Marine Pollution Bulletin xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Trace element compartmentation in the seagrass *Posidonia oceanica* and biomonitoring applications

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ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 19 December 2016
Accepted 31 December 2016
Available online xxxx

Keywords:
Posidonia oceanica
Trace elements
Marine pollution
Sediments
Sicily
Mediterranean Sea

ABSTRACT

This study investigated the trace element bioaccumulation capacity of the Mediterranean seagrass *Posidonia oceanica*, and its suitability as a bioindicator of contamination in water and sediments. Results showed that *P. oceanica* leaves accumulate higher concentrations of Ni and Zn. Since *P. oceanica* regenerates its leaves periodically, the higher concentrations in aerial organs may suggest a "removal" strategy according to which *P. oceanica* accumulates greater concentrations of trace elements in its temporary organs. In turn, *P. oceanica* seems to adopt an exclusion strategy for toxic non-essential elements (As, Cr, Pb). Results showed also that *P. oceanica* organs are correlated with As, Cd, Cu, Ni, and Zn concentrations in sediments. No significant relationship was found between *P. oceanica* and water. This study showed that *P. oceanica* may adopt different tolerance strategies compared to mainland-rooted macrophytes, and its possible use as a bioindicator of trace elements in sediments should be considered.

1. Introduction

The human impact on marine ecosystems has intensified in the last few decades as a consequence of the last industrial revolution, and the consequent demographic boom with over 7 billion people currently living in the world (Jackson et al., 2001; Stachowitsch, 2003; Millennium Ecosystem Assessment, 2005; Halpern et al., 2008; UN Department of Economic and Social Affairs, 2015). Coastal environments are especially vulnerable as they host rich marine biodiversity, important urban settlements and numerous human activities (Small and Nicholls, 2003; Halpern et al., 2008). Metals, in particular, are among the most ubiquitous pollutants increasingly entering marine ecosystems, both from natural and anthropogenic sources whose resulting contamination can range from local to global scale (UNEP, 2005; Boudouresque et al., 2009; Serrano et al., 2011). Trace elements, most of which are metals, occur naturally in the environment, and although some elements act as fundamental micronutrients for plants (Cu, Zn, Fe, Mn, and Ni), the same elements may have toxic effects at high concentrations (Kabata-Pendias and Pendias, 2001). Other non-essential elements are toxic to organisms even at low concentrations, such as Hg, Pb, Al, As, Cd and Cr (Prasad, 2004).

Trace elements are regarded as dangerous pollutants in the aquatic ecosystems because of their toxicity, their persistence in the environment, and their ability to accumulate in living organisms (Bargagli, 1998). Trace elements bioaccumulation into the different trophic levels may thus have damaging effects on humans and important economic consequences (Rainbow, 2007; Roberts et al., 2008). However, the ecological risks provoked by trace elements are difficult to assess, due to their complex behaviour in marine waters (Guilizzoni, 1991; Greger, 2004). In turn, through the monitoring of trace element concentrations in tissues of living organisms (biomonitoring), it is possible not only to gain direct information on the bioavailable fraction of contaminants, but also to detect early signs of environmental disturbance before upper trophic levels are affected (Rainbow, 1995). Bioaccumulation studies led to the adoption of the bio-indicator concept (Langston and Spence, 1995), and over time, the use of aquatic plants as bio-indicators became an irreplaceable tool for investigation in ecological research applied to the conservation of littoral ecosystems (Rainbow and Phillips, 1993; Bonanno and Lo Giudice, 2010, Bonanno, 2011). Seagrasses, in particular, are often considered as sentinel species because any change in their distribution (e.g. a reduction in the maximum depth limit or a loss of covered areas) implies an environmental change (Schlacher-Hoenlinger and Schlacher, 1998; Pergent-Martini and Pergent, 2000; Ferrat et al., 2003a; Ralph et al., 2006; Orth et al., 2006). In addition, marine angiosperms generally have a high trace metal bioaccumulation capacity since they interact directly with both the water column (through the leaves) and the pore water (through the roots), as both leaves and roots are sites of ionic uptake (Romero et al., 2006; Ralph et al., 2006; Bonanno and Di Martino, 2016). In particular, macrophytes contribute significantly to the primary production of water bodies, in the littoral zone being a fundamental part of the trophic

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http://dx.doi.org/10.1016/j.marpolbul.2016.12.081
0025-326X/

structure of aquatic ecosystems and an important link in the recycling of nutrients. Consequently, they can incorporate large amounts of metals from the environment (Jackson, 1998; Kaldy, 2006).

The marine phanerogam *Posidonia oceanica* (L.) Delile is an endemic Mediterranean species, often considered as a potential trace element bioindicator given its bathymetric range (0–40 m depth) and consequent direct exposure to coastal pollution (Warnau et al., 1995; Pergent-Martini and Pergent, 2000; Campanella et al., 2001; Lafabrie et al., 2008). *P. oceanica* forms dense communities (meadows) widely distributed throughout the Mediterranean of which occupies c. 1.5% of its surface (c. 35,000 km^2) (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). However, although several studies suggest the possible use of *P. oceanica* as a trace element bioindicator, the practical use of this species is still not sufficiently recognized and integrated in the monitoring campaigns aimed to assess the impact of metallic pollutants on marine ecosystems. This may depend on several factors such as results based on few samples (Catsiki and Panayotidis, 1993) and small sampling areas (Malea et al., 1994; Conti et al., 2007). But probably what stops the regular use of *P. oceanica* as a trace element bioindicator concerns mainly the conflicting results on which *P. oceanica* organs act as better bioindicators and for which elements.

This study aimed to shed further light on the use of *P. oceanica* as a trace element bioindicator. Specifically, this study conducted a large-scale analysis of the trace element concentrations in the roots, rhizomes and leaves of different *P. oceanica* populations distributed along the island of Sicily (Italy), located in the middle of the Mediterranean Sea and at the crossroads of international maritime traffics. The ultimate goal of this study was to contribute to make *P. oceanica* a standard tool for monitoring trace element contamination in coastal waters.

2. Materials and methods

2.1. Biology and ecology of Posidonia oceanica

Seagrasses of the genus *Posidonia* are restricted to the Mediterranean and coastal waters of Australia. They form dense infralittoral populations that frame the so-called *Posidonia* meadow ecosystems, and include a total of nine species of which only *Posidonia oceanica* is endemic to the Mediterranean (Short et al., 2001). *Posidonia oceanica* L. (Delile) is the most common seagrass species in the Mediterranean Sea, and forms extensive coastal meadows from the surface to 30–40 m depth, depending on water transparency and temperature (Boudouresque et al., 2012). *P. oceanica* meadows are climax ecosystems, and over time, this long-lived plant builds up a set of rhizomes and roots whose interstices, filled in by sediment, form a structure called 'matte'. The plant can reproduce both sexually and asexually but its growth is very slow (1 ÷ 6 cm/year). Consequently, the colonization of new areas and the recolonization of lost areas, via seeds, vegetative fragments or marginal spread of the meadow, are extremely slow, thus making each loss almost irreversible (Marbà et al., 1996; Boudouresque et al., 2009). After the death of the plant, the deterioration of rhizomes is also very slow, leading to a dead matte that may persist for millennia (Arnaud-Haond et al., 2012). Because of the important ecological (nursery, spawning, feeding, oxygenation) and economic roles (coastal protection and sediment trapping) (Borum et al., 2004; Boudouresque et al., 2012), *P. oceanica* is protected by EU legislation (Habitats Directive), the Bern and Barcelona Conventions, national laws, and is currently classified as a Least Concern species on the IUCN Red List (IUCN, 2015).

2.2. Study area

This study was carried out in six coastal locations of Sicily (Italy), and included two nature reserves and four seaside resorts (Fig. 1; Table 1). The coast of some sites was sandy; in other sites the substrate was rocky. The nature reserves, selected as control sites for their pristine conditions, are important wetlands and marine sanctuaries where trace element inputs should be considered negligible and, in case, mainly of natural origin. Seaside resorts, instead, were supposed to be enriched in trace elements due to human activities, especially untreated municipal wastewaters and polluting spills from maritime traffic (e.g. tourist ships). No signs of eutrophication were found in the anthropogenic sites, and tidal phenomena are generally negligible in all sites. Freshwater inputs are relatively low and mainly associated with rainy events. Wet and mild winters and dry summers characterize the maritime climate of the study areas. The annual mean temperature is 18 °C, whereas precipitation ranges between 400 and 600 mm yearly, and mainly occurs from October to December. *P. oceanica* meadows were fairly abundant around the study sites, and formed dense monospecific stands occupying a bathymetric fringe from near the surface to a maximum depth of 10 m.

2.3. Sampling

The sampling of *P. oceanica* took place during December 2015, and January, February and March 2016. Sampling was conducted during stable weather conditions; in particular, days were sunny with smooth sea, no wind and no recent rains. Sampling took place in the earlier hours of the day between 7 and 10 am to benefit from light conditions. Sampling activities were carried out in a once-off trip per site, and all samples were transported to laboratory on the same day of collection within 2 h from the end of sampling. In each collection site, sampling was carried out within the area occupied by dense meadows of *P. oceanica*, whose distance from the coast varied from 1 to 100 m, and with a sea depth of 1 ÷ 10 m. The size of *P. oceanica* meadows was quite variable, and ranged from 5 × 5 m to 100 × 100 m.

Three kinds of samples were collected in the field: water and sediment samples, and individuals of *P. oceanica*. Specifically, plant individuals consisted of roots, rhizomes and mature leaves. Moreover, as metals can be mobilised from sediments during disturbance (Stoddern, 2003), plant individuals and water were collected before sediments were sampled. In each sampling site, 20 samples per typology were collected. Each of these 20 samples was obtained by mixing subsamples. Regarding *P. oceanica*, the generic analytical sample was obtained by mixing 10 mature individuals randomly and manually harvested within a subplot of average size of 5 × 5 m. In the same subplot, 10 subsamples of sediment and 10 of water were also randomly collected. The generic analytical sample of sediment and water was obtained by mixing such subsamples into a representative composite sample. This protocol was repeated twenty times for every kind of sample in each collection site (N = 20). Plant individuals were collected one by one and put in a landing net provided with sealing system. Once ashore, plant individuals were gently shaken to remove gross particles, rinsed with distilled water (to remove epiphytic organisms and minor sediment particles), and dried with a clean linen cloth. Then, the 10 plant individuals of each subplot were sealed in one sterilized and airtight plastic bag. Regarding sediment collection, subsamples were collected through a Plexiglas corer (internal diameter 10 cm). Only the upper layer of sediment (about the first 5 cm) was collected because it corresponds to the sediment part generally affected by the root system. Finally, water subsamples were collected at mid-height between seabed and water surface. Sediment and water samples were put in 0.5-L polyethylene bottles. All samples were stored in PVC containers and kept at a constant temperature of 4 ± 1 °C until laboratory analysis.

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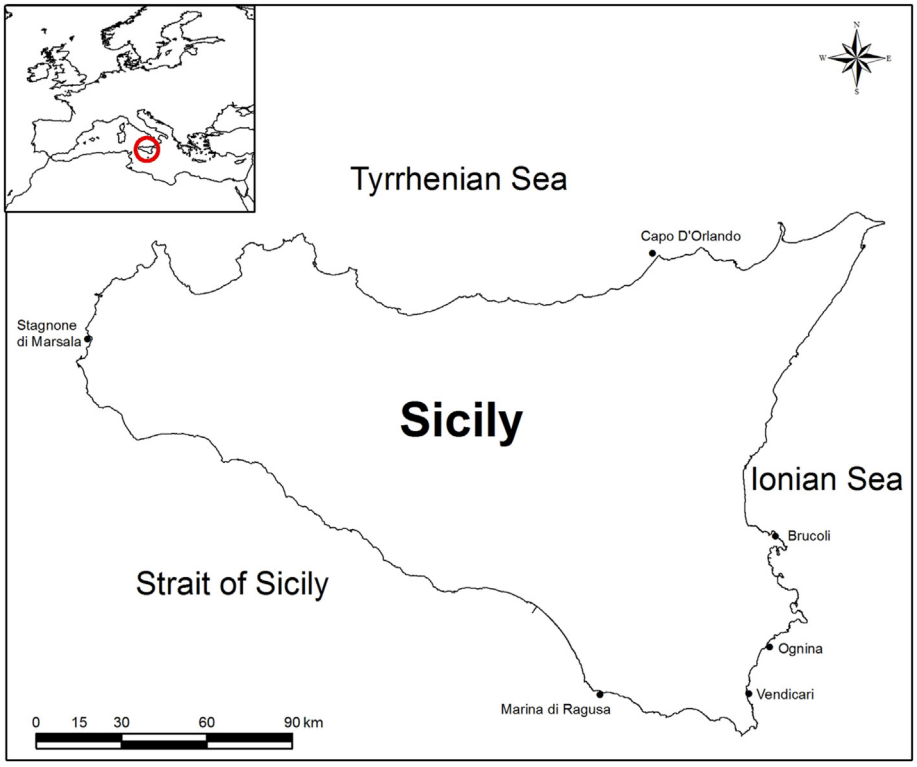

Fig. 1. Sampling sites.

2.4. Chemical analysis

The total concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb, Zn were determined in water, sediment and *P. oceanica* organs. Once in the laboratory, the individuals of *P. oceanica* were first washed thoroughly in running tap water to avoid any surface contamination, and then rinsed with bidistilled water, obtained through a Milli-Q® Gradient distiller, to remove any further residual material adhering to the surface. After that, the plant samples were dissected into roots, rhizomes and leaves, and together with sediment samples, were put in a refrigerator at 4 °C until processing. The average weight of root, rhizome, leaf and sediment samples was 0.50, 0.80, 1.0, 2.0 kg, respectively. After defrosting, leaves, roots, rhizomes and sediment were dried to constant weight at room temperature because higher temperatures (>60 °C) may interfere in some element analyses (Llagostera et al., 2011). Once dry, plant samples were ground and homogenized in an agate mortar to ensure a powder with uniform element distribution. Sediment samples were instead passed through a 1 mm diameter sieve. These homogenous powder and sieved sediment were then weighed at 0.1 ± 0.05 g, and oven-digested at 90 °C overnight (microwave oven Mars 6, CEM Corporation) in an acid solution (H_2O_2/HNO_3, 2:3 ratio; Carlo Erba) in Teflon digestion vessels. Regarding water, the samples were acidified with 63% HNO_3 to pH ≤ 2, before being filtered through a filter paper 2.0 μm (Whatman® GF/A glass microfiber filters). After digestion, the solid residue of plant and sediment samples was separated by centrifugation before being filtered through a filter paper 2.0 μm. Finally, supernatants were diluted with ultrapure Milli-Q water to a final volume of 25 mL (into a volumetric flask), and transferred for chemical analysis via ICP-MS (Cd, Cr, Cu, Ni, Pb, Zn), ICP-OES (Al, Fe) and FAAS (As and Hg) (respectively through PerkinElmer Elan® 6000, PerkinElmer® Optima™ 8000, PerkinElmer® AAnalyst™ 400 AA Spectrometer). The elements used as internal standards were yttrium (Y), rhenium (Re) and rhodium (Rh). Quality control was performed through stability of instrumental recalibration and using analytical blanks. The instruments were cyclically checked against the low level standards (once every five samples) and recalibrated either when signs of drift were noted or after every 10 samples. The validity and precision of the analytical procedures

Table 1
Sampling sites, degree of human pressure and coordinates.

Site name	Site type	Number of inhabitants	Maritime traffic	Human impact	Coordinates
Brucoli	Seaside resort	1000	High	High	37°16′56″N 15°11′34″E
Ognina	Seaside resort	1000	Moderate	Moderate	36°58′48″N 15°15′37″E
Vendicari	Nature reserve	Negligible	Negligible	Negligible	36°47′33″N 15°06′09″E
Marina di Ragusa	Seaside resort	3500	High	High	36°47′02″N 14°31′31″E
Stagnone di Marsala	Nature reserve	Negligible	Negligible	Negligible	37°51′27″N 12°28′29″E
Capo d'Orlando	Seaside resort	13,000	Negligible	High	38°09′25″N 14°45′54″E

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were assessed by analyzing (with the same protocol of field-collected samples) the standard reference material *Ulva lactuca* (B.C.R. reference material No. 279/504). Student's *t*-test ($\alpha = 0.05$) was also used to assess whether analyzed values for the reference material were in good agreement (not statistically different) with the certified values. The percent recovery showed values between 80 and 110%. Three replicates were prepared for each sample in order to evaluate the reproducibility of measurements. Instrument detection limits were expressed as three times the standard deviation from the mean blank. Hg values were not reported because below detection limits (<0.02 mg/kg).

2.5. Statistical processing

The factors of translocation and bioconcentration were calculated as follows:

- Translocation Factors (TFs):

$C_{rhizome}/C_{root}$

C_{leaf}/C_{root}

$C_{leaf}/C_{rhizome}$

where C_{root}, $C_{rhizome}$ and C_{leaf} are, respectively, the concentrations (mg/kg, dry weight) of the target element in roots, rhizomes and leaves of *P. oceanica*. TF expresses the target element mobility within the plant, and a larger value of TF implies higher translocation capability (Deng et al., 2004);

- Bioconcentration Factors (BCFs):

$C_{root}/C_{sediment}$

where $C_{sediment}$ and C_{root} are respectively the concentrations of the target element in sediment and roots (mg/kg, dry weight) of *P. oceanica*. BCF expresses the efficiency of a plant species to uptake and accumulate an element in its tissues. A larger value of BCF implies better bioaccumulation capability (Soda et al., 2012).

The Shapiro-Wilk test was performed to determine the normal distribution of the data sets whereas the Levene's test was used to check the hypothesis of homoscedasticity. If these two tests were accepted, one-way analysis of variance (ANOVA) would be used to assess the existence of significant differences between trace element concentrations in water, sediment and plant organs of *P. oceanica*. Tukey post-hoc test was then performed to identify which specific mean pairs differ significantly. In case of rejection of ANOVA conditions, data were log-transformed. To find a possible relationship between trace elements in water, sediment and plant organs, the Pearson's *r* coefficient was performed. A probability of 0.05 or lower was considered significant. All statistical analyses were performed with the statistical package IBM SPSS Version 22.0.

Table 2
Translocation and bioconcentration factors in the organs of *P. oceanica*.

Element	BCF	TF		
	C_{root} / $C_{sediment}$	$C_{rhizome}$ / C_{root}	C_{leaf} / $C_{rhizome}$	C_{leaf} / C_{root}
As	1.06	0.75	1.04	0.77
Cd	2.68	0.56	2.20	1.22
Cr	0.12	0.69	1.05	0.73
Cu	6.93	0.47	1.42	0.66
Ni	0.37	0.62	4.61	2.85
Pb	1.21	0.50	1.24	0.62
Zn	4.81	0.51	2.43	1.24
Mean	2.45	0.58	2.0	1.16

3. Results and discussion

The results of this study showed that the element mobility in *P. oceanica* varies remarkably according to the element and the source (Table 2). Specifically, the highest mean mobility was found from sediment to roots (BCF = 2.45), whereas the lowest translocation was between roots and rhizomes (mean TF = 0.58). This may suggest that trace element penetration in *P. oceanica* from sediment is more effective than internal mobility, which is particularly low in the rhizosphere. However, the trend of internal mobility reversed in the superior organs (leaves) where translocation is higher, especially from rhizomes to leaves (mean TF = 2.0). Water and sediment showed different decreasing trends of element concentrations, respectively Cu > Ni > Zn > Cr > As > Pb > Cd and Cr > Zn > Ni > Pb > As > Cu > Cd (Table 3). In turn, roots, rhizomes and leaves showed almost coinciding bioaccumulation trends, respectively Zn > Cu > Ni > Pb > As > Cr > Cd, Zn > Cu > Ni > As > Pb > Cr > Cd and Zn > Ni > Cu > Pb > As > Cr > Cd. In general, trace element trends in plant organs showed similarities with sediment. No significant relationship was found between plant organs and water; as a result, *P. oceanica* seems to have low capacities to capture nutrients from the water column. Positive correlations were instead found between organs and sediment for As, Cd, Cu, Ni, and Zn (Tables 4). In particular, Ni and Zn were the only element for which a positive correlation was found between sediment and plant organs in all sites. These results are encouraging for a potential use of *P. oceanica* as a bioindicator of contamination in marine sediments.

The elements Cr and Pb in sediment showed no correlation with *P. oceanica* organs, and As, Cd, and Cu were correlated only for some organs (especially roots) and in some sampling sites. Several factors could mask the potential significant relationships between concentrations in *P. oceanica* and bottom sediments. First, this study analyzed the total element concentrations in sediment, but only one fraction of the total content might be available to biota. Second, as suggested by Lafabrie et al. (2011) for the seagrass *Vallisneria neotropicalis*, the absence of a relationship between element concentrations in *P. oceanica* organs and those found in sediment, could reflect an important foliar contribution to contaminant bioaccumulation. Indeed, *P. oceanica*, as a rooted aquatic species, can absorb elements both through its leaves, via the surrounding water, and through its roots, via sediment. However, an important trace element uptake from the water column seems unlikely to occur because sediment has a greater potential pool of contaminants than the overlying water column, and rooted submerged plants with well-developed root systems, would primarily extract elements from sediment with subsequent translocation to above-ground tissues (Jackson, 1998; Harguinteguy et al., 2016). Biomass and growth rates are other two factors that may have affected the performance of *P. oceanica* as a trace element bioindicator of sediments. Indeed, high growth rates could lead to a "biological dilution" of contaminants in plant tissues, thus masking possible significant correlations between plant species and sediment (Hudon, 1998; Ferrat et al., 2003b; Duman et al., 2006). However, the sampling strategy of this study was designed to minimize the impact of environmental factors on *P. oceanica* growth rate (i.e., collection of mature individuals, same sampling periods, etc.).

The concentration values in *P. oceanica* organs decreased according to two trends: root > leaf > rhizome (As, Cr, Cu, Pb), and leaf > root > rhizome (Cd, Ni, Zn). This result diverges from the general bioaccumulation trend in mainland rooted macrophytes (freshwater and brackish), whose underground organs (roots and rhizomes) show a predominant exclusion strategy by accumulating higher element concentrations than aerial organs (stems and leaves), thus allowing the plant to protect itself against the adverse effects of toxic concentrations in photosynthetic processes (e.g., Stoltz and Greger, 2002; Matthews et al., 2005; Reboreda and Caçador, 2007; Willis et al., 2010; Bonanno, 2012, 2013). In this study, indeed, the leaves of *P. oceanica* showed higher concentrations than rhizomes, and in 3/7 elements, even higher than roots. These results suggest that *P. oceanica* may follow different

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Table 3
Mean concentrations of trace elements in water, sediment and organs of *P. oceanica*.

	As	Cd	Cr	Cu	Ni	Pb	Zn
Water [μg/L]	1.70 ± 0.22	0.20 ± 0.02	5.89 ± 0.62	79.5 ± 9.52	19.6 ± 2.65	0.24 ± 0.01	13.6 ± 1.41
Sediment [mg/kg]	3.32 ± 0.42	0.41 ± 0.05	28.5 ± 3.15	2.54 ± 0.27	19.1 ± 3.21	4.27 ± 0.55	19.2 ± 2.15
Root [mg/kg]	3.53 ± 0.36	1.10 ± 0.08	3.40 ± 0.41	17.6 ± 2.31	7.05 ± 0.98	5.16 ± 0.63	92.4 ± 8.53
Rhizome [mg/kg]	2.63 ± 0.31	0.61 ± 0.07	2.35 ± 0.28	8.19 ± 0.95	4.36 ± 0.55	2.59 ± 0.31	47.4 ± 6.32
Leaf [mg/kg]	2.73 ± 0.18	1.34 ± 0.10	2.47 ± 0.32	11.6 ± 1.88	20.1 ± 2.56	3.22 ± 0.42	115 ± 17.8

tolerance mechanisms compared to the exclusion strategy of mainland aquatic rooted species (Bonanno et al., 2017). Since *P. oceanica* regenerates its leaves periodically (leaf life span varies from 15 to 60 weeks; Wittmann, 1984), the higher concentration in these superior organs may suggest a "removal" strategy according to which *P. oceanica* stores greater concentrations of trace elements in its temporary organs. This suggests that uptake kinetics and passive absorption properties of leaves may differ from those of roots (Llagostera et al., 2011). This removal strategy of *P. oceanica* seems to find confirmation in *Cymodocea nodosa*, an ecologically similar seagrass often living in meadows adjacent to *P. oceanica*. In particular, Malea and Haritonidis (1999) reported active mobilization of toxic metals, such as Cd, Ni and Pb from roots to shoots in the seagrass *Cymodocea nodosa*, thus facilitating metal loss due to the high turnover rates of leaves. In general, *P. oceanica* may reflect the trace element concentrations in sediment, and its leaves should be considered as potential bioindicators, at least for short-term monitoring campaigns (Gosselin et al., 2006).

Arsenic concentrations in *P. oceanica* organs are scarcely reported in literature. For instance, Gosselin et al. (2006) found As values of 14–21 mg/kg in *P. oceanica* populations off North Corsica (France), a higher content compared to the findings of this study (1.40–5.65 mg/kg). However, in agreement with our study, Gosselin et al. (2006) found that *P. oceanica* may be used as a biological monitor of As. The fact the *P. oceanica* reflects As content in sediments may imply that the higher As concentrations found by Gosselin et al. (2006) in *P. oceanica* is a result of a higher As presence in sediments. Regarding this study, As concentrations in sediments (<0.90–7.23 mg/kg) were lower than the Italian limits of sediment quality set at 12 mg/kg (GURI, 2011), thus a potential sediment toxicity from study areas should be considered generally negligible. Cadmium, another non-essential element, showed concentrations in *P. oceanica* organs (0.31–2.02 mg/kg) in line with several studies conducted off the Mediterranean coasts, which reported values ranging from 0.40 to 2.40 mg/kg (Costantini et al., 1991; Warnau et al., 1995; Baroli et al., 2001; Campanella et al., 2001; Gosselin et al., 2006; Conti et al., 2007; Khaled et al., 2014). Our study reported higher Cd concentrations in sediments than the polluted sites investigated by Lafabrie et al. (2007). This, however, did not reflect in *P. oceanica* bioaccumulation whose Cd concentrations (0.31–2.02 mg/kg) were in line with

Table 4
Concentrations in samples of water, sediment and organs of *P. oceanica*.

Element	Kind of sample	Sampling sites					
		Brucoli	Ognina	Vendicari	Marina di Ragusa	Stagnone di Marsala	Capo d'Orlando
As	Water [μg/L]	3.52 ± 0.27	1.56 ± 0.12	<0.10	3.03 ± 0.41	<0.10	1.89
	Sediment [mg/kg]	5.85 ± 0.42	<0.90	<0.90	7.23 ± 1.21	<0.90	4.15
	Root [mg/kg]	$4.77 \pm 0.52^{a,b,1}$	$3.08 \pm 0.44^{a,b}$	$2.25 \pm 0.35^{a,b}$	$5.65 \pm 0.85^{a,b,1}$	$2.15 \pm 0.31^{a,b}$	$3.25 \pm 0.40^{a,b,1}$
	Rhizome [mg/kg]	$1.40 \pm 0.21^{a,b}$	$4.43 \pm 0.51^{a,a,b}$	$1.89 \pm 0.22^{a,b}$	$3.46 \pm 0.47^{a,b}$	$1.67 \pm 0.21^{a,b}$	$2.89 \pm 0.32^{a,b,b,1}$
	Leaf [mg/kg]	$1.70 \pm 0.18^{a,b}$	$2.36 \pm 0.31^{a,b}$	$2.31 \pm 0.29^{a,b}$	$5.14 \pm 0.65^{a,b,1}$	$1.70 \pm 0.14^{a,b}$	$3.15 \pm 0.51^{a,b,1}$
Cd	Water [μg/L]	0.14 ± 0.02	<0.10	<0.10	0.55 ± 0.06	<0.10	0.20 ± 0.02
	Sediment [mg/kg]	0.16 ± 0.02	<0.30	<0.30	1.12 ± 0.15	<0.30	0.25 ± 0.03
	Root [mg/kg]	$0.36 \pm 0.04^{a,b,1}$	$1.53 \pm 0.18^{a,b}$	$1.82 \pm 0.15^{a,b}$	$1.46 \pm 0.17^{a,a,b,1}$	$0.55 \pm 0.07^{a,b}$	$0.84 \pm 0.09^{a,b,1}$
	Rhizome [mg/kg]	$0.42 \pm 0.03^{a,b}$	$0.47 \pm 0.05^{a,b}$	$0.89 \pm 0.10^{a,b,b}$	$1.10 \pm 0.15^{a,b}$	$0.44 \pm 0.07^{a,b}$	$0.31 \pm 0.04^{a,b}$
	Leaf [mg/kg]	$1.24 \pm 0.10^{a,b,B}$	$1.99 \pm 0.21^{a,b}$	$1.45 \pm 0.18^{a,a,b}$	$2.02 \pm 0.24^{a,b}$	$0.78 \pm 0.08^{a,b,B}$	$0.53 \pm 0.06^{a,b}$
Cr	Water [μg/L]	8.54 ± 0.10	5.45 ± 0.64	2.15 ± 0.24	9.89 ± 1.12	3.45 ± 0.41	5.87 ± 0.87
	Sediment [mg/kg]	75.8 ± 8.85	4.37 ± 0.55	5.67 ± 0.67	65.3 ± 7.65	4.43 ± 0.44	15.6 ± 2.21
	Root [mg/kg]	$2.70 \pm 0.25^{a,b}$	$5.28 \pm 0.43^{a,b}$	$3.56 \pm 0.27^{a,b,b}$	$3.76 \pm 0.44^{a,b,b}$	$3.34 \pm 0.33^{a,b,b}$	$1.98 \pm 0.24^{a,b}$
	Rhizome [mg/kg]	$2.40 \pm 0.25^{a,b}$	$3.27 \pm 0.37^{a,b}$	$1.12 \pm 0.20^{a,b}$	$2.89 \pm 0.32^{a,b}$	$2.00 \pm 0.32^{a,b}$	$2.43 \pm 0.31^{a,b}$
	Leaf [mg/kg]	$2.47 \pm 0.25^{a,b}$	$2.44 \pm 0.31^{a,b}$	$1.99 \pm 0.15^{a,b}$	$2.56 \pm 0.28^{a,b}$	$3.43 \pm 0.45^{a,b}$	$1.95 \pm 0.13^{a,b}$
Cu	Water [μg/L]	112 ± 18.3	85.1 ± 10.5	44.5 ± 6.54	123 ± 15.6	33.7 ± 4.45	78.6 ± 9.56
	Sediment [mg/kg]	5.72 ± 0.65	0.30 ± 0.05	0.20 ± 0.03	7.65 ± 0.67	0.23 ± 0.01	1.15 ± 0.24
	Root [mg/kg]	$26.3 \pm 3.54^{a,1}$	16.2 ± 2.34^{a}	14.6 ± 2.14^{a}	$24.5 \pm 3.45^{a,1}$	10.2 ± 1.56^{a}	13.6 ± 1.98^{a}
	Rhizome [mg/kg]	$11.4 \pm 1.67^{a,1}$	4.87 ± 0.65^{a}	7.12 ± 0.98^{a}	$12.5 \pm 1.87^{a,1}$	8.7 ± 1.02^{a}	4.56 ± 0.32^{a}
	Leaf [mg/kg]	$14.9 \pm 1.87^{A,1}$	11.1 ± 1.14^{A}	10.5 ± 1.95^{a}	$15.7 \pm 2.04^{a,1}$	8.9 ± 1.25^{a}	8.24 ± 0.72^{A}
Ni	Water [μg/L]	25.7 ± 3.34	19.4 ± 2.85	8.51 ± 0.95	33.6 ± 4.56	7.60 ± 0.88	22.6 ± 3.55
	Sediment [mg/kg]	32.3 ± 4.15	4.93 ± 0.65	3.23 ± 0.32	45.4 ± 5.65	6.45 ± 0.85	22.1 ± 3.41
	Root [mg/kg]	$6.91 \pm 0.85^{a,b,1}$	$8.14 \pm 0.66^{a,b,1}$	$5.12 \pm 0.54^{a,b,1}$	$8.24 \pm 1.10^{a,b,1}$	$5.56 \pm 0.71^{a,b,1}$	$8.32 \pm 0.93^{a,b,1}$
	Rhizome [mg/kg]	$7.01 \pm 0.76^{a,b,1}$	$3.82 \pm 0.43^{a,b,B,1}$	$2.34 \pm 0.31^{a,b,1}$	$5.23 \pm 0.62^{a,b,B,1}$	$4.21 \pm 0.53^{a,b,1}$	$3.56 \pm 0.55^{a,b}$
	Leaf [mg/kg]	$22.6 \pm 3.15^{a,b,B,1}$	$31.8 \pm 2.85^{A,b,1}$	$9.54 \pm 1.14^{A,B,1}$	$27.6 \pm 3.86^{a,b,b,1}$	$10.5 \pm 1.44^{a,B,1}$	$17.7 \pm 2.03^{A,B,1}$
Pb	Water [μg/L]	0.30 ± 0.02	<0.20	<0.20	0.35 ± 0.05	<0.20	<0.20
	Sediment [mg/kg]	4.30 ± 0.55	4.32 ± 0.64	2.22 ± 0.31	8.45 ± 1.04	1.89 ± 0.22	4.45 ± 0.51
	Root [mg/kg]	$2.81 \pm 0.35^{a,b}$	$6.56 \pm 0.84^{a,b,B}$	$2.56 \pm 0.32^{a,b}$	$8.49 \pm 0.95^{a,b}$	$4.21 \pm 0.51^{a,B}$	$6.32 \pm 0.72^{a,b,B}$
	Rhizome [mg/kg]	$0.79 \pm 0.09^{a,b}$	$5.41 \pm 0.73^{a,b}$	$1.14 \pm 0.15^{a,b}$	$4.67 \pm 0.67^{a,b,B}$	$3.21 \pm 0.44^{a,B}$	$0.34 \pm 0.03^{a,B}$
	Leaf [mg/kg]	$2.05 \pm 0.31^{a,b}$	$1.69 \pm 0.21^{a,b}$	$2.01 \pm 0.26^{a,b}$	$5.21 \pm 0.64^{a,b}$	$3.87 \pm 0.25^{a,b,B}$	$3.12 \pm 0.41^{A,B}$
Zn	Water [μg/L]	18.6 ± 2.24	10.9 ± 2.23	7.63 ± 1.12	25.6 ± 3.35	8.5 ± 0.95	10.6 ± 1.85
	Sediment [mg/kg]	26.8 ± 3.12	12.7 ± 2.15	10.5 ± 1.45	35.6 ± 5.21	12.5 ± 1.65	15.6 ± 1.55
	Root [mg/kg]	$58.3 \pm 7.65^{a,b,1}$	$120 \pm 18.7^{a,b,1}$	$44.3 \pm 6.51^{a,b,1}$	$146 \pm 18.2^{a,b,1}$	$75.7 \pm 8.43^{a,B,1}$	$110 \pm 13.4^{a,b,1}$
	Rhizome [mg/kg]	$43.2 \pm 5.34^{a,b,b,B,1}$	$58.9 \pm 7.43^{a,b,B,1}$	$32.5 \pm 4.45^{a,b,B,1}$	$65.4 \pm 8.20^{a,B,1}$	$45.2 \pm 5.21^{a,b,B,1}$	$38.9 \pm 4.41^{a,B,1}$
	Leaf [mg/kg]	$157 \pm 18.6^{A,b,1}$	$108 \pm 13.6^{a,b,1}$	$55.7 \pm 6.41^{a,B,1}$	$165 \pm 19.2^{a,b,1}$	$95.6 \pm 10.2^{A,b,1}$	$102 \pm 13.4^{a,b,1}$

Different forms of letter "a" mean significant differences between organs for one specific element in the same site. Different forms of letter "b" mean significant differences between the same kinds of organ for one specific element in all sampling sites. The number "1" means positive correlation between organs and sediment for one specific element in the same site.

Please cite this article as: Bonanno, G., Di Martino, V., Trace element compartmentation in the seagrass *Posidonia oceanica* and biomonitoring applications, Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2016.12.081

Lafabrie et al. (2007), who found 2.10 ± 0.10 mg/kg in Porto Torres (Sardinia, Italy), an industrial town subject to polluting inputs of hydrocarbons and petrochemical products (De Luca et al., 2004). Similarly to Lafabrie et al. (2007), this study did not find any significant correlation between Cd in leaves and sediment. However, this study found a positive sediment-root correlation in Cd, consequently, some organs of *P. oceanica* may act as potential bioindicators of Cd in sediments. A stronger root-sediment correlation, indeed, may be favored by a direct contact, thus, despite the results of this study, we cannot exclude *P. oceanica* leaves as Cd bioindicators.

Chromium showed relatively comparable values in *P. oceanica* (1.12–5.28 mg/kg) with previous findings (e.g. Warnau et al., 1995; Campanella et al., 2001; Conti et al., 2007). In particular, this study agreed with Lafabrie et al. (2007), who detected 1.23 ± 0.23 mg/kg in *P. oceanica* meadows living off the sea village of Canari (Corsica, France), located near a former asbestos mine. The high Cr contamination in sediments (1194 ± 282 mg/kg) found by Lafabrie et al. (2007), and the low content in *P. oceanica*, corroborate one of the findings of this study according to which *P. oceanica* may implement a Cr-exclusion strategy. Indeed, not only did our results show that *P. oceanica* does not reflect Cr values in sediment (no significant correlation found) but also this study reported the lowest element mobility from sediment to roots BCF = 0.12 for Cr). In particular, according to the Italian guideline limits of sediment quality (D.M. 260/2010, GURI, 2011), only two study sites passed the Cr threshold of 50 mg/kg (Brucoli and Marina di Ragusa, respectively with 75.8 and 65.3 mg/kg). Copper is an essential micronutrient for the growth and metabolism of plants (Malea and Haritonidis, 1989), and this may sometimes explain its hyperaccumulation by some species (Smillie, 2015). In this study, indeed, *P. oceanica* showed a Cu-hyperaccumulation tendency with the highest mobility of Cu (among all elements) from sediment to roots (BCF = 6.93). Results showed also that Cu concentrations in *P. oceanica* were in the range of 4.56–26.3 mg/kg, essentially in line with previous findings that reported a range of 0.30–59 mg/kg (Catsiki and Panayotidis, 1993; Warnau et al., 1995; Baroli et al., 2001, Campanella et al., 2001; Gosselin et al., 2006; Conti et al., 2007; Khaled et al., 2014). Our results suggest a possible use of *P. oceanica* as Cu bioindicator since this seagrass seems to reflect Cu content in the environment, especially in case of high concentrations in sediments.

Ni concentrations in *P. oceanica* showed the highest values in the leaves (9.54–31.8 mg/kg), in line with the *P. oceanica* populations off the industrial towns of Porto Torres and Livorno (Italy) (Lafabrie et al., 2007). Roots and rhizomes reported instead lower ranges of Ni concentrations (respectively 5.12–8.32 and 2.34–7.01 mg/kg), relatively in line with Khaled et al. (2014) from Egypt. Overall, the results of this study agreed with previous findings of Ni concentrations in *P. oceanica* (e.g., Catsiki and Panayotidis, 1993; Gosselin et al., 2006). Ni content in sediment passed the Italian quality limits of 30 mg/kg (GURI, 2011) in the anthropogenic sites of Brucoli and Marina di Ragusa (respectively 32.3 and 45.4 mg/kg). In particular, Ni concentration found in the latter study sites (a relatively small sea resort) are comparable with the results found by Lafabrie et al. (2007) in Livorno (40 mg/kg), which is one of the largest Italian ports. Ni content in *P. oceanica* organs showed also a positive relationship with Ni content in sediment from all study sites. Moreover, Ni showed low mobility from sediment to roots and in the rhizosphere, whereas Ni showed the highest translocation from rhizomes to leaves (TF = 4.61). This may suggest that, unlike Cr, *P. oceanica* adopts internal detoxification mechanisms that allow accumulating high Ni concentrations, especially in leaves (Marschner, 1995).

This study found significantly low Pb mean concentrations in sediment (4.27 ± 0.55 mg/kg), compared to the Italian quality limits of 30 mg/kg (GURI, 2011). Pb values in *P. oceanica* organs are, however, in line with the previous findings of Lafabrie et al. (2007) in highly Pb polluted sites. This may suggest a predominant exclusion strategy that aims to reduce the transit of toxic Pb values into the internal organs of *P. oceanica*. The fact that *P. oceanica* may adopt a Pb-exclusion strategy finds further support in the lack of significant correlation with sediments from all study sites. Lafabrie et al. (2007) found a similar conclusion for *P. oceanica*, which showed non-significant relations with highly Pb-polluted sediments. In particular, Lafabrie et al. (2007) argues that the absence of correlations between Pb concentrations in *P. oceanica* and in sediment, would imply that Pb in *P. oceanica* tissues may reflect Pb in the water column. This hypothesis, according to Lafabrie, is reinforced when considering laboratory studies that showed aquatic plants can remove Pb from the surrounding water (e.g. Axtell et al., 2003). Our study, however, did not find any significant correlation between *P. oceanica* and surrounding water, and given the probable Pb-exclusion strategy of *P. oceanica*, it seems unlikely that *P. oceanica* can uptake significant concentrations of Pb from water.

Zn concentrations in *P. oceanica* showed the highest values compared to the other elements. Specifically, the mean values in leaves, roots and rhizomes were respectively 115, 92.4 and 47.4 mg/kg, and generally in agreement with previous studies that reported a range of 27–140 mg/kg (Malea et al., 1994; Warnau et al., 1995; Campanella et al., 2001; Conti et al., 2007; Khaled et al., 2014). It is well known that Zn is essential for the growth and metabolism of plants because it plays an important role as enzyme activator, and is involved in the biosynthesis of some enzymes and growth hormones (Malea and Haritonidis, 1989). This is likely to result in hyperaccumulated levels of Zn, which may explain the high mobility from sediment to *P. oceanica* (BCF = 4.81) as found in this study (and as seen in Cu). *P. oceanica* may thus avoid a Zn-exclusion strategy because, like plant species in general, *P. oceanica* needs to uptake Zn for important physiological reasons. Zn values were also below the limits of sediment quality in all study sites (Long et al., 1995; MacDonald et al., 1996). Anyway, *P. oceanica* showed a positive correlation with Zn in sediments, thus, its use as a bioindicator of contamination should be considered possible.

4. Conclusions

Examining trace element concentrations in *P. oceanica* has important consequences because numerous foodwebs originate from the consumption of *P. oceanica* leaves and their epiphytes. This study made it possible to draw general conclusions concerning the possible use of *P. oceanica* as a bioindicator of trace element contamination in marine sediments. Results showed, in particular, that bioaccumulation is a basically a compartmentation process that is organ- and element-specific. A still not suitably investigated aspect concerns the possible use of *P. oceanica* as a trace element bioindicator of marine waters. To better understand element uptake and bioindication capacity of *P. oceanica*, future studies should thus focus on the role water column in the bioaccumulation of trace elements.

Acknowledgement

The authors wish to thank all the people involved in field sampling and laboratory analysis. This study was financed by EcoStat Srl – Spin-off of Catania University, by the Italian Ministry of Education, University and Research, and by the Italian National Research Council (CNR-ISAFOM Prot. n. 0003614/2016).

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Please cite this article as: Bonanno, G., Di Martino, V., Trace element compartmentation in the seagrass *Posidonia oceanica* and biomonitoring applications, Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2016.12.081

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Please cite this article as: Bonanno, G., Di Martino, V., Trace element compartmentation in the seagrass *Posidonia oceanica* and biomonitoring applications, Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2016.12.081