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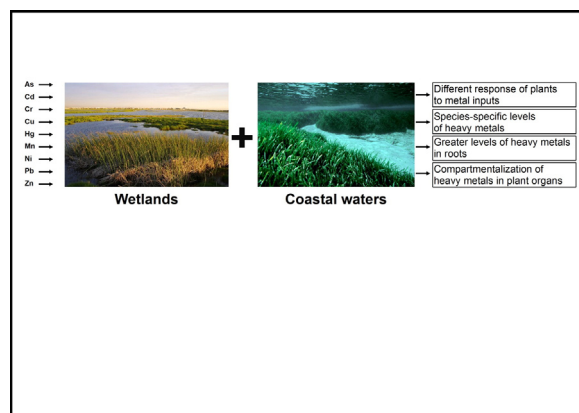
Levels of heavy metals in wetland and marine vascular plants and their biomonitoring potential: A comparative assessment

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HIGHLIGHTS

- Plants respond differently to metal inputs, despite similar ecology and anatomy.
- Bioaccumulation, internal translocation and bioindication are species-specific.
- Total metal concentrations are generally species-specific.
- Plants share high metal levels in roots and organ element compartmentalization.
- *P. australis* was the best bioaccumulator and bioindicator species.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study investigated the levels of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn in the seagrasses *Posidonia oceanica* and *Cymodocea nodosa*, and in the wetland macrophytes *Phragmites australis*, *Arundo donax*, *Typha domingensis*, *Apium nodiflorum*, and *Nasturtium officinale*. Results showed that the bioaccumulation capacity from sediments, translocation, total levels in plant tissues, and bioindication of metals in sediments, are generally species-specific. In particular, the patterns of metals in the aquatic plants studied were overall independent of ecology (coasts vs wetlands), biomass, anatomy (rhizomatous vs non rhizomatous plants), and life form (hemicryptophytes vs hydrophytes). However, marine phanerogams and wetland macrophytes shared some characteristics such as high levels of heavy metals in their below-ground organs, similar capacity of element translocation in the rhizosphere, compartmentalization of metals in the different plant organs, and potential as bioindicators of Cu, Mn and Zn levels in the substratum. In particular, the present findings indicate that, despite ecological and morphological similarities, different plant species tend to respond differently to exposure to heavy metals. Furthermore, this seems to result from the species individual ability to accumulate and detoxify the various metals rather than being attributed to differences in their ecological and morpho-anatomical characteristics.

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1. Introduction

Heavy metals in air, soil and water have become a global issue as a consequence of the increasing human impact in the last few decades

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(Nriagu, 1996; Charlesworth et al., 2011). Because of their toxicity, accumulative and non-biodegradable nature, heavy metals are potentially hazardous to terrestrial and aquatic ecosystems, and thus to human and animal life (Tchounwou et al., 2012). Heavy metals are present in the environment as a result of natural sources and human activities (He et al., 2005; Li et al., 2009). In natural systems, heavy metals originate from rocks, ore minerals, volcanoes, and release of metals during weathering leading to soil formation (Szyzewski et al., 2009). On the other hand, anthropogenic causes of heavy metals are mostly related to urban development, generation of electricity, and the metal industry including mining, extraction, and refining processes (Alloway, 1995; Kabata-Pendias and Mukherjee, 2007; Norgate et al., 2007). Heavy metals are generally considered as inhibitors of life processes, although their inhibiting potential depends on several factors such as levels present, ability to form complexes, and degree of oxidation (Lin and Zhang, 1990; Szyzewski et al., 2009). Heavy metals fall within two basic categories: essential and non-essential. Essential metals or micronutrients, such as Cr, Co, Cu, Mn, Mo, Fe, Se and Zn, are necessary for the optimal functioning of biological and biochemical processes in organisms (including humans) that include redox reactions and formation of pigments and enzymes (Babula et al., 2008). In turn, non-essential metals, such as As (metalloid, strictly speaking), Cd, Hg, and Pb, have no known biological function and exert their toxicity by competing with essential elements for active enzyme or membrane protein sites (Torres et al., 2008). However, essential metals may also have detrimental effects to species and whole ecosystems when these are exposed to high levels (Nagajyoti et al., 2010).

Aquatic ecosystems, such as wetlands and coastal waters, are particularly vulnerable to heavy metal inputs (Mitsch and Gosselink, 2007; Halpern et al., 2008). The risks of heavy metal pollution are of great concern for the ecosystem services affected (Verhoeven et al., 2006), and also difficult to assess because of the elements complex behavior and interactions in aquatic ecosystems (Guilizzoni, 1991; Greger, 2004). Unlike most organic pollutants, indeed, heavy metals are typically not removed from aquatic ecosystems by natural processes (Bargagli, 1998). Once accumulated in bottom sediments, they begin to move up the food chain, often biomagnifying at higher trophic levels and ultimately causing potential disorders in humans and animals (Barwick and Maher, 2003; Roberts et al., 2008). Coastal ecosystems, in particular, are affected by a wide range of pollutants, among which heavy metals are particularly widespread and increasingly affecting marine habitats (Faganelli et al., 1997; Ralph et al., 2006; Boudouresque et al., 2009). Similarly, heavy metals may adversely affect the precarious stability of wetlands whose ecological importance for nutrient cycling and pollution control is widely recognized (Mitsch and Gosselink, 2007).

Plants have the ability to absorb all metals, especially those essential for their growth and development (Kabata-Pendias, 2011). Macrophytes, in particular, play a fundamental role in wetland geochemistry because they are the principal living accumulators of heavy metals through active and passive absorption (Vodyanitskii and Shoba, 2015). In terms of biomass, macrophytes are the predominant organisms in highly productive, littoral ecosystems, such as wetlands and shallow coastal areas (Brix and Schierup, 1989). Rooted macrophytes are also stationary and continuously exposed to contaminants such as metals (Jackson, 1998). Macrophytes, compared with other plant and animal species, have been reported to have a larger or similar capacity for metal accumulation (Jana, 1988; Albers and Camardese, 1993). Similarly, seagrasses have a high metal bioaccumulative capacity since they interact directly with both the water column (through the leaves) and the sediment pore water (through the roots), as both leaves and roots are sites of ionic uptake (Romero et al., 2006; Ralph et al., 2006). In particular, seagrasses contribute significantly to the primary production of aquatic ecosystems in the littoral zone, since they have a fundamental trophic role in aquatic ecosystems and an important link in the recycling of nutrients. Consequently, they can extract large amounts of metals from the environment (Kaldy, 2006).

Knowing patterns of metal levels in macrophytes, including seagrasses and wetland plants, and in sediments and soils, is important for ecological restoration, management and monitoring. In particular, knowing the relationship of a given plant species with specific heavy metals, may help implement tailored applications of ecological engineering aimed to regain the natural functions of impacted wetlands and coastal marine habitats. However, studies comparing the patterns of heavy metals between wetland and coastal marine vascular plants are generally lacking. For example, it is not clear yet whether wetland and marine ecosystems determine a different distribution of heavy metals in the respective primary producer species, and whether wetland and marine species have a similar capacity for heavy metals bio-monitoring. The potential of metal uptake largely varies with plant species, and carrying out a comparative analysis between wetland and marine plant species may help identify general and specific patterns in species that differ ecologically and morphologically.

The marine phanerogam *Posidonia oceanica* (L.) Delile is an endemic Mediterranean species that forms dense communities (meadows), with bathymetric range of 0–40 m depth, widely distributed throughout the Mediterranean of which occupies c. 3% of its surface (c. 35,000 km²) (IUCN, 2015). It is now well established that *P. oceanica* meadows hold a central position in the ecology of the Mediterranean being not only one of the most important contributors to coastal primary production but also acting as spawning areas, nurseries, and permanent habitats for numerous plant and animal species (Bay, 1984; Hemminga and Duarte, 2000). The marine phanerogam *Cymodocea nodosa* (Ucria) Asch., known as Lesser Neptune Grass, is a coastal seagrass of tropical origin, nowadays restricted to the Mediterranean Sea and some locations in the North Atlantic, from southern Portugal and Spain to Senegal, including the Canary Islands and Madeira (Green and Short, 2003; OSPAR, 2010). Generally, it forms mono-specific meadows, and can be found in deep waters (40 m) (Mazzella et al., 1993). *C. nodosa* is considered a pioneer species that can quickly colonize bare areas of the sea floor, with its rhizomes growing several meters per years (Duarte and Sand-Jensen, 1990; Borum and Greve, 2004).

Phragmites australis (Cav.) Trin. ex Steud., *Arundo donax* L., and *Typha domingensis* Pers., are worldwide distributed emergent and partially submerged macrophytes. Such species are perennial herbaceous and rhizomatous plants that form dense monospecific stands in natural wetlands characterized by shallow and stagnant water, and muddy sediment (Pignatti, 1982). *P. australis* (common reed) is a large grass with stems up to 6 m, and can survive extreme environmental conditions, including high concentrations of toxic contaminants such as heavy metals (Batty and Younger, 2004; Bragato et al., 2009). *A. donax* (giant reed) is another perennial rhizomatous grass (Poaceae family), native to the freshwater regions of Eastern Asia but nowadays worldwide distributed (Quinn and Holt, 2008; Gordon et al., 2011). Able to reach the height of 8 m, *A. donax* is among the fastest growing terrestrial plants (Mirza et al., 2010). *T. domingensis* (southern cattail) is ecologically similar to *P. australis*, and can survive in highly contaminated sites (Maddison et al., 2009). *Apium nodiflorum* (L.) Lag. (fool's-watercress) and *Nasturtium officinale* W. T. Aiton (watercress) are two non-rhizomatous, herbaceous, and perennial plants, with a prostrate habitus (total height < 1 m), and preference for the stagnant waters of ponds, ditches and streams (Pignatti, 1982). Relatively few studies have focused on heavy metal concentrations in *A. nodiflorum* and *N. officinale* (Zurayk et al., 2001).

The main aim of the present work was to analyze the levels of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn in sediments, in two Mediterranean seagrasses *P. oceanica* and *C. nodosa*, and in five common wetland plant species, *P. australis*, *A. donax*, *T. domingensis*, *A. nodiflorum* and *N. officinale*. The present study also aimed to shed further light on the role of ecology, biomass, anatomy and life form in influencing the levels of heavy metals in wetland and marine plants, and to assess the bio-monitoring potential of the targeted species.

2. Materials and methods

2.1. Study sites

Sampling was carried out at eight different locations in Sicily (Italy), four of which were shallow water coastal sites used for seagrasses collection, and another four sites located inland and on coastal wetlands for macrophytes collection (Fig. 1; Table 1). Sampling sites had different levels of human impact, where heavy metals inputs were mainly due to untreated municipal wastewaters, farming and pollution from marine traffic. Specifically, San Vito Lo Capo and San Leonardo are mainly affected by human activities at the adjacent seaside resorts; Marina di Palma and Villarosa are affected by small human settlements, the latter site also receiving agricultural chemicals. Ancipa and Tellaro are each part of protected areas; similarly, anthropogenic impact is relatively negligible in Portopalo di Capopassero and Cefalù. The mean annual values of temperature and rainfall are quite variable in the study sites, ranging from 12 to 18 °C, and from 400 to 1000 mm (higher values in inland sites) respectively.

2.2. Sampling

Sampling was carried out bimonthly during 2014 and 2015. During sampling, environmental conditions were stable; specifically, days were sunny, not windy, without recent rains, with calm sea (in marine sites), and with regular water flow (in wetland sites). *P. oceanica* and *C. nodosa* meadows were relatively abundant in the study sites, and formed dense monospecific stands ranging in size between 5 m × 5 m to 20 m × 20 m down to a depth of 10 m within 100 m from the shore. Samples of the two seagrasses were collected on the same day at the same site. At each sampling site, a total of 20 sediment samples and 20 seagrass shoots were collected. Each batch of 20 samples was obtained by mixing subsamples. For seagrasses, 10 individual shoots were

collected manually at random within a subplot measuring 5 m × 5 m. In the same subplot, 10 samples of sediment were also collected at random. Sediment samples were collected from the top 5 cm of the upper layer using a Plexiglas corer with an internal diameter of 10 cm. The samples of seagrass and sediment for analyses were obtained by mixing the respective subsamples to obtain a representative composite sample. This procedure was repeated twenty times for each sample at each collection site (N = 20). After collection, plant individuals were carefully shaken to remove large attached particles, rinsed with distilled water to remove smaller sediment particles, and then dried lightly with a clean linen cloth to remove excess seawater. The 10 seagrass shoots from each subplot were sealed in a sterilized and airtight plastic bag. Sediment samples were transferred to sterilized 0.5 L polyethylene bottles.

Sampling of adlittoral macrophytes was carried out by randomly collecting 10 plant individuals per species from each sampling site. A sediment sample was also taken from the vicinity of the collected plant individual at a depth of 10–50 cm from within a circular area having a radius of around 0.5 m and with the collected plant located at its center. Macrophyte individuals were carefully removed to ensure collection of the entire rhizome/root system. To avoid contamination by metals, the macrophytes were rooted out with stainless steel tools. After collection, macrophyte individuals were delicately shaken to remove large soil particles, cleaned lightly with a linen cloth, and then placed in airtight plastic bags. Sediment samples from wetlands were placed in sterilized 1-L polyethylene bottles. All samples were transported in PVC containers at a temperature of 4 ± 1 °C, and taken to the laboratory on the same day of collection.

2.3. Chemical analysis

In the laboratory, the plant samples were first washed under running tap water to remove large particles, and then rinsed with bidistilled

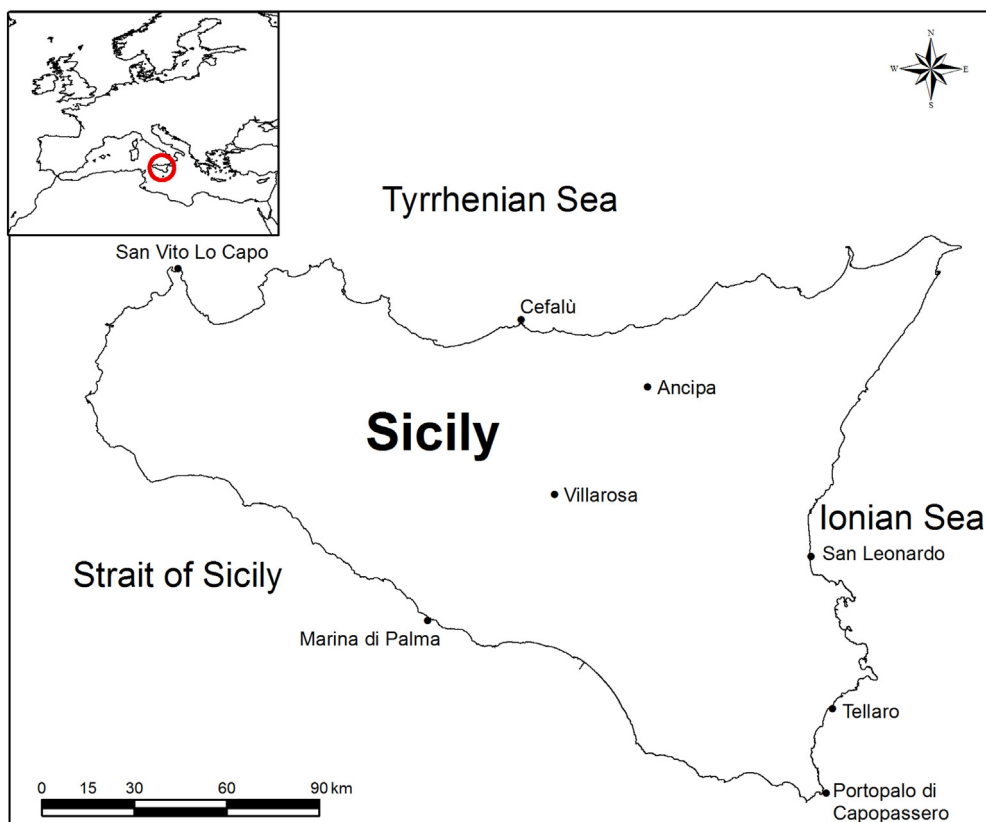


Fig. 1. Location of sampling sites.

Table 1
Sampling sites, level of impact, geographical coordinates, and collected species (X).

Study site	Impact	Coordinates	P.o.	C.n.	P.a.	A.d.	T.d.	A.n.	N.o.
Portopalo di Capopassero	Low	36°40'5.09"N 15°7'58.54"E	X	X	–	–	–	–	–
Marina di Palma	Moderate	37°10'10.33"N 13°43'33.01"E	X	X	–	–	–	–	–
San Vito Lo Capo	High	38°10'39.55"N 12°44'45.38"E	X	X	–	–	–	–	–
Cefalù	Low	38°1'36.95"N 14°3'0.24"E	X	X	–	–	–	–	–
Ancipa	Low	37°50'18.91"N 14°33'37.04"E	–	–	X	X	X	X	X
Villarosa	Moderate	37°35'00.83"N 14°12'11.40"E	–	–	X	X	X	X	X
San Leonardo	High	37°20'38.25"N 15°05'28.25"E	–	–	X	X	X	X	X
Tellaro	Low	36°50'17.94"N 15°06'05.44"E	–	–	X	X	X	X	X

Note: P.o. = *P. oceanica*; C.n. = *C. nodosa*; P.a. = *P. australis*; A.d. = *A. donax*; T.d. = *T. domingensis*; A.n. = *A. nodiflorum*, N.o. = *N. officinale*.

water to remove any remaining fine residual material. Seagrasses were dissected into roots, rhizomes and leaves; rhizomatous macrophytes into roots, rhizomes, stems and leaves; and non-rhizomatous macrophytes into roots, stems and leaves. Given the lower levels of element concentrations present in the inferior parts of seagrass leaves (Di Leo et al., 2013), the basal part of leaf samples of *P. oceanica* and *C. nodosa* (bottom 5 cm) was removed to reduce the distortion during the analysis of heavy metal levels. Plant organs were cut off using a stainless steel scissors, and kept at 2 °C until analysis. Plant organs and sediment were then dried to constant weight at room temperature to avoid potential undesirable effects of higher temperatures (>60 °C) on the analytical results for some of the heavy metals. Once dry, plant samples were ground and homogenized in an agate mortar, while the sediment samples were passed through a 1 mm diameter sieve. The plant and sediment samples were then weighed to the nearest 0.1 ± 0.05 g, and oven-digested at 90 °C overnight (microwave oven Mars 6, CEM Corporation) in an acid solution (H₂O₂/HNO₃, 2:3 ratio; Carlo Erba). After digestion, the plant and sediment samples were diluted with ultrapure Milli-Q water to a final volume of 25 mL, and analyzed using ICP-MS (Cd, Cr, Cu, Mn, Ni, Pb, Zn), and FAAS (As and Hg) (respectively using a PerkinElmer Elan® 6000 and PerkinElmer® AAnalyst™ 400 AA Spectrometer). Rhodium (Rh) was used as internal standard. Quality control was performed through stability of instrumental recalibration, and using analytical blanks. The instruments were regularly checked against low level standards (once every five samples), and recalibrated either when signs of drift were noted or after every 10 samples. The standard reference materials *Ulva lactuca* (B.C.R. reference material No. 279/504) and *Virginia Tobacco leaves* (CTA-VTL-2) were analyzed in order to assess the validity and accuracy of the analytical procedures. Student's *t*-test ($\alpha = 0.05$) was performed to ascertain good agreement between experimental and certified values. The percent recovery was within 15% of the certified values and ranged between 90 and 105% (Table 2). All the analyses were carried out in three replicates, and instrument detection limits were expressed as three times the standard deviation from the mean blank.

Table 2
Analysis of certified reference materials (mean values ± 95% confidence interval).

Elements	<i>Ulva lactuca</i> (BCR-279) (mg kg ⁻¹)			<i>Virginia tobacco leaves</i> (CTA-VTL-2) (µg g ⁻¹)		
	Certified	Experimental	Recovery (%)	Certified	Experimental	Recovery (%)
As	3.09 ± 0.20	3.02 ± 0.25	97.7	0.97 ± 0.07	0.90 ± 0.08	92.8
Cd	0.27 ± 0.02	0.25 ± 0.02	92.6	1.52 ± 0.17	1.45 ± 0.21	95.4
Cr	10.7 ± 0.90 ^a	10.1 ± 1.12	94.4	1.87 ± 0.16	1.96 ± 0.19	105
Cu	13.1 ± 0.37	13.5 ± 0.44	103	18.2 ± 0.9	17.3 ± 1.15	95.1
Hg	0.05 ± 0.003 ^a	0.05 ± 0.003	99.2	0.05 ± 0.01	0.05 ± 0.01	99.3
Mn	2090 ± 35.6 ^a	1890 ± 31.4	90.4	79.7 ± 2.60	81.2 ± 3.67	102
Ni	15.9 ± 0.40 ^a	14.8 ± 0.64	93.1	1.98 ± 0.21	1.85 ± 0.32	93.4
Pb	13.5 ± 0.40	12.7 ± 0.56	94.1	22.1 ± 1.20	20.3 ± 1.41	91.8
Zn	51.3 ± 1.20	53.5 ± 1.46	104	43.3 ± 2.10	40.1 ± 3.07	92.6

^a Indicative values.

2.4. Statistical processing

Following analyses, values were determined for bioconcentration and translocation factors to assess element mobility in the study species. The values obtained were based on the following:

$$\text{Bioconcentration Factor (BCF)} = C_{\text{root}}/C_{\text{sediment}}$$

where C_{sediment} and C_{root} are respectively levels (mg kg⁻¹ DW) of a specific element in sediment and roots of the study species. BCF expresses the efficiency of a plant species to take up from the sediment and accumulate a specific element in its tissues. Higher BCF values imply a greater bioaccumulation capability (EPA, 2007).

Translocation factors (TF):

$$C_{\text{rhizome}}/C_{\text{root}}$$

$$C_{\text{stem}}/C_{\text{rhizome}}$$

$$C_{\text{leaf}}/C_{\text{rhizome}}$$

$$C_{\text{stem}}/C_{\text{root}}$$

$$C_{\text{leaf}}/C_{\text{stem}}$$

$$C_{\text{leaf}}/C_{\text{root}}$$

where C_{root} , C_{rhizome} , C_{stem} and C_{leaf} are, respectively, levels (mg kg⁻¹ DW) of a given element in roots, rhizomes, stems and leaves of the study species. Plant species that do not have rhizomes or stems were not analyzed for levels of heavy metals in these organs. TF expresses the mobility of a given element within the plant species, where higher TF values result in a greater translocation capability (Deng et al., 2004).

All data sets were checked for normality and variance homogeneity prior to the statistical analyses, using Shapiro-Wilk and Levene tests respectively. In case of non-acceptance of normality and variance homogeneity, data were normalized through log-transformation. A one-way ANOVA was used to test for significant differences in levels of heavy metals between sediments in the different study sites, between different plant organs of the same species, and between the same organs of different species. Student's *t*-test was used to detect possible correlations between levels of the elements in sediments and organs. To

Table 3
Levels (mean values \pm SD) of metals in sediments [mg kg^{-1}].

	Study sites							
	Portopalo di Capopassero	Marina di Palma	San Vito Lo Capo	Cefalù	Ancipa	Villarosa	San Leonardo	Tellaro
As	1.02 \pm 0.10 ^a	3.13 \pm 0.36 ^b	4.02 \pm 0.55 ^b	2.07 \pm 0.32 ^c	0.95 \pm 0.15 ^a	2.55 \pm 0.35 ^c	6.43 \pm 0.92 ^d	0.56 \pm 0.06 ^e
Cd	0.25 \pm 0.03 ^{a,d}	0.41 \pm 0.05 ^b	0.50 \pm 0.06 ^b	0.15 \pm 0.02 ^c	0.22 \pm 0.03 ^a	0.32 \pm 0.04 ^d	0.85 \pm 0.09 ^e	0.21 \pm 0.02 ^a
Cr	6.13 \pm 0.73 ^a	7.23 \pm 0.95 ^{a,d}	17.4 \pm 2.95 ^b	7.22 \pm 0.85 ^{a,d}	5.15 \pm 0.72 ^c	8.15 \pm 1.12 ^{a,d}	20.6 \pm 4.55 ^e	5.21 \pm 0.62 ^c
Cu	9.38 \pm 1.21 ^a	8.77 \pm 1.05 ^a	25.3 \pm 5.21 ^b	15.3 \pm 3.49 ^c	6.35 \pm 0.89 ^d	16.4 \pm 2.96 ^c	34.6 \pm 7.23 ^e	5.34 \pm 0.79 ^f
Hg	0.05 \pm 0.01 ^a	0.06 \pm 0.01 ^a	0.10 \pm 0.01 ^b	0.06 \pm 0.01 ^a	0.03 \pm 0.01 ^c	0.05 \pm 0.01 ^a	0.06 \pm 0.01 ^a	0.04 \pm 0.01 ^{a,c}
Mn	780 \pm 95.5 ^a	868 \pm 110 ^{a,b}	846 \pm 95.2 ^{a,b}	924 \pm 120 ^{b,c}	742 \pm 85.7 ^a	896 \pm 135 ^{b,c}	989 \pm 117 ^c	802 \pm 75.2 ^a
Ni	4.65 \pm 0.42 ^a	8.12 \pm 1.01 ^b	13.5 \pm 2.51 ^c	8.78 \pm 1.12 ^b	3.32 \pm 0.43 ^d	6.13 \pm 0.96 ^a	18.5 \pm 4.31 ^e	4.02 \pm 0.65 ^a
Pb	2.56 \pm 0.33 ^a	4.25 \pm 0.65 ^b	11.5 \pm 1.57 ^c	5.56 \pm 0.73 ^b	1.05 \pm 0.21 ^d	2.21 \pm 0.36 ^a	17.2 \pm 4.58 ^e	1.85 \pm 0.41 ^a
Zn	4.46 \pm 0.54 ^a	5.49 \pm 0.61 ^a	23.4 \pm 5.32 ^b	4.54 \pm 0.56 ^a	4.19 \pm 0.32 ^a	8.42 \pm 1.33 ^c	38.6 \pm 8.36 ^d	3.32 \pm 0.44 ^e

Note: The superscript different letters indicate a significant difference in levels of the particular metal between different study sites (one-way ANOVA, $p < 0.05$).

identify the source of significant differences between sample pairs, Tukey post hoc tests were carried out. When performing multiple significance tests, the possibility of a Type I error increases. Therefore, the value of significance α , which was initially set at 0.05, was adjusted according to the Bonferroni formula $\alpha_B = \alpha/k$, where α_B is the adjusted level of α , and k is the number of comparisons. Statistical analyses were carried out using IBM SPSS Version 22.0.

3. Results

Levels of heavy metals in sediments were significantly different across study sites (Table 3). High levels of Cr, Cu, Ni, Pb and Zn were recorded from sites under the influence of anthropogenic disturbance. Mean levels of Hg and Mn in sediments were 0.06 and 850 mg kg^{-1} respectively, while those for the other heavy metals were within the range of 0.50–15 mg kg^{-1} . The results for uptake of heavy metals from sediments to plants (BCF) and for translocation within plants (TF) indicated significant differences in levels between different species and for different elements (Tables 4, 5, 6, 7, 8). Mean values of BCF ranged from 0.13 (Mn) to 4.86 (Hg). In particular, Hg was the element for which values of BCF varied most, between 0.39 (in *A. nodiflorum*) and 13.7 (in *T. domingensis*) (Table 4). BCF values varied greatly with species, the minimum value (0.10) in *A. nodiflorum* and the maximum (2.31) in *P. australis*. On the other hand, values of mobility in the rhizosphere ($\text{TF}_{\text{rhizome/root}}$) were within a relatively narrow range with regard to both the specific heavy metal (minimum 0.43 in As, maximum 0.65 in Cu) and species (minimum 0.40 in *C. nodosa*, maximum 0.68 in *T. domingensis*) (Table 5). Values of heavy metal translocation from rhizome to leaf were highest for Ni (1.80) and lowest for Hg (0.61) (Table 5). Values of $\text{TF}_{\text{leaf/rhizome}}$ for the different species were highest for *C. nodosa* (2.49) and lowest for *T. domingensis* (0.34) (Table 5). Values for heavy metal stem/root translocation varied appreciably for the different elements (minimum of 0.22 for As and a maximum of 0.61 for Zn) and between species (minimum of 0.15 in *A. donax* and a maximum of 0.52 in *T. domingensis*) (Table 6). Values of leaf/stem translocation of heavy metals were within the range 1.06–1.58 (Table 6), except for Mn for which the value was much higher (2.51).

Table 4
Values of bioconcentration factor (BCF) ($C_{\text{root}}/C_{\text{sediment}}$).

	<i>P. oceanica</i>	<i>C. nodosa</i>	<i>P. australis</i>	<i>T. domingensis</i>	<i>A. donax</i>	<i>N. officinale</i>	<i>A. nodiflorum</i>	Mean
As	0.72	2.12	1.22	0.91	0.10	0.06	0.08	0.74
Cd	5.21	1.89	3.27	1.45	0.27	0.19	0.23	1.72
Cr	0.12	0.23	0.28	0.38	0.10	0.04	0.02	0.16
Cu	4.15	2.96	0.35	0.27	0.18	0.05	0.05	1.12
Hg	5.25	2.87	12.4	13.7	0.89	0.46	0.39	4.86
Mn	0.15	0.10	0.48	0.20	0.01	0.02	0.03	0.13
Ni	0.52	0.28	0.39	2.21	0.17	0.04	0.04	0.48
Pb	0.71	0.84	0.26	0.30	0.06	0.05	0.03	0.30
Zn	2.78	2.03	1.62	1.12	0.05	0.05	0.04	1.06
Mean	2.14	1.44	2.31	2.22	0.19	0.11	0.10	

The values of $\text{TF}_{\text{leaf/stem}}$ were more variable between species, with a maximum value of 2.62 and a minimum of 0.67 recorded respectively for *P. australis* and *T. domingensis* (Table 6). Values of leaf/root translocation of heavy metals were comparable with respect to both elements (minimum of 0.29 for Pb and maximum of 0.77 for Zn) and species (minimum of 0.24 in *T. domingensis* and maximum of 1.04 in *P. oceanica*) (Table 7). Similar values were recorded for stem/rhizome translocation with respect to both heavy metals (minimum of 0.12 for Pb and maximum of 0.67 for Hg) and species (minimum 0.27 in *A. donax* and maximum 0.69 in *T. domingensis*) (Table 8).

Values of heavy metals recorded in the different plant organs for the different study species are shown in Table 9. Results show that the levels of heavy metals recorded from the different plant species decreased significantly in the following order (ANOVA Results also indicated the following trend of decrease in levels of heavy metals in the different organs recorded for the different plant species (ANOVA $p < 0.05$):

- root > rhizome > leaf > stem in *P. australis* and *A. donax*;
- root > rhizome > stem > leaf in *T. domingensis*;
- root > leaf > rhizome in *P. oceanica* and *C. nodosa*;
- root > leaf > stem in *N. officinale* and *A. nodiflorum*.

Overall, the seven plant species reflected the levels of heavy metals present in sediments (Table 9). However, the bioindicator potential was more prominent in *P. australis* and *T. domingensis*, followed by *P. oceanica* and *C. nodosa*. On the other hand, the bioindicator potential of *N. officinale* and *A. nodiflorum* was restricted to Cu, Mn and Zn in sediments, and that of *A. donax* to Cr and Ni.

4. Discussion

The present findings indicate that *P. oceanica* is more efficient in accumulating metals from sediments, and the rate of internal translocation of the elements also appears to be higher, compared to *C. nodosa*. Translocation of elements from sediment to roots and within plant tissues is related to numerous factors including pH,

Table 5
Values of rhizome/root and leaf/rhizome translocation factors.

	$C_{\text{rhizome}}/C_{\text{root}}$						$C_{\text{leaf}}/C_{\text{rhizome}}$					
	<i>P.o.</i>	<i>C.n.</i>	<i>P.a.</i>	<i>T.d.</i>	<i>A.d.</i>	Mean	<i>P.o.</i>	<i>C.n.</i>	<i>P.a.</i>	<i>T.d.</i>	<i>A.d.</i>	Mean
As	0.75	0.12	0.53	0.42	0.35	0.43	0.75	4.89	0.32	0.26	0.53	1.33
Cd	0.65	0.34	0.88	0.67	0.43	0.55	2.18	4.04	0.90	0.22	0.36	1.52
Cr	0.74	0.27	0.48	0.61	0.51	0.48	0.81	2.55	0.65	0.41	0.82	1.03
Cu	0.51	1.14	0.50	0.77	0.30	0.65	1.17	1.10	1.21	0.35	0.73	0.89
Hg	0.68	0.62	0.49	0.62	0.41	0.51	1.03	0.78	0.57	0.43	0.31	0.61
Mn	0.57	0.63	0.21	0.51	0.59	0.52	1.27	0.92	3.15	0.51	0.68	1.29
Ni	0.68	0.28	0.36	0.80	0.76	0.56	3.87	3.43	0.72	0.36	0.72	1.80
Pb	0.74	0.19	0.72	0.55	0.19	0.45	0.70	3.07	0.24	0.16	0.34	0.89
Zn	0.50	0.45	0.31	0.90	0.65	0.58	2.45	2.11	1.06	0.26	0.60	1.27
Mean	0.63	0.40	0.48	0.68	0.46		1.56	2.49	0.97	0.34	0.54	

Note: *P.o.* = *P. oceanica*; *C.n.* = *C. nodosa*; *P.a.* = *P. australis*; *T.d.* = *T. domingensis*; *A.d.* = *A. donax*; *A. nodiflorum* and *N. officinale* were not included because not rhizomatous species.

reduction potential, temperature, salinity, organic matter content and levels of other elements present (Greger, 1999; Yang and Ye, 2009). Similarly, other factors such as seasonal variation in physiology and compartmentalization potential, may also contribute to bioaccumulation and internal translocation capacities (Bargagli, 1998; Obarska-Pempkowiak et al., 2005). However, *P. oceanica* and *C. nodosa* showed similar values for some of the estimates; namely BCF and $TF_{\text{leaf/rhizome}}$ which were <1, and $TF_{\text{rhizome/root}}$ and $TF_{\text{leaf/root}}$ which were around 0.5 and 1.0 respectively (Tables 4, 5, 7). Overall, *P. oceanica* had higher levels of heavy metals in its tissues compared to *C. nodosa* (Table 9). In terms of potential as bioindicators, *P. oceanica* and *C. nodosa* were both good bioindicators of trace element pollution in marine sediments. Positive correlations were recorded between levels of heavy metals in the sediment and seagrass tissue, except for Cr and Hg, and partly for As and Pb (Student *t*-test). In particular, roots were the organs that correlated most with levels of heavy metals in the sediment. Therefore, the choice of the organ to be used is important when establishing a biomonitoring campaign using seagrasses. Roots are more suitable for long-term monitoring periods, whereas leaves, given their periodical regeneration, should be considered for short-term periods of 6–12 months (Llagostera et al., 2011).

Levels of heavy metals in the different seagrasses organs differed according to the general trend: root > leaf > rhizome and, to a lesser extent, leaf > root > rhizome (ANOVA, Table 9). These results show that rhizomes are generally the organs that have lower levels, while roots and leaves have, alternately, the highest levels of heavy metals. This observed alternation of leaves and roots serving as main bioaccumulator organs of heavy metals suggests that *P. oceanica* and *C. nodosa* may adopt two possible tolerance strategies: a compartmentalization strategy that leads to accumulation of the bulk of elements in the roots, and a removal strategy that favors accumulation of elements in temporary organs such as the leaves. The compartmentalization strategy is common in wetland species, and basically is aimed at storing the highest levels of heavy metals in the underground organs (e.g. roots and rhizomes) as a

defensive mechanism to protect the species against the harmful effects of toxic levels for the photosynthetic processes (e.g., Weis et al., 2004; Gratao et al., 2005; Willis et al., 2010; Phillips et al., 2015). Such a strategy may result in differences in heavy metal levels between wetland and marine species, with the former plants generally showing the trend: root > rhizome > leaf, and the latter: root > leaf > rhizome and sometimes leaf > root > rhizome, as noted in the present study. The results obtained also indicate the different role of rhizomes as important bioaccumulators in wetland species, and as organs supporting transient high levels of heavy metals in seagrasses *P. oceanica* and *C. nodosa*. These findings are in agreement with those from previous studies, which indicated lower levels of heavy metals in the rhizomes of *P. oceanica* and *C. nodosa* (Lewis and Devereux, 2009; Malea and Kevrekidis, 2013). Another possible tolerance strategy, which we refer to as 'removal strategy', is based on accumulation of heavy metals in the leaves of *P. oceanica* and *C. nodosa*, which are periodically lost and regenerated with high turnover (e.g. 15–60 weeks in *P. oceanica*; Wittmann, 1984). The finding from the present study of a possible removal strategy for Cd and Ni by *Posidonia* is noteworthy. Specifically, *P. oceanica* may adopt internal detoxification mechanisms that allow the accumulation of high Ni levels, e.g. through Ni complexation with organic acids in the cell vacuoles (Marschner, 1995). According to various authors (e.g. Malea and Haritonidis, 1999; Llagostera et al., 2011), this removal strategy may be related to active mobilization of toxic metals from roots to leaves, thus facilitating element loss during periodical leaf regeneration in seagrasses.

The present findings indicate that compartmentalization of heavy metals in plant organs appears to be widespread in marine and wetland species, with higher levels of the elements generally accumulated in roots (Table 9). In particular, wetland macrophytes may accumulate higher levels of heavy metals in the rhizosphere as a consequence of the higher internal detoxification capacity of their below-ground organs. Previous studies reported that roots and rhizomes can store higher levels of heavy metals as a result of the large intercellular air spaces that

Table 6
Values of stem/root and leaf/stem translocation factors.

	$C_{\text{stem}}/C_{\text{root}}$						$C_{\text{leaf}}/C_{\text{stem}}$					
	<i>P.a.</i>	<i>T.d.</i>	<i>A.d.</i>	<i>N.o.</i>	<i>A.n.</i>	Mean	<i>P.a.</i>	<i>T.d.</i>	<i>A.d.</i>	<i>N.o.</i>	<i>A.n.</i>	Mean
As	0.05	0.16	0.11	0.53	0.34	0.22	2.13	0.56	2.29	1.25	1.70	1.55
Cd	0.62	0.33	0.08	0.42	0.56	0.41	1.49	0.53	1.91	1.68	1.39	1.36
Cr	0.10	0.62	0.25	0.27	0.21	0.27	2.31	0.38	1.55	0.78	1.05	1.20
Cu	0.19	0.70	0.12	0.79	0.78	0.53	2.86	0.40	1.80	1.21	1.10	1.42
Hg	0.37	0.88	0.08	0.31	0.29	0.37	0.80	0.32	1.93	1.04	1.01	1.06
Mn	0.05	0.37	0.15	0.41	0.39	0.29	6.65	0.74	2.90	1.20	0.75	2.51
Ni	0.18	0.86	0.26	0.25	0.28	0.35	2.32	0.42	2.58	1.18	1.17	1.52
Pb	0.12	0.04	0.11	0.39	0.50	0.22	1.84	2.54	1.64	1.10	0.84	1.58
Zn	0.15	0.94	0.28	0.80	0.82	0.61	2.93	0.30	1.58	1.15	1.12	1.44
Mean	0.20	0.52	0.15	0.44	0.47		2.62	0.67	2.08	1.15	1.11	

Note: *P.a.* = *P. australis*; *T.d.* = *T. domingensis*; *A.d.* = *A. donax*; *N.o.* = *N. officinale*; *A.n.* = *A. nodiflorum*; *P. oceanica* and *C. nodosa* were not included because their stems were not analyzed.

Table 7
Values of leaf/root translocation factor (C_{leaf}/C_{root}).

	<i>P. oceanica</i>	<i>C. nodosa</i>	<i>P. australis</i>	<i>T. domingensis</i>	<i>A. donax</i>	<i>N. officinale</i>	<i>A. nodiflorum</i>	Mean
As	0.63	0.55	0.12	0.10	0.27	0.71	0.62	0.41
Cd	1.34	1.08	0.85	0.17	0.19	0.75	0.80	0.72
Cr	0.69	0.72	0.31	0.27	0.43	0.19	0.26	0.42
Cu	0.54	1.11	0.53	0.29	0.23	0.81	0.86	0.64
Hg	0.71	0.51	0.32	0.24	0.17	0.31	0.30	0.35
Mn	0.84	0.72	0.61	0.32	0.44	0.42	0.35	0.55
Ni	3.03	0.75	0.27	0.33	0.67	0.21	0.25	0.76
Pb	0.50	0.43	0.17	0.05	0.07	0.43	0.48	0.29
Zn	1.27	1.13	0.38	0.25	0.47	0.85	0.90	0.77
Mean	1.04	0.75	0.42	0.24	0.31	0.51	0.55	

characterize their cortex parenchyma (e.g., Sawidis et al., 1995). Root cell walls may also prove suitable sites of accumulation of the elements, thus leading to higher levels of heavy metals accumulated in roots compared to other organs (Mishra et al., 2008). Compartmentalization itself should be considered as a tolerance strategy whereby plant species can reduce translocation of heavy metals from roots to shoots without causing any toxic effect to the photosynthetic tissue. Metal tolerance is mainly a function of plant phenology, vigor, and growth, as well as being influenced by metal speciation and water chemistry (Deng et al., 2004; Madejón et al., 2007; Yang and Ye, 2009). Some species have also been found to develop tolerant ecotypes that are either able to withstand higher levels of heavy metals accumulated in their tissues, or have developed efficient mechanisms to exclude metal ions from their tissue (Dunbabin and Bowmer, 1992). Overall, roots are the main pathway of heavy metal uptake to plants, and consequently rooted species tend to reflect the levels of the elements in sediments (Obarska-Pempkowiak and Klimkowska, 1999; Kumar et al., 2006; Mucha et al., 2008).

The internal mobility of heavy metals in wetland plants appears to be variable, and depends on organ and species (Tables 4–8). The lowest mobility recorded from the present study is for element translocation from the roots to the higher organs (stems and leaves), which is in line with the general tendency of rooted macrophytes to accumulate the bulk of trace elements in their roots. The important role of below-ground organs as bioaccumulators of heavy metals also supports the general notion that wetland plant species are very useful for phytostabilization. The present results indicate that the wetland species *P. australis* and *T. domingensis* are the best performers in terms of heavy metal phytostabilization compared to the seagrasses *P. oceanica* and *C. nodosa*, which in turn appear to have a higher potential for phytoextraction given their recorded higher internal mobility of elements.

The present results for heavy metal levels in the plant species studied indicate that, overall, seagrasses and wetland species have comparable values (Table 9). In particular, similar patterns in levels of heavy metals were noted among *P. oceanica*, *C. nodosa*, *P. australis* and *T. domingensis*. The present findings on levels of As in seagrasses corroborate the results from previous studies which indicate the potential of *P. oceanica* and *C. nodosa* to accumulate high levels of As (Gosselin et al., 2006). Levels of As in *P. australis* and *A. donax* are similar to those in seagrasses but were lower in *A. donax*, *N. officinale* and *A. nodiflorum*. Previous studies have reported that in highly As-contaminated sites, macrophytes can accumulate high levels of As without showing symptoms of toxicity (Mirza et al., 2010). In the present study, levels of heavy metals in organs of *P. australis* (all four organs), *T. domingensis* (roots and rhizome), *P. oceanica* (roots) and *C. nodosa* (roots) reflected well levels of As in the sediment, as has been also reported in other studies (e.g. Gosselin et al., 2006; Bonanno, 2013). The use of macrophytes as indicators of levels of As in sediments is important for preventing and monitoring environmental pollution given that it is a non-essential metalloid and considered one of the most toxic elements due to its persistence in the environment and tendency

to bioaccumulate (Kapaj et al., 2006). Similarly, Cd, Cr, Hg, Ni and Pb are non-essential metals, known for being highly toxic since they affect the growth, metabolism and physiology of plants (Kabata-Pendias, 2011). Our findings indicate that levels of these heavy metals in marine and wetland species are in general similar to those recorded from previous studies (e.g., Conti et al., 2007; Lewis and Devereux, 2009; Llagostera et al., 2011; Bonanno, 2013; Bonanno and Di Martino, 2016). Findings from the present study also confirm the usefulness of the studied macrophytes as bioindicators of heavy metal levels in the environment, which is also in agreement with the results from other works (e.g., Lafabrie et al., 2007; Bonanno and Lo Giudice, 2010; Malea and Kevrekidis, 2013). However, the present findings indicate that the bioindicator potential varies between different species, such that no clear trend among the studied species or between marine and wetland plants was identified (Table 9). Specifically, a relationship between levels of heavy metals (Cd, Cr, Hg, Ni, and Pb) in plants and sediment was identified for *P. australis* and *T. domingensis* but not for *N. officinale* and *A. nodiflorum*, while levels of Cr, Ni, and partly Pb (roots only) in *A. donax* were significantly correlated with levels in the substratum. Levels of Cr and Hg in *P. oceanica* and *C. nodosa* were not related with levels of these two elements in the sediment; in the case of *C. nodosa*, lack of relationship was also not evident for Pb. These results are generally in line with those of other studies which show that seagrasses may significantly reduce accumulation of some toxic elements such as Cr at highly polluted sites (Nicolaidou and Nott, 1998; Lafabrie et al., 2007). The adoption of exclusion mechanisms for specific heavy metals by seagrasses may affect the bioindicator potential of such macrophytes; hence usefulness of *P. oceanica* and *C. nodosa* as bioindicators of Cr, Hg and Pb should be further assessed. Similarly, when levels of Cd are high in sediments, several plant species tend to adopt exclusion mechanisms that may affect their bioindicator potential (Ralph and Burchett, 1998). However, results from the present study showed that levels of Cd in *C. nodosa* (in all organs) and *P. oceanica* (mainly in roots) reflected well levels of this element in sediments, as noted also by other scholars (Marín-Guirao et al., 2005). Similarly, *P. australis* and *T. domingensis* had high values of BCF for Cd; a finding

Table 8
Values of stem/rhizome translocation factor ($C_{stem}/C_{rhizome}$).

	<i>P. australis</i>	<i>T. domingensis</i>	<i>A. donax</i>	Mean
As	0.15	0.42	0.25	0.27
Cd	0.60	0.45	0.22	0.41
Cr	0.24	0.92	0.47	0.52
Cu	0.35	0.85	0.35	0.50
Hg	0.58	1.34	0.15	0.67
Mn	0.41	0.56	0.25	0.39
Ni	0.36	0.87	0.22	0.47
Pb	0.12	0.06	0.21	0.12
Zn	0.32	0.92	0.41	0.53
Mean	0.33	0.69	0.27	

Note: *P. oceanica* and *C. nodosa* were not included because their stems were not analyzed; *N. officinale* and *A. nodiflorum* were not included because they are not rhizomatous species.

Table 9
Levels (mean values \pm SD) of heavy metals [mg kg⁻¹] in root, rhizome and leaf compartments.

Species	Organs	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn
<i>P. oceanica</i>	root	3.44 \pm 0.45 ^{a,*1}	0.45 \pm 0.05 ^{a,*1}	2.56 \pm 0.32 ^{a1}	32.7 \pm 5.15 ^{a,*1}	0.37 \pm 0.05 ^{a1}	126 \pm 20.3 ^{a,*1}	8.56 \pm 1.45 ^{a,*1}	4.52 \pm 0.55 ^{a,*1}	63.3 \pm 8.12 ^{a,*1}
	rhizome	1.78 \pm 0.21 ^{b,1}	0.39 \pm 0.05 ^{a,1}	2.31 \pm 0.19 ^{a,1}	27.6 \pm 3.72 ^{a,*1}	0.25 \pm 0.04 ^{b,1}	79.3 \pm 11.5 ^{b,*1}	8.29 \pm 1.78 ^{a,*1}	1.83 \pm 0.24 ^{b,1}	51.5 \pm 6.68 ^{b,*1}
	leaf	1.99 \pm 0.25 ^{b,1}	0.86 \pm 0.11 ^{b,1}	2.68 \pm 0.25 ^{a,1}	18.7 \pm 2.46 ^{b,*1}	0.27 \pm 0.04 ^{b,1}	105 \pm 14.5 ^{c,*1}	19.3 \pm 3.52 ^{b,*1}	1.96 \pm 0.35 ^{b,1}	124 \pm 18.3 ^{c,*1}
<i>C. nodosa</i>	root	4.21 \pm 0.60 ^{a,*2}	0.35 \pm 0.04 ^{a,*2}	5.32 \pm 0.55 ^{a,2}	6.77 \pm 0.83 ^{a,*2}	0.19 \pm 0.03 ^{b,2}	94.6 \pm 13.2 ^{a,*2}	3.89 \pm 0.53 ^{a,*2}	4.37 \pm 0.52 ^{a,1}	50.4 \pm 7.12 ^{a,*2}
	rhizome	2.59 \pm 0.31 ^{b,2}	0.22 \pm 0.03 ^{b,*2}	2.41 \pm 0.26 ^{b,1}	3.41 \pm 0.40 ^{b,*2}	0.13 \pm 0.02 ^{b,2}	70.2 \pm 10.5 ^{b,*2}	2.78 \pm 0.41 ^{b,*2}	1.87 \pm 0.22 ^{b,1}	31.7 \pm 4.56 ^{b,*2}
	leaf	3.16 \pm 0.35 ^{b,2}	0.31 \pm 0.03 ^{a,*2}	3.67 \pm 0.45 ^{c,2}	5.89 \pm 0.61 ^{c,*2}	0.10 \pm 0.02 ^{c,2}	66.4 \pm 8.57 ^{c,*2}	3.05 \pm 0.34 ^{b,*2}	2.42 \pm 0.27 ^{c,2}	62.9 \pm 9.42 ^{c,*2}
<i>P. australis</i>	root	3.17 \pm 0.42 ^{a,*1}	1.36 \pm 0.18 ^{a,*3}	4.12 \pm 0.62 ^{a,*3}	18.2 \pm 3.04 ^{a,*3}	0.91 \pm 0.11 ^{a,*3}	558 \pm 84.3 ^{a,*3}	4.78 \pm 0.67 ^{a,*3}	7.78 \pm 1.35 ^{a,*2}	144 \pm 26.4 ^{a,*3}
	rhizome	1.21 \pm 0.24 ^{b,*3}	0.88 \pm 0.12 ^{b,*3}	2.36 \pm 0.44 ^{b,*1}	10.8 \pm 2.21 ^{b,*3}	0.74 \pm 0.09 ^{b,*3}	157 \pm 24.6 ^{b,*3}	3.89 \pm 0.56 ^{b,*3}	5.14 \pm 0.95 ^{b,*2}	60.7 \pm 8.31 ^{b,*3}
	stem	0.17 \pm 0.03 ^{c,*1}	0.57 \pm 0.07 ^{c,*1}	0.73 \pm 0.09 ^{c,*1}	4.75 \pm 0.65 ^{c,*1}	0.27 \pm 0.03 ^{c,*1}	44.5 \pm 7.23 ^{c,*1}	0.79 \pm 0.10 ^{c,*1}	0.35 \pm 0.06 ^{c,*1}	15.3 \pm 2.85 ^{c,*1}
<i>T. domingensis</i>	leaf	0.36 \pm 0.05 ^{d,*3}	0.81 \pm 0.11 ^{b,*1}	1.69 \pm 0.30 ^{d,*3}	11.3 \pm 2.55 ^{b,*3}	0.54 \pm 0.06 ^{d,*3}	336 \pm 56.2 ^{d,*3}	2.59 \pm 0.18 ^{d,*3}	1.25 \pm 0.21 ^{d,*3}	53.2 \pm 8.21 ^{b,*3}
	root	2.56 \pm 0.34 ^{a,*3}	1.12 \pm 0.21 ^{a,*4}	3.73 \pm 0.58 ^{a,*4}	16.3 \pm 3.52 ^{a,*3}	1.05 \pm 0.15 ^{a,*4}	162 \pm 23.4 ^{a,*4}	25.7 \pm 3.88 ^{a,*4}	5.67 \pm 0.75 ^{a,*3}	121 \pm 20.2 ^{a,*4}
	rhizome	1.06 \pm 0.02 ^{b,*3}	0.79 \pm 0.10 ^{b,*3}	1.87 \pm 0.31 ^{b,*2}	10.0 \pm 2.02 ^{b,*3}	0.89 \pm 0.10 ^{b,*4}	90.4 \pm 12.5 ^{b,*4}	17.6 \pm 2.46 ^{b,*4}	3.05 \pm 0.45 ^{b,*3}	105 \pm 16.6 ^{b,*4}
<i>A. donax</i>	stem	0.46 \pm 0.06 ^{c,2}	0.36 \pm 0.06 ^{c,2}	1.66 \pm 0.26 ^{b,*2}	12.5 \pm 2.39 ^{b,*2}	0.68 \pm 0.08 ^{b,*2}	59.8 \pm 8.78 ^{c,*2}	18.7 \pm 2.90 ^{b,2}	0.89 \pm 0.10 ^{c,2}	110 \pm 17.5 ^{b,*2}
	leaf	0.25 \pm 0.04 ^{d,4}	0.20 \pm 0.04 ^{d,3}	1.09 \pm 0.10 ^{c,*4}	5.68 \pm 1.05 ^{c,*2}	0.45 \pm 0.21 ^{c,4}	45.9 \pm 7.27 ^{d,*4}	8.05 \pm 1.13 ^{c,4}	0.73 \pm 0.09 ^{c,4}	57.8 \pm 8.15 ^{c,*3}
	root	0.24 \pm 0.04 ^{a,4}	0.11 \pm 0.02 ^{a,5}	1.23 \pm 0.21 ^{a,*5}	6.76 \pm 0.85 ^{a,*2}	0.33 \pm 0.07 ^{a,5}	18.2 \pm 3.43 ^{a,*5}	2.36 \pm 0.25 ^{a,*5}	1.04 \pm 0.15 ^{a,*4}	5.68 \pm 0.71 ^{a,*5}
<i>N. officinale</i>	rhizome	0.11 \pm 0.02 ^{b,4}	0.05 \pm 0.01 ^{b,4}	0.86 \pm 0.11 ^{b,*3}	4.01 \pm 0.55 ^{b,*4}	0.18 \pm 0.03 ^{b,5}	9.58 \pm 1.58 ^{b,*5}	1.65 \pm 0.19 ^{b,*5}	0.31 \pm 0.04 ^{b,4}	4.04 \pm 0.56 ^{b,*5}
	stem	0.03 \pm 0.01 ^{c,3}	0.01 \pm 0.002 ^{c,3}	0.18 \pm 0.03 ^{c,3}	0.96 \pm 1.46 ^{c,*3}	0.03 \pm 0.01 ^{c,3}	2.67 \pm 0.45 ^{c,*3}	0.33 \pm 0.05 ^{c,3}	0.07 \pm 0.01 ^{c,3}	1.32 \pm 0.26 ^{c,*3}
	leaf	0.07 \pm 0.02 ^{d,5}	0.02 \pm 0.004 ^{d,4}	0.66 \pm 0.09 ^{d,*5}	3.09 \pm 0.18 ^{d,*4}	0.06 \pm 0.01 ^{d,5}	7.14 \pm 0.95 ^{d,*5}	1.16 \pm 0.15 ^{d,*5}	0.14 \pm 0.02 ^{d,5}	3.48 \pm 0.34 ^{b,*4}
<i>A. nodiflorum</i>	root	0.18 \pm 0.03 ^{a,5}	0.09 \pm 0.01 ^{a,6}	0.12 \pm 0.03 ^{a,6}	2.52 \pm 0.36 ^{a,*4}	0.03 \pm 0.01 ^{a,6}	7.35 \pm 1.10 ^{a,*6}	0.74 \pm 0.01 ^{a,6}	0.34 \pm 0.05 ^{a,5}	6.75 \pm 0.98 ^{a,*6}
	stem	0.10 \pm 0.02 ^{b,4}	0.04 \pm 0.01 ^{b,4}	0.03 \pm 0.01 ^{b,4}	1.75 \pm 0.26 ^{b,*4}	0.01 \pm 0.002 ^{b,4}	4.24 \pm 0.62 ^{b,*4}	0.25 \pm 0.03 ^{b,4}	0.13 \pm 0.02 ^{b,4}	3.02 \pm 0.42 ^{b,*4}
	leaf	0.13 \pm 0.02 ^{c,6}	0.07 \pm 0.01 ^{c,5}	0.07 \pm 0.01 ^{c,6}	2.23 \pm 0.30 ^{a,*5}	0.01 \pm 0.002 ^{c,6}	6.75 \pm 0.85 ^{a,*6}	0.18 \pm 0.06 ^{c,6}	0.19 \pm 0.03 ^{c,6}	5.16 \pm 0.67 ^{c,*5}
<i>A. nodiflorum</i>	root	0.22 \pm 0.04 ^{a,6}	0.12 \pm 0.02 ^{a,7}	0.18 \pm 0.03 ^{a,7}	2.15 \pm 0.26 ^{a,*5}	0.03 \pm 0.01 ^{a,6}	6.56 \pm 0.76 ^{a,*6}	0.59 \pm 0.08 ^{a,7}	0.27 \pm 0.05 ^{a,6}	5.75 \pm 0.85 ^{a,*5}
	stem	0.08 \pm 0.02 ^{b,5}	0.07 \pm 0.01 ^{b,5}	0.05 \pm 0.01 ^{b,4}	1.23 \pm 0.18 ^{b,5}	0.01 \pm 0.002 ^{b,5}	3.86 \pm 0.45 ^{b,*4}	0.21 \pm 0.04 ^{b,4}	0.10 \pm 0.02 ^{b,4}	4.35 \pm 0.61 ^{b,*5}
	leaf	0.14 \pm 0.04 ^{c,6}	0.10 \pm 0.02 ^{c,6}	0.09 \pm 0.01 ^{c,6}	1.65 \pm 0.15 ^{c,6}	0.01 \pm 0.002 ^{c,6}	4.45 \pm 0.55 ^{b,*7}	0.12 \pm 0.01 ^{c,7}	0.07 \pm 0.01 ^{b,7}	4.10 \pm 0.52 ^{b,*6}

Note: Different letters mean significant differences between the various organs of the same species for one element (one-way ANOVA, $p < 0.05$); ** means significant correlation with sediments (t -test, $p < 0.05$); different numbers mean significant differences between the same organs of the various study species for one element (one-way ANOVA, $p < 0.05$).

that corroborates the results from other studies, which indicated significant tolerance to Cd by these same species (e.g., [Ederli et al., 2004](#); [Carranza-Álvarez et al., 2008](#)). Compared to wetland plants, seagrasses also showed a higher potential for accumulating Pb from sediments. For this element, the low bioindication potential was consistent for all the marine and wetland species studied, with the exception of *P. australis* (all four organs), and to a lesser extent, *T. domingensis* (roots and rhizomes), *A. donax* (roots), and *P. oceanica* (roots). In general, as already noted for several heavy metals, plant species usually limit accumulation of Pb by adopting exclusion mechanisms ([Sharma and Dubey, 2005](#)). *P. australis* and *T. domingensis* were the only species which showed the highest values of BCF for Hg and comparability of levels of this element with those present in the sediment, which suggests that these two macrophytes are not only useful as bioindicators of Hg but also for phytoremediation, as has been found in other studies (e.g. [Lominchar et al., 2015](#)).

In the case of Cu, Mn and Zn, the present results indicate that all study species reflected the levels of these elements in the sediment. This was expected given that Cu, Mn and Zn are important micronutrients for plant growth and metabolism ([Kabata-Pendias, 2011](#)), and are therefore retained within the plant tissue. However, this may result also in high bioaccumulated levels of Cu, Mn and Zn in some species ([Smillie, 2015](#)). Consequently, micronutrients may end up being more toxic at high levels compared to non-essential elements as a result of active uptake mechanisms for the former and tolerance strategies for the latter ([Ralph and Burchett, 1998](#)). *P. oceanica* and *C. nodosa* showed a higher bioaccumulation potential for Cu and Zn from the sediment, whereas *P. australis* and *T. domingensis* showed higher potential for Mn ([Table 4](#)). No trend for internal mobility of micronutrients was identified, with translocation rates being overall comparable between marine and wetland species. However, high levels of micronutrients in leaves were a common finding for the study species, and in some cases levels of the elements were higher in leaves compared to roots ([Table 9](#)). The observed high levels of Cu, Mn and Zn in plant tissue, particularly in the leaves, may be attributed to the important role of these micronutrients in photosynthesis ([Memon et al., 2001](#)). Overall, levels of micronutrients differed significantly among the different species, with the highest mean levels of Cu, Mn and Zn recorded respectively in *P. oceanica*, *P. australis*, and *T. domingensis*. Levels of Cu, Mn and Zn recorded in the present work are, overall, similar to those reported in other studies (e.g., [Gosselin et al., 2006](#); [Conti et al., 2007](#); [Bonanno, 2013](#)).

Overall, the present study showed that, for heavy metals, the bioaccumulator potential of marine and wetland macrophytes, uptake and translocation between organs, bioindicator potential and total levels present within the plant tissue, are species-specific. In particular, the present findings indicate that levels of heavy metals in the species studied are, overall, independent of ecological (coasts vs wetlands), biomass (large vs small herbaceous plants), anatomical (rhizomatous vs non rhizomatous plants), and life form (hemicryptophytes vs hydrophytes) characteristics. However, for the heavy metals considered in the present work, all study species shared some general common trends, such as high levels in their below-ground organs, similar translocation in the rhizosphere, compartmentalization in plant organs, and high levels and bioindicator potential for micronutrients (Cu, Mn, Zn). In particular, the present study identifies three sub-groups with highly similar trends for levels of heavy metal: (i) *P. oceanica* and *C. nodosa*; (ii) *P. australis* and *T. domingensis*; and (iii) *A. donax*, *N. officinale* and *A. nodiflorum*. Identification of these sub-groups would seem to indicate that species having similar ecological and morphological characteristics tend to possess similar mechanisms and process to deal with heavy metals in their organs and tissues, although this should not be generalized since related species may have totally different patterns of levels of heavy metals, as noted for *P. australis* and *T. domingensis* compared with *A. donax*. In turn, morphologically different species may show similar patterns of levels of heavy metals, as noted for *A. donax*, *N. officinale* and *A. nodiflorum*. Another noteworthy finding from the present study

is that plants, which have different ecological and morphological characteristics, may have a similar bioaccumulation potential for heavy metals from sediments, as noted for the seagrass *P. oceanica*, and the wetland species *P. australis* and *T. domingensis*. In general, uptake of elements by plants depends on numerous biotic and abiotic factors and interactions thereof ([Yang and Ye, 2009](#)). Despite the potentially numerous factors that affect levels of heavy metals in the environment, the present results suggest that the study species respond differently to metal inputs, and that this appears to be more dependent on the plant species specific ability to accumulate and detoxify the different elements, rather than on ecological and morpho-anatomical characteristics.

5. Conclusions

Shallow coastal areas and terrestrial wetlands support different ecosystems that share fundamental ecological roles but are both under the influence of increasing amounts of heavy metals in the environment. Investigating the accumulation and distribution of heavy metals in wetland and marine plant communities may reveal how primary producers respond to toxic element inputs, and whether these responses follow specific patterns. This has undoubtedly important implications for pollution control and monitoring, and for implementing ecological engineering projects such as phytoremediation. The present study showed that ecologically and morphologically different plant species share common patterns such as high heavy metal concentrations in roots and bioindication capacity of trace elements in sediments. However, this study also showed that patterns of levels of heavy metals cannot be generalized for different plant species as these respond differently and specifically to metal inputs, while such responses appear to be independent of ecology, biomass, anatomy, and life form.

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