738.2 ± 88.3^{b}	757.4 ± 90.8	0.0 ± 0.0
Zn	74.8 ± 16.9^{a}	2637.2 ± 258.9^{b}	2712.0 ± 264.1	0.8 ± 0.1
TOTAL	405.9 ± 90.5^{a}	170486.0 ± 15576.1^{b}	170891.9 ± 15602.8	21.8 ± 1.4

Table 4 Metals accumulated in leaves, stems, rhizomes and roots (g m^{-2}) in totally colonized areas of *Spartina maritima* 28 months after transplanting in the Odiel Marshes (n = 10). Different coefficients indicate significant differences between organs (analysis of variance, $P < 0.05$). (* measurements under the detection threshold)

Metal	Leaves	Stems	Rhizomes	Roots	TOTAL
Al	0.4826 ± 0.0465^{a}	0.2210 ± 0.0314^{bc}	0.1263 ± 0.0163^{b}	0.2559 ± 0.0210^{c}	1.0858 ± 0.0898
As	0.0020 ± 0.0001^{a}	0.0009 ± 0.0001^{b}	0.0014 ± 0.0001^{b}	0.0056 ± 0.0013^{ab}	0.0099 ± 0.0015
Cd	0.0001 ± 0.0000^{a}	0.0005 ± 0.0001^{ab}	0.0004 ± 0.0000^{ab}	0.0005 ± 0.0001^{b}	0.0015 ± 0.0002
Cr	0.0013 ± 0.0001^{a}	0.0011 ± 0.0001^{ab}	0.0006 ± 0.0000^{c}	0.0007 ± 0.0001^{bc}	0.0037 ± 0.0003
Cu	0.0295 ± 0.0018^{a}	0.0336 ± 0.0040^{a}	0.0314 ± 0.0042^{a}	0.0668 ± 0.0112^{b}	0.1613 ± 0.0168
Fe	0.5384 ± 0.0486^{ab}	0.2523 ± 0.0208^{a}	0.2696 ± 0.3523^{a}	0.7975 ± 0.1812^{b}	1.8579 ± 0.2174
Ni	0.0354 ± 0.0032^{a}	0.1108 ± 0.0107^{b}	0.0845 ± 0.0084^{b}	0.0470 ± 0.0094^{a}	0.2778 ± 0.0189
Pb	0.0016 ± 0.0002^{a}	*	0.0001 ± 0.0000^{b}	0.0011 ± 0.0005^{a}	0.0028 ± 0.0005
Zn	0.0363 ± 0.0033^{a}	0.0273 ± 0.0100^{a}	0.0205 ± 0.0059^{a}	0.0370 ± 0.0104^{a}	0.1211 ± 0.0242
TOTAL	1.1274 ± 0.0976^{a}	0.6478 ± 0.0563^{b}	0.5348 ± 0.0439^{b}	1.2121 ± 0.1885^{a}	3.5219 ± 0.2314

7.46 ± 1.35 kg DW m^{-2} by Castillo *et al*. 2008a,2008b). These differences seemed to be related to the slower development of BGB in relation to AGB in transplanted populations of *S. maritima* (Castillo *et al*. 2008a). Then, even more metals would be captured by BGB during the maturation of the restored marshes.

The total recorded metal pool in *S. maritima* areas 28 months after transplanting, including their sediments in the first 20 cm deep, was 1430.5 t (170.91 t ha^{-1}), corresponding only by 0.013% to vegetation; ca. 4.4 t metals (0.53 t ha^{-1}) were added annually by sedimentation and ca. 0.1 t metals yr^{-1} was sequestered by *S. maritima* expansion (NBPP of 264 ± 42 g DW m^{-2} yr^{-1} and a NAPP of 553 ± 83 g DW m^{-2} yr^{-1}).

Our results showed that *S. maritima* can be a useful biotool for phytoremediation projects in European polluted salt marshes at low elevations in the tidal gradient. *S. maritima* may be used for phytoextraction and phytostabilization since it promotes sedimentation at the same time that concentrates metals in its rhizosediments. In phytoextraction, sediments adhered to the roots should be extracted together with the plants, since they are rich in metals (as proposed by Almeida *et al*. (2004) for *Juncus maritimus* Lam). Although phytoextraction using *Spartina maritima* can provide some environmental benefits, this strategy will not be a complete solution in the Odiel Marshes because the salt marshes continuously receive sediments with high metal loads coming from the Iberian Pyrite Belt transported along Odiel and Tinto rivers (Nieto *et al*. 2007). This is the first study quantifying the phytoextraction and phytostabilization capacity of *S. maritima* plantations, which may be very helpful for phytoremediation projects in polluted European estuaries.

ACKNOWLEDGMENTS

We thank the Port Authority of Huelva for their sponsorship of the monitoring ecological engineering project in the Odiel Marshes. We also thank microanalysis service of CITIUS for collaboration and Dr. Brenda Grewell for the language editing.

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