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Heavy metal accumulation in *Halimione portulacoides*: Intra- and extra-cellular metal binding sites

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Abstract

Salt marsh plants can sequestrate and inherently tolerate high metal concentrations found in salt marsh sediments. This work intended to understand the *Halimione portulacoides* (L.) Aellen strategies to prevent metal toxicity, by investigating the metal location in different plant organs and in the cell. A sequential extraction was performed on leaves, stems and roots of *H. portulacoides* in order to determine and compare the metal (Zn, Pb, Co, Cd, Ni and Cu) concentration in several fractions of the plant material (ethanolic, aqueous, proteic, pectic, polissacaridic, lenhinic and cellulosic). This study shows that all plant organs of *H. portulacoides* mostly retain metals in the cell wall (65% is the average for all studied metals stored in the root cell wall, 55% in the stems and 53% in the leaves), and the metal content in the intracellular compartment is much lower (21% in roots, 25% in stems and 32% in leaves). High levels of heavy metal in the sedimentary environment do not cause toxicity to *H. portulacoides*, because *H. portulacoides* immobilizes them in different cell compartments (cell wall + proteic fraction + intracellular) outside key metabolic sites.

Keywords: Compartmentation; Phytoremediation; Phytotoxicity; Salt marsh; Sequential extraction

1. Introduction

Estuarine salt marshes are frequently highly contaminated with metals, due to human and industrial activities occurring in the estuaries and adjacent areas. However, these contaminants must be in an available form for them to be taken up by salt marsh plants (Greger, 2004), which are known to tolerate and accumulate high levels of heavy metals (e.g. Matthews et al., 2005). It seems there is an innate tolerance to metals in wetland plants (McCabe et al., 2001), eventually explained by the biogeochemistry of the rhizosphere (Otte et al., 2004). The solubility and availability of metals for plants may be affected by several factors such as their loading rate, chemical characteristics, pH, redox potential, soil texture, clay content and organic matter content, cation exchange capacity, etc. (Greger, 2004), which determine the different uptake by different plant species and at different locations. Salt marsh plants have the ability to uptake metals. Metals are then translocated within the plant, at different concentrations in different organs. Salt marsh plants generally accumulate different percentages of metals in the below- and above-ground parts, with a higher percentage of metals in the roots rather than in the above-ground part (Fitzgerald et al., 2003; Matthews et al., 2004). Metal translocation can occur in the phloem, via the apoplast, and via the xylem, acropetally (Greger, 1999). Therefore, metal translocation and storage capacity differs with plant species and with metal (e.g. Stoltz and Greger, 2002).

In order to survive in metal contaminated salt marshes, salt marsh plants may have mechanisms to regulate (and

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distribute) internal and cell wall metal concentrations, according to their tolerance capacity, which determines their survival. Metal tolerance by plants, and heavy metal detoxification may be achieved through metal complexation with ligands such as organic acids, amino acids and some members of the mugineic acids which exist in plant tissues, and also by compartmentation (Hall, 2002; see Carrier et al., 2003 and references therein). So, metals can be stored/accumulated either in cell walls (e.g. Lozano-Rodriguez et al., 1997; Carrier et al., 2003), cytoplasm (Rauser and Ackerley, 1987; Carrier et al., 2003) or in cell vacuoles (e.g. for Cd see Carrier et al., 2003). In order to maximize their detoxification and/or transport, plants control both the oxidation state and coordination environment of specific metallic elements (Salt et al., 2002). Direct coordination of the element (e.g. cadmium, nickel and zinc) by the plant, through the most chemically appropriate ligand leads to stable non-toxic complexes, and this is one of the mechanisms used for detoxification of metals and metalloids (Salt et al., 2002). Moreover, ectomycorhizas can be efficient in diminishing the toxicity effect on the host plant, usually in trees and shrubs (review in Hall, 2002; Liu and Kottke, 2003). Other mechanisms consist of binding metals in soil as highly insoluble metal sulfides, through the precipitation of metals such as zinc, lead and cadmium (Otte et al., 2004). Metals can also be mobilized in the rhizosphere through adsorption and co-precipitation with iron oxy-/hydroxides which circulate as far as the iron plaque (it functions as a metal sink), and are immobilized near the root surface (Otte et al., 2004). Additionally, metals can adsorb to organic matter within the sediments (Fritioff and Greger, 2006) forming metal quelates or complexes (Sauvé et al., 2000; Mellis et al., 2004), also conditionating its bioavailability.

The Tagus estuary is located near a highly populated and industrialized city (Lisbon). According to previous works (Caçador et al., 1996, 2000) the estuary receives discharges from industries (e.g. chemicals and steelmaking) and effluents from anthropogenic sources (including metals) incorporating them in the sediment. Its salt marshes are colonized by several halophyte species, namely *Halimione portulacoides* (L.) Aellen. These salt marshes retain heavy metals in their sediments, which are largely sequestrated and tolerated by these plants (Caçador et al., 2000; Reboreda and Caçador, 2007). However, the toleration mechanism is not yet completely understood, not even the exact location of metal accumulation in the plants and cells.

Considering the capacity of salt marsh plants to accumulate high concentrations of heavy metal and its useful employment in phytoremediation processes (e.g. Reboreda and Caçador, 2007), the aim of this work is to understand the molecular/cellular mechanisms that control the uptake and detoxification of metals by *H. portulacoides* (a salt marsh plant), and the metal compartmentation and location within the plant cell.

Regarding the previously mentioned effluents and discharges into the Tagus estuary and salt marshes, the most abundant heavy metals at this site were analyzed in this study (zinc, lead, cobalt, cadmium, nickel and copper).

2. Materials and methods

2.1. Sampling site description

The Tagus estuary, located on the western coast of Europe, has a shallow bay covering an area of about 320 km^2. Intertidal mudflats in the Tagus salt marshes are colonized by several halophyte species, with *H. portulacoides* (Caryophyllales: Chenopodiaceae) as one of the most representative, corresponding to 25% of covered area in the salt marsh plant community. This study was carried out in the Rosário salt marsh (in the Tagus estuary), located near urbanised and industrial areas, thus receiving effluent discharges from these sources, which unequivocally affect these habitats. This salt marsh sediment presented the following heavy metal concentrations among the roots of *H. portulacoides*: 72.9 ± 14.7 μg g^{-1} DW of Cu, 461.8 ± 219.5 μg g^{-1} DW of Pb, 3.6 ± 0.4 μg g^{-1} DW of Cd (Reboreda and Caçador, 2007), 749.3 ± 84.1 μg g^{-1} DW of Zn, 49.6 ± 0.04 μg g^{-1} DW of Ni, 59.6 ± 0.05 μg g^{-1} DW of Co (Caçador, unpublished data). According to Reboreda and Caçador (2007) and Caçador (unpublished data), metals dissolved in porewater at this salt marsh presented the following concentrations: 60.6 ± 8.5 μg g^{-1} of Cu, 247.2 ± 56.7 μg g^{-1} of Pb, 95.4 ± 6.6 μg g^{-1} of Cd, 98.5 ± 15.1 μg g^{-1} of Zn, 15.3 ± 2 μg g^{-1} of Ni and 13.1 ± 1 μg g^{-1} of Co.

2.2. Sampling strategy and laboratorial processing

Samples of *H. portulacoides* plants were collected from monotypic stands in the Rosário salt marsh, in the Tagus estuary. Three squares of 0.3×0.3 m^2 were sampled, wherein the above-ground material was collected by harvesting it, and the below-ground material was collected by taking sediment cores on exactly the same area. Afterwards, the samples were brought to the Institute of Oceanography – FCUL laboratory and were processed. *H. portulacoides* (above- and below-ground material) was carefully rinsed with demineralised water, and dried during 48 h (until it reached a constant weight) at 60 °C. Leaves, stems and belowground material were separated.

2.3. Heavy metal extraction procedure

A sequential extraction was performed (adapted from Farago and Pitt, 1977), in order to assess the metal content in cellular constituents of *H. portulacoides*. Vegetal material from different plant organs (leaves, stems and roots; 1 g DW; $n = 3$), previously homogenized, was processed individually in a soxhlet by successive extractions. The extracting agent used first was ethanol 80% (p.a., Merck, 150 ml) in reflux in a soxhlet for 12 h; then, the residue was placed in 150 ml of demineralised water and subjected

to reflux for 12 h. In the third extraction step, the residue was put in a solution of 100 ml demineralised water (pH 7.5; temperature 37 °C) with 0.2 g pronase E (from Streptomyces griseus, Merck) plus 0.03 g chloramphenicol (⩾98%, TLC) and subjected to continuous shaking for 24 h. Later, the same residue was added to 100 ml of a pectinase solution (1% P5146, Sigma; pH 4, temperature 25 °C) and shaken for 24 h. The following step consisted of a reflux of the residue in 150 ml NaOH solution (0.5 M) (p.a. ⩾98%, Sigma) for 12 h, and after that, another continuous reflux with 100 ml HCl 5% (prepared from HCl fumant 37% p.a., Merck) was performed for 12 h at 25 °C. Lastly, an acid digestion of the plant residue was performed in Teflon bombs with $HNO_3/HClO_4$ (7:1, v:v) (HNO_3 65% p.a., Merck; $HClO_4$ 70% p.a. ACS-ISO, Panreac) and put into the oven at 110 °C for 3 h. After cooling, all extracts/fractions (ethanolic, aqueous, proteic, pectic, polissacaridic, lenhinic and cellulosic) were filtered through Whatman 42 filters (pore Ø 2.5 μm) and diluted to 10 ml with demineralised water.

Metals bound to pectic, polissacaridic, lenhinic and cellulosic fractions are those bound to the cell wall, since these are constituents of the cell wall. The different types of proteins can not be determined using this extraction method, which implies that its exact location in the cell can not be defined. The metals bound to some amino acids, chlorophyll, low weight compounds (all extracted by ethanol) and those extracted in the aqueous fraction were designated soluble metal (Farago and Pitt, 1977).

2.4. Analytical procedures

Metal concentrations in the *H. portulacoides* samples were determined by air-acetylene flame atomic absorption spectroscopy (VARIAN Spectr AA-50) and a manual microinjection method. The metal concentrations are reported in $\mu g\,g^{-1}$ dry weight (DW). Quality assurance was performed through stability of instrumental recalibration and using analytical blanks. Moreover, one certified reference material – CRM (Community Bureau of Reference – BCR 62, *Olea europeae*) was analysed to assess the validity and precision of the analytical procedures. The BCR was randomly allocated within the sample measurements. The analysed values for the reference material were in good agreement (not statistically different from the certified ones, t student; $\alpha = 0.05$), with the certified values and blanks proving to be negligible. The detection limits of the AAS analysis were in $mg\,kg^{-1}$ dry weight for: Zn (0.33), Pb (0.32), Co (0.13), Cd (0.03), Ni (0.15), Cu (0.03).

2.5. Statistical analyses and calculations

Two-way ANOVA (analysis of variance) was performed for each metal to test for differences in metal concentration between plant organs (three levels) and extracted fractions (seven levels). Dixon's test was performed to detect outliers. Data were log-, log $(x+1)$-, $1/(x+0.5)$- or x^2-transformed when necessary, to achieve the homogeneity of variances (Cochran's Q test). Normality of the data was also assured (Kolmogorov-Smirnov test). Post-hoc comparisons were performed using the Newman-Keuls test at $\alpha = 0.05$ significance level. Analyses were performed with the STATISTICA 7.0 software package.

The translocation factor (TF) was calculated by the ratio of $[metal]_{leaves}/[metal]_{roots}$ and also by the ratio $[metal]_{stems}/[metal]_{roots}$, expressing the metal's translocation within the plant, from the roots to the leaves and the stems (Deng et al., 2004). The TF from the sediment to the roots was also calculated.

3. Results

Total metal concentrations (sum of metals from all extracted fractions) from different organs (roots, stems and leaves) of *H. portulacoides* show a common pattern: Zn > Pb > Cu > Ni > Co > Cd, ranging between 290.89 $\mu g\,g^{-1}$ DW of Zn in the roots to 5.10 $\mu g\,g^{-1}$ DW of Cd in the leaves (Table 1 and Fig. 1). Zn presents five to twenty seven times higher concentration than the other metals, both in the roots and in the above-ground material.

The roots present significantly higher metal concentrations than the stems and the leaves, for all studied metals (two-way ANOVA, $p < 0.001$; Newman-Keuls test for post-hoc) (Fig. 1 and Table 2). Cd was the only metal where metal concentration in the leaves was significantly lower than in the stems, with all other metals presenting statistically the same concentrations in the leaves and the stems. The translocation of metals from the roots to the leaves can be expressed by the translocation factor (TF), and varied from 0.35 ± 0.20 (Cu) to 0.47 ± 0.19 (Zn) (Table 3). Instead, if we consider the TF for metals from the roots to the stems, it varied from 0.48 ± 0.17 for Co to 0.59 ± 0.26 for Zn, 0.59 ± 0.27 for Pb and 0.59 ± 0.46 for Cd. The TF range from the sediment to the roots is from 0.11 ± 0.05 (Pb) to 0.81 ± 0.20 (Ni), and there is an extreme value of 3.04 ± 0.47 (Cd). As was expected, the sediment presents higher metal concentrations than do the roots, with the exception of Cd. According to the pore-water metal concentrations (the metals really available to the plant), Zn and Pb presented the highest concentrations in the sediment. Considering all plant material (leaves, stems and roots), Zn and Cd are the metals with the highest TFs and Cu and Co with the lowest ones. Cd is the metal with the highest mobility also from the sediment to the roots, opposing Co with the lowest TF.

Regarding metal compartmentation in cell constituents, there was no statistically significant interaction between the plant organ and extracted fraction for each metal (two-way ANOVA, $p > 0.05$; Table 2). Significantly higher Zn concentrations were present in the proteic fraction, and the lowest concentration was detected in cellulosis (Fig. 2). The highest Pb percentage occurs in the ethanolic and polissacaridic fractions. Co, Cd and Ni were mostly accumulated in the polissacaridic fraction in all plant organs,

Table 1
Metal concentrations (μg g^{-1} DW) (average ± SD; $n = 3$) on different fractions of *Halimione portulacoides* leaves, stems and roots, corresponding to extra- and intra-cellular location

Plant organ	Fraction	Metal (μg g^{-1} DW) (average ± SD)					
		Zn	Pb	Co	Cd	Ni	Cu
Roots	Ethanolic	28.34 ± 11.84	7.27 ± 1.07	1.55 ± 0.46	0.97 ± 0.05	2.84 ± 0.63	1.83 ± 1.53
	Aqueous	33.37 ± 26.01	10.27 ± 2.09	2.01 ± 0.35	1.14 ± 0.13	3.11 ± 0.90	2.74 ± 1.68
	Proteic	77.70 ± 35.27	5.30 ± 1.81	1.56 ± 0.73	1.15 ± 0.54	2.12 ± 1.09	7.70 ± 3.61
	Pectic	53.40 ± 32.27	8.29 ± 2.59	1.76 ± 0.49	1.26 ± 0.52	2.27 ± 0.65	9.89 ± 7.52
	Polissacaridic	19.44 ± 7.31	16.40 ± 7.75	9.58 ± 4.90	5.12 ± 2.66	11.95 ± 5.38	6.46 ± 4.00
	Lignin	66.30 ± 12.35	7.57 ± 1.40	1.57 ± 0.50	1.30 ± 0.26	2.07 ± 0.67	6.43 ± 3.57
	Cellulosis	12.33 ± 9.01	0.01 ± 0.007	1.47 ± 1.25	0.04 ± 0.07	2.30 ± 0.00	1.55 ± 0.58
	Total	290.89	55.13	19.50	10.99	26.65	36.60
Stems	Ethanolic	13.82 ± 3.42	5.23 ± 3.10	1.19 ± 0.67	0.89 ± 0.45	1.73 ± 0.71	1.92 ± 0.31
	Aqueous	15.93 ± 11.21	4.99 ± 2.40	1.18 ± 0.60	0.85 ± 0.44	1.97 ± 0.43	1.79 ± 0.98
	Proteic	78.90 ± 44.55	4.45 ± 1.72	1.01 ± 0.17	0.81 ± 0.34	1.48 ± 0.47	3.66 ± 1.62
	Pectic	15.73 ± 4.43	4.13 ± 1.49	0.77 ± 0.23	0.63 ± 0.18	1.31 ± 0.37	1.56 ± 1.64
	Polissacaridic	11.70 ± 5.98	8.61 ± 3.24	4.31 ± 1.80	2.69 ± 0.90	5.30 ± 4.26	3.38 ± 0.95
	Lignin	26.43 ± 9.46	4.63 ± 2.27	0.93 ± 0.61	0.89 ± 0.60	1.50 ± 0.76	1.59 ± 1.29
	Cellulosis	3.68 ± 1.86	0.00 ± 0.00	0.06 ± 0.10	0.29 ± 0.36	0.87 ± 0.55	0.70 ± 0.36
	Total	166.19	32.04	9.47	7.04	14.14	14.60
Leaves	Ethanolic	13.63 ± 5.65	5.27 ± 1.02	1.85 ± 0.60	1.15 ± 0.33	2.00 ± 0.55	3.08 ± 1.85
	Aqueous	9.32 ± 0.59	3.98 ± 1.23	0.94 ± 0.33	0.65 ± 0.23	1.22 ± 0.44	0.97 ± 0.43
	Proteic	46.15 ± 24.16	3.49 ± 0.007	0.71 ± 0.17	0.54 ± 0.13	1.00 ± 0.24	1.57 ± 0.47
	Pectic	14.89 ± 5.32	2.80 ± 0.34	0.60 ± 0.08	0.44 ± 0.07	0.75 ± 0.001	2.29 ± 2.06
	Polissacaridic	27.79 ± 34.27	2.56 ± 2.75	2.78 ± 2.71	1.69 ± 1.53	3.95 ± 3.03	2.61 ± 2.00
	Lignin	16.62 ± 13.44	3.38 ± 0.71	0.73 ± 0.21	0.54 ± 0.16	0.99 ± 0.27	0.81 ± 0.26
	Cellulosis	8.63 ± 6.30	0.51 ± 0.46	0.36 ± 0.29	0.10 ± 0.02	1.27 ± 1.68	0.73 ± 0.24
	Total	137.03	21.99	7.96	5.10	11.18	12.05

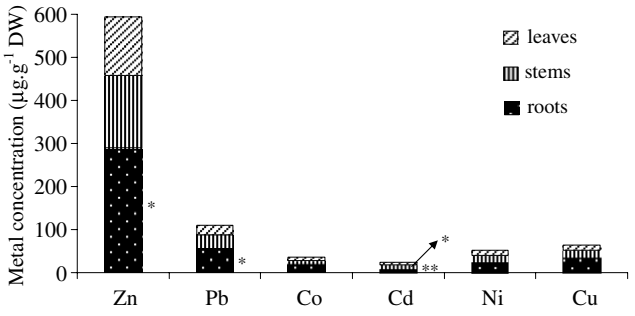

Fig. 1. Metal concentrations (μg g^{-1} DW) on *H. portulacoides* roots, stems and leaves, as a sum of all extracted fractions. (∗ and ∗∗ mean statistically different metal concentrations; two-way ANOVA, $p > 0.05$ and Newman-Keuls' test for post-hoc).

and cellullosis was the fraction with the lowest concentration of these metals. Cu was mostly accumulated in ethanolic, proteic and pectic fractions.

Considering the extracted fractions, metal location in the plant can be divided into three sections: cell wall, proteic fraction and intracellular location (the soluble metals). Cell wall includes the pectic, polissacaridic, lenhinic and cellulosic fractions. The proteins exact location in the cell can not be determined by this extraction method. Intracellular metals include those extracted by ethanol and demineralised water (Figs. 3 and 4). Roots accumulate on average 21% of metals intracellularly (sum of the metal extracted with ethanol and demineralised water; average

Table 2
Two-way ANOVA: effects of plant organ and extracted fraction on Zn, Pb, Co, Cd, Ni and Cu extracted

Metal	Source	DF	MS	F	p
Zn	Plant organ	2	0.804	10.323	0.0002
	Fraction	6	0.827	10.624	<0.0001
	Plant organ × fraction	12	0.059	0.758	0.6885
	Residuals	42	0.078		
Pb	Plant organ	2	31354.7	6.692	0.0032
	Fraction	6	15378.4	3.282	0.0106
	Plant organ × fraction	12	8232.2	1.757	0.0925
	Residuals	38	4685.2		
Co	Plant organ	2	0.259	15.848	<0.0001
	Fraction	6	0.280	17.093	<0.0001
	Plant organ × fraction	12	0.023	1.408	0.2006
	Residuals	42	0.016		
Cd	Plant organ	2	0.098	9.140	0.0005
	Fraction	6	0.271	25.268	<0.0001
	Plant organ × fraction	12	0.016	1.490	0.1664
	Residuals	42	0.011		
Ni	Plant organ	2	0.308	12.101	0.0001
	Fraction	6	0.223	8.737	<0.0001
	Plant organ × fraction	12	0.014	0.545	0.8712
	Residuals	40	0.025		
Cu	Plant organ	2	1.179	11.372	0.0001
	Fraction	6	0.387	3.733	0.0046
	Plant organ × fraction	12	0.151	1.453	0.1810
	Residuals	42	0.104		

Zn and Cu data were log-transformed, Co and Ni data were log $(x + 1)$-transformed, Cd data were $1/(x + 0.5)$-transformed and Pb data were x^2-transformed, to achieve ANOVA assumptions in Ni two outliers were excluded, and in Pb four outliers were excluded (according to Dixon's test).

Table 3
Translocation factors (TF) for metals within *Halimione portulacoides* plants from Tagus estuary ($n = 3$; average ± SD)

Translocation factor (TF)	Metal					
	Zn	Pb	Co	Cd	Ni	Cu
$[metal]_{leaves}/[metal]_{roots}$	0.47 ± 0.19	0.41 ± 0.11	0.38 ± 0.16	0.43 ± 0.09	0.43 ± 0.17	0.35 ± 0.20
$[metal]_{stems}/[metal]_{roots}$	0.59 ± 0.26	0.59 ± 0.27	0.48 ± 0.17	0.59 ± 0.46	0.55 ± 0.24	0.50 ± 0.30
$[metal]_{roots}/[metal]_{sediment}$	0.51 ± 0.07	0.13 ± 0.05	0.11 ± 0.05	3.04 ± 0.47	0.81 ± 0.20	0.51 ± 0.25

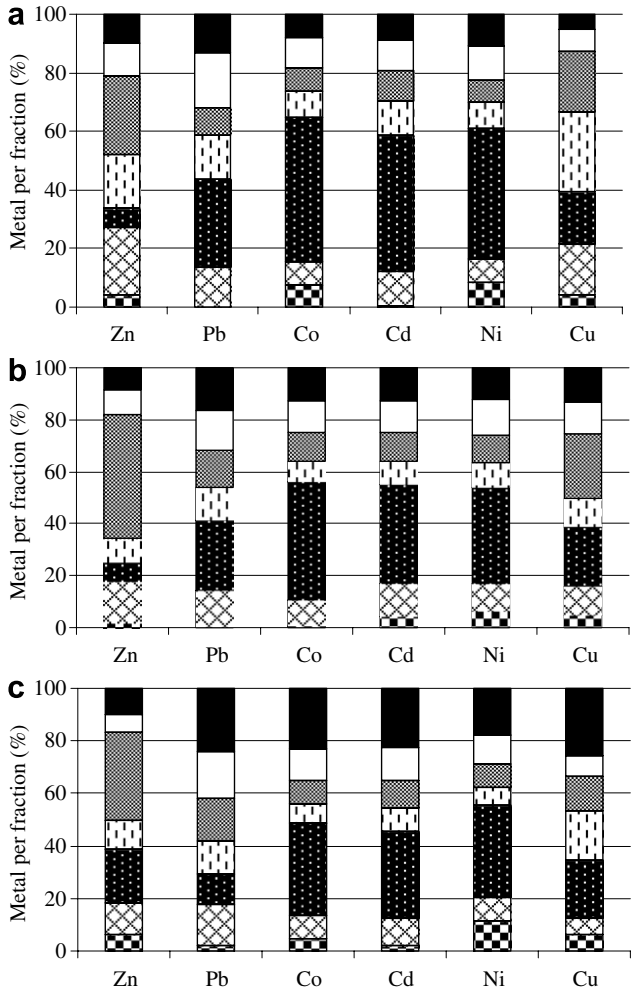

Fig. 2. Metal concentrations (%) (average; $n = 3$) on different fractions of *H. portulacoides* roots (a), stems (b), and leaves (c). The fractions, from top to down, are ethanolic (■), aqueous (□), proteic (▩), pectic (▨), polissacaridic (▩), lenhinic (▨) and cellulosic (▩).

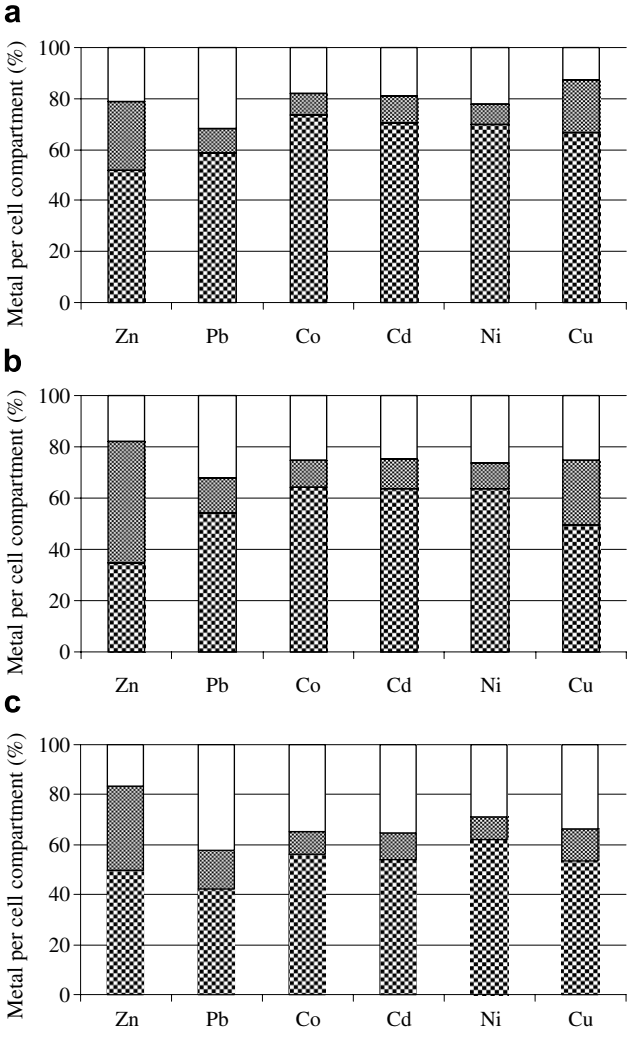

Fig. 3. Metal organic ligands (%) (average; $n = 3$) located intracellularly (□) (ethanolic + aqueous fraction), on the proteic fraction (▩) and on the cell wall (▩) (pectic + polissacaridic + lenhinic + cellulosic fractions) of *H. portulacoides* roots (a), stems (b) and leaves (c), corresponding to extra- and intra-cellular location.

for all metals), 14% is retained in the proteic fraction and 65% is in the cell wall (sum of metals quantified in the pectic, polissacaridic, lenhinic and cellullosic fractions). In the stems, on average 25% of metals are retained inside the cell, 20% the proteic fraction and the cell wall retain 55%. The highest percentage of metals accumulated intracellularly occurs in the leaves (32% average for all metals) and the proteic fraction presents 15%. The cell walls of the leaves retain 53% of metals. Co, Cd and Ni are the metals whose highest percentage is located in the cell wall of the leaves, stems and roots, whereas Pb presented the highest intracellular percentage. Zn was the metal presenting the highest percentage stored in the proteic fraction (Fig. 3).

4. Discussion

The results show that the roots (sum of all extracted fractions) of *H. portulacoides* accumulate much more metals than the above-ground parts (leaves and stems), which is in accordance with previous works on the Tagus estuary

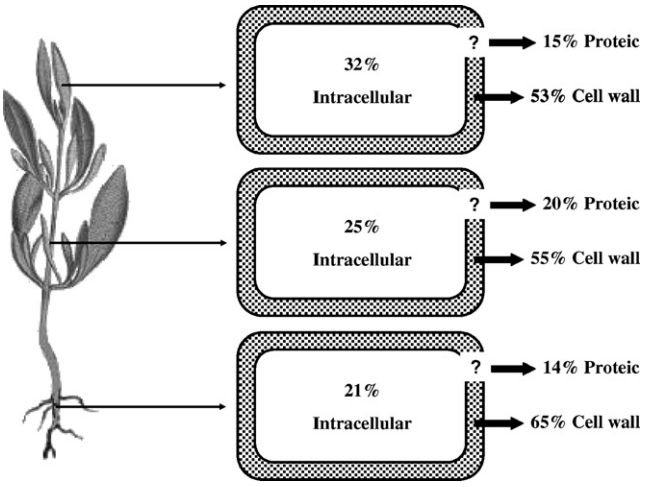

Fig. 4. Metal distribution and compartmentation (cell wall (▩), proteic fraction, and intracellular location (□) in *Halimione portulacoides* leaves, stems and roots.

(Caçador et al., 2000; Reboreda and Caçador, 2007). These results were also registered for Cd, Cu, Zn and Pb in several plant species by Stoltz and Greger (2002) and in other aquatic macrophytes from Australia (Cardwell et al., 2002). In Ireland, *Spartina* spp. present higher Cu and Pb concentrations in the below-ground material than in the above-ground (Fitzgerald et al., 2003); and the same results were obtained for zinc in a wetland grass (*Glyceria fluitans*) of five European populations (Matthews et al., 2004). In the Thames estuary *H. portulacoides*, *Spartina* spp. *Salicornia* spp. and *Aster tripolium* presented, in most cases, higher metal concentrations in the roots than in the above-ground (except for the most chemically mobile metals, Cd, Mn and Zn) (Williams et al., 1994). The Zn, Cu, Cd and Pb accumulated by the aquatic macrophyte *Potamogeton natans* were higher in the roots than in the stems or leaves (Fritioff and Greger, 2006). Nevertheless, metal concentration in the plant shoots can be higher than in the roots and, depending on the metals and on the plant species, there is a high variability. For instance, *Paspalum distichum* and *Cynodon dactylon* presented higher Pb, Zn and Cu concentrations in the shoots rather than in the roots, when colonizing mine tailings contaminated sites as well as normal soils (Shu et al., 2002). Perronnet et al. (2003) showed that *Thlaspi caerulescens* shoots accumulate higher concentrations of Zn and Cd than its roots, which accumulate less than 20% of the hyperaccumulated metal in the plant.

The limited mobility of the metals once inside the salt marsh plant (Deng et al., 2004), which was observed in this study, may be the explanation for the fact that metals are essentially accumulated in the below-ground rather than in the above-ground part of salt marsh plants. This is also shown by translocation factors lower than 1, which was seen in *H. portulacoides* for all the studied metals. The high mobility and bioavailability of Zn and Cd (Kiekens, 1995) may explain the highest translocation factors observed for these two metals, when considering the allocation of metals from the roots to the leaves and from the roots to the stems. The lowest TFs registered for Cu, which is a relatively immobile metal in plants (Baker and Senft, 1995), render the higher accumulation of this metal in the roots, instead of being translocated to the leaves of *H. portulacoides*.

The sediment of the studied salt marsh, in relation to other contaminated sediments (e.g. Freitas et al., 2004), presented higher metal concentrations than *H. portulacoides* plant (Caçador et al., 2000; Reboreda and Caçador, 2007; this study). This is also rendered by the calculated TF from the sediment to the roots.

Uptake and accumulation of metals by salt marsh plants depend on many factors such as the plant species, the age and growth stage of the plants, seasonal variations, the existence of iron plaques on the roots, the level of metal contamination in a specific local, soil properties, tidal inundations, salinity; and then metal characteristics influence the absorption, accumulation and translocation of metals (Fitzgerald et al., 2003; Deng et al., 2004). Accordingly, plants have developed several strategies to survive in heavy metal contaminated soils. Tolerance mechanisms for Zn and Ni have been explained by its complexation with organic acids in the cell vacuoles (Marschner, 1995). In the well known hyperaccumulator *Thlaspi caerulescens*, the roots accumulate Cd both in the apoplast (binding to cell wall components) and inside the cells, as a Cd-detoxification mechanism; the leaves use vacuoles as the main compartment for Cd storage and detoxification (Wójcik et al., 2005). Phytochelatins had been defended as the main mechanism for metal detoxification in some plants (e.g. Cobbett, 2000), but the role of phytochelatins has been questioned by several authors (de Knecht et al., 1994, 1995; Ernst et al., 2000; Ebbs et al., 2002). On the other hand, according to Ramos et al. (2002) and Zornoza et al. (2002), lettuce leaves and lupin leaves, respectively, presented the most cellular Cd bound to cell wall fraction. In *Brassica napus,* Carrier et al. (2003) reported that the Cd is preferentially stored in the vacuoles and the cell walls, reducing the Cd toxicity in the leaves. Previous studies demonstrated that *T. caerulescens* (Salt et al., 1999), presents approximately half of the metal content as cell wall-bound, which was recently reinforced by Fritioff and Greger (2006), where *T. caerulescens* stored 24–59% of Zn, Cu, Cd and Pb in the cell wall-bound fraction.

In *H. portulacoides* all studied metals were found with a higher percentage bind to cell wall compounds, in roots, stems and leaves. However, the distribution of metals in different cell wall extracted fractions varied with the metal, which may be related to the properties of the metal themselves. It has been suggested that this higher accumulation of metals in cell walls works as a protection barrier against harmful effects by diminishing the metal concentration in the cytoplasm (Ramos et al., 2002; Zornoza et al., 2002).

This work shows that metal content in the intracellular compartment of *H. portulacoides* is much lower than the

total metal content retained by the plant. Considering these data, it can be stated that compartmentation and detoxifying mechanisms are crucial for *H. portulacoides* to be able to tolerate high levels of heavy metals. Results show that the high levels of heavy metal in the salt marsh sediment, as well as those dissolved in the porewater (Reboreda and Caçador, 2007; Caçador, unpublished data) do not causes toxicity to the plants because the plant immobilizes them outside key metabolic sites. Thus, metal compartmentation in *H. portulacoides* constitutes a key mechanism of resistance in the plant. Metals are preferentially stored/sequestered in compartments (vacuoles) and in the cell wall, away from metabolic active sites/compartments (such as cytoplasm, chloroplast, mithochondria) reducing the metal's toxicity in the plant (Frey et al., 2000; Psaras et al., 2000; Küpper et al., 2001; Psaras and Manetas, 2001; this study).

As a whole, metals are accumulated intracellularly in the plant and in different cell compartments, bound to different cell compounds (cell wall + proteic fraction + intracellular). The fact that the main percentage of metals is bound to the cell wall rather than located intracellullarly, may have crucial significance as a detoxifying mechanism in *H. portulacoides* leaves, stems and roots. This compartmentation may contribute to, and may be crucial to the survival of salt marsh plants in salt marshes that are highly contaminated by metal, since metals are immobilized outside metabolic active sites in the cell. This study gives an insight into the different compartmentation of Zn, Pb, Cu, Ni, Co and Cd inside *H. portulacoides* cells and plant organs (leaves, stems and roots) from the Tagus estuary. Thus, considering the *H. portulacoides* heavy metal storage capacity and its ability to immobilize metals in different compartments within the cell (functioning as a detoxification mechanism), this plant can be considered an important tool in phytoremediation processes.

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References

Baker, D.E., Senft, J.P., 1995. Copper. In: Alloway, B.J. (Ed.), Heavy Metals in Soils, second ed. Blackie Academic and Professional, London, UK, pp. 179–205.

Caçador, I., Vale, C., Catarino, F., 1996. The influence of plants on concentration and fractionation of Zn, Pb, Cu in salt marsh sediments (Tagus estuary, Portugal). J. Aquat. Ecos. Health 5, 193–198.

Caçador, I., Vale, C., Catarino, F., 2000. Seasonal variation of Zn, Pb, Cu and Cd concentrations in the root-sediment system of *Spartina maritima* and *Halimione portulacoides* from Tagus estuary salt marshes. Mar. Environ. Res. 49, 279–290.

Cardwell, A.J., Hawker, D.W., Greenway, M., 2002. Metal accumulation in aquatic macrophytes from southeast Queensland, Austrália. Chemosphere 48, 653–663.

Carrier, P., Baryla, A., Havaux, M., 2003. Cadmium distribution and microlocalization in oilseed rape (Brassica napus) after long-term growth on cadmium-contaminated soil. Planta 216, 939–950.

Cobbett, C.S., 2000. Phytochelatins and their roles in heavy metal detoxification. Plant Physiol. 123, 825–832.

De Knecht, J.A., Van Dillen, M., Koevoets, P.M., Schat, H., Verkleij, J.A.C., Ernst, W.H.O., 1994. Phytochelatins in cadmium-sensitive and cadmium-tolerant *Silene vulgaris*. Plant Physiol. 104, 255–261.

De Knecht, J.A., van Baren, N., Ten Bookum, W.M., Wong Fong Sang, H.W., Koevoets, P.L.M., Schat, H., Verkleij, J.A.C., 1995. Synthesis and degradation of phytochelatins in cadmium-sensitive and cadmium-tolerant *Silene vulgaris*. Plant Sci. 106, 9–18.

Deng, H., Yea, Z.H., Wong, M.H., 2004. Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. Environ. Pollut. 132, 29–40.

Ebbs, S., Lau, I., Ahner, B., Kochian, L., 2002. Phytochelatin synthesis is not responsible for Cd tolerance in the Zn/Cd hyperaccumulator *Thlaspi caerulescens* (J and C Presl). Planta 214, 635–640.

Ernst, W.H.O., Nelissen, H.J.M., Bookum, W.M.T., 2000. Combination toxicology of metal-enriched soils: physiological responses of a Zn- and Cd-resistant ecotype of *Silene vulgaris* on polymetallic soils. Environ. Exp. Bot. 43, 55–71.

Farago, M.E., Pitt, M.J., 1977. Plants which accumulate metals. Part II. An investigation of the soluble zinc containing extracts from two Australian species. Inorg. Chim. Acta 24, 127–130.

Fitzgerald, E.J., Caffrey, J.M., Nesaratnam, S.T., McLoughlin, P., 2003. Copper and lead concentrations in salt marsh plants on the suir Estuary, Ireland. Environ. Pollut. 123, 67–74.

Freitas, H., Prasad, M.N.V., Pratas, J., 2004. Plant community tolerant to trace elements growing on the degraded soils of São Domingos mine in the south east of Portugal: environmental implications. Environ. Int. 30, 65–72.

Frey, B., Zierold, K., Brunner, I., 2000. Extracelular complexation of Cd in the hartig net and cytosolic Zn sequestration in the fungal mantle of Picea abies – Hebeloma crustuliniforme ectomycorrhizas. Plant Cell Environ. 23, 1257–1265.

Fritioff, A., Greger, M., 2006. Uptake and distribution of Zn, Cu, Cd and Pb in an aquatic plant *Potamogeton natans*. Chemosphere 63, 220–227.

Greger, M., 1999. Metal availability and bioconcentration in plants. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy Metal Stress in Plants: From Molecule to Ecosystems. Springer-Verlag, Heidelberg, Berlin, Germany.

Greger, M., 2004. Metal availability, uptake, transport and accumulation in plants. In: Prasad, M.N.V. (Ed.), Heavy Metal Stress in Plants – From Biomolecules to Ecosystems, second ed. Springer-Verlag, Heidelberg, Berlin, Germany, pp. 1–27.

Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 53 (366), 1–11.

Kiekens, L., 1995. Zinc. In: Alloway, B.J. (Ed.), second ed., Heavy Metals in Soils Blackie Academic and Professional, London, UK, pp. 284–305.

Küpper, H., Lombi, E., Zhao, F., Wieshammer, G., McGrath, S.P., 2001. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. J. Exp. Bot. 52, 2291–2300.

Liu, D., Kottke, I., 2003. Subcellular localization of Cd in the root cells of Allium sativum by electron energy loss spectroscopy. J. Biosci. 28, 471–478.

Lozano-Rodriguez, E., Hernandéz, L.E., Bonay, P., Carpena-Ruiz, R.O., 1997. Distribution of cadmium in shoot and root tissues of maize and pea plants: physiological disturbances. J. Exp. Bot. 48, 123–128.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, second ed. Academic Press Limited, London, UK.

Matthews, D.J., Moran, B.M., McCabe, P.F., Otte, M.L., 2004. Zinc tolerance, uptake, accumulation and distribution in plants and protoplasts of five European populations of the wetland grass *Glyceria fluitans*. Aquat. Bot. 80, 39–52.

Matthews, D.J., Moran, B.M., Otte, M.L., 2005. Screening the wetland plant species *Alisma plantago-aquatica*, *Carex rostrata* and *Phalaris arundinacea* for innate tolerance to zinc and comparison with *Eriophorum angustifolium* and *Festuca rubra* merlin. Environ. Pollut. 134, 343–351.

McCabe, O.M., Baldwin, J.L., Otte, M.L., 2001. Metal tolerance in wetland plants? Minerva Biotech. 13, 141–149.

Mellis, E.V., Cruz, M.C.P., Casagrande, J.C., 2004. Nickel adsoprtion by soils in relation to pH, organic matter, and iron oxides. Sci. Agric. (Piracicaba, SP) 61, 190–195.

Otte, M.L., Matthews, D.J., Jacob, D.L., Moran, B.M., Baker, A.J.M., 2004. Biogeochemistry of metals in the rhizosphere of wetland plants – an explanation for ''innate'' metal tolerance? In: Wong, M.H. (Ed.), Developments in ecosystems, vol. 1. Elsevier, pp. 87–94 (Chapter 6).

Perronnet, K., Schwartz, C., Morel, J.L., 2003. Distribution of cadmium and zinc in the hyperaccumulator *Thlaspi caerulescens* grown on multicontaminated soil. Plant Soil 249, 19–25.

Psaras, G.K., Constantinidis, Th., Cotsopoulos, B., Manetas, Y., 2000. Relative abundance of nickel in the leaf epidermis of eight hyperaccumulators: evidence that the metal is excluded from both guard cells and trichomes. Ann. Bot (London) 86, 73–78.

Psaras, G.K., Manetas, Y., 2001. Nickel localization in seeds of the metal hyperaccumulator *Thlaspi pindicum* Hausskn. Ann. Bot. (London) 88, 513–516.

Ramos, I., Esteban, E., Lucena, J.J., Gárate, A., 2002. Cadmium uptake and subcellular distribution in plants of *Lactuca* sp. Cd−Mn interaction. Plant. Sci. 162, 761–767.

Rauser, W.E., Ackerley, C.A., 1987. Localization of cadmium in granules within differentiating and mature root cells. Can. J. Bot. 65, 643–646.

Reboreda, R., Caçador, I., 2007. Halophyte vegetation influences in salt marsh retention capacity for heavy metals. Environ. Pollut. 146, 147–154.

Salt, D.E., Prince, R.C., Baker, A.J.M., Raskin, I., Pickering, I.J., 1999. Zinc ligands in the metal hyperaccumulator Thlaspi caerulescens as determined using X-ray absorption spectroscopy. Environ. Sci. Technol. 33, 713–717.

Salt, D.E., Prince, R.C., Pickering, I.J., 2002. Chemical speciation of accumulated metals in plants: evidence from X-ray absorption spectroscopy. Microchem. J. 71, 255–259.

Sauvé, S., Hendershot, W., Allen, H.E., 2000. Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. Environ. Sci. Technol. 34, 1125–1131.

Shu, W.S., Ye, Z.H., Lan, C.Y., Zhang, Z.Q., Wong, M.H., 2002. Lead, zinc, and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodon dactylon*. Environ. Pollut. 120, 445–453.

Stoltz, E., Greger, M., 2002. Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. Environ. Exp. Bot. 47, 271–280.

Williams, T.P., Bubb, J.M., Lester, J.N., 1994. The occurrence and distribution of trace metals in halophytes. Chemosphere 28, 1189–1199.

Wójcik, M., Vangronsveld, J., D'Haen, J., Tukiendorf, A., 2005. Cadmium tolerance in *Thlaspi caerulescens*. II. Localization of cadmium in *Thlaspi caerulescens*. Environ. Exp. Bot. 53, 163–171.

Zornoza, P., Vázquez, S., Esteban, E., Fernández-Pascual, M., Carpena, R., 2002. Cadmium-stress in nodulated white lupin: strategies to avoid toxicity. Plant Physiol. Biochem. 40, 1003–1009.