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The role of the seagrass *Posidonia oceanica* in the cycling of trace elements

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Abstract. The aim of this study was to investigate the role of the seagrass *Posidonia oceanica* on the cycling of a wide set of trace elements (Ag, As, Ba, Bi, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Ni, Pb, Rb, Sr, Tl, V and Zn). We measured the concentration of these trace elements in different compartments of *P. oceanica* (leaves, rhizomes, roots and epiphytes) in a non-polluted seagrass meadow representative of the Mediterranean and calculated the annual budget from a mass balance. We provide novel data on accumulation dynamics of many trace elements in *P. oceanica* compartments and demonstrate that trace element accumulation patterns are mainly determined by plant compartment rather than by temporal variability. Epiphytes were the compartment, which showed the greatest concentrations for most trace elements. Thus, they constitute a key compartment when estimating trace element transfer to higher trophic levels by *P. oceanica*. Trace element translocation in *P. oceanica* seemed to be low and acropetal in most cases. Zn, Cd, Sr and Rb were the trace elements that showed the highest release rate through decomposition of plant detritus, while Cs, Tl and Bi showed the lowest. *P. oceanica* acts as a sink of potentially toxic trace elements (Ni, Cr, As and Ag), which can be sequestered, decreasing their bioavailability. *P. oceanica* may have a relevant role in the cycling of trace elements in the Mediterranean.

1 Introduction

Seagrass meadows are considered one of the most valuable habitats in coastal areas (Orth et al., 2006) and rank among the most productive habitats (Pergent et al., 1997; Duarte and Chiscano, 1999). *Posidonia oceanica* is the most abundant seagrass in the Mediterranean playing a key role in the cycling of matter in Mediterranean coasts (Pergent et al., 1994).

P. oceanica biomass can have very different fate according to the part of the plant. While around 29 % of its produced biomass, mainly rhizomes and roots, is buried in the sediment, the rest of it, mainly leaves, is mineralized, either in situ or in adjacent ecosystems (Pergent et al., 1994). Together with leaves, the epiphytes that grow on *P. oceanica* leaves account for a substantial part of the primary production of the seagrass meadows (Lepoint et al., 1999). Epiphytes constitute a considerable and preferential food resource for herbivores (Tomas et al., 2005), and along with leaves, rhizomes, and roots is a main compartment of the plant in matter fluxes of this ecosystem.

Marine coastal systems are areas under pressure of many anthropogenic activities (Turner et al., 1996) and represent a sink for potential pollutants (Sanz-Lázaro and Marin, 2009), such as trace elements (Islam and Tanaka, 2004). Trace elements are elements that occur naturally in very low concentrations in the environment and can be either essential (e.g. Co, Cu, Fe, Mn, Ni, Rb and V) or non essential (e.g. Li, Cd, Sr, Ba, Tl, Ag, Ga, Pb, Bi and Cs) to living

organisms (Alloway, 1995). Trace elements are not necessarily toxic but many anthropogenic activities increase their natural concentrations causing pollution.

Seagrasses take up trace elements through leaves and roots (Schroeder and Thorhaug, 1980), which can be translocated among the parts of the plant. Since, seagrasses show different element accumulation patterns among their compartments (Lewis and Devereux, 2009), they may act as storage compartments and biological filters, favouring the decrease of toxic substances (Kaldy, 2006). They also can be introduced into higher trophic levels of the ecosystem, through grazing and decomposition of leaves and epiphytes (Lewis and Devereux, 2009).

To understand trace element cycling in seagrasses, it is important to study the accumulation trends in all plant compartments. Most of the studies dealing with uptake and accumulation of trace elements in *P. oceanica* and in other seagrasses focused on using these plants as bioindicators of the water column (Pergent-Martini and Pergent, 2000). Hence, these studies have mainly analyzed element concentration in leaves and rhizomes (Pergent-Martini and Pergent, 2000). To the best of our knowledge, only Sanchiz et al. (2000) and Schlacher-Hoenlinger and Schlacher (1998) analyzed trace element concentration in roots, and roots and epiphytes, respectively. Furthermore, most of these studies have focused on few trace elements, mainly Cd, Cr, Cu, Fe, Ni, Pb and Zn. Nevertheless, other trace elements that are essential and/or may be also toxic have been barely studied (Ag, As, Ba, Bi, Co, Cs, Li, Mn and Tl) (Pergent-Martini and Pergent, 2000 and references therein; Tovar-Sanchez et al., 2010) or not at all (Ga, Rb, Sr and V).

P. oceanica is expected to play a major role in the cycling of trace elements in the coastal areas of the Mediterranean, due to its wide abundance, high productivity and capacity to accumulate trace elements.

The aim of this study was to investigate the role of *P. oceanica* in the cycling of a wide set of trace elements (Ag, As, Ba, Bi, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Ni, Pb, Rb, Sr, Tl, V and Zn). Trace element concentrations were quantified in different compartments of *P. oceanica* (leaves, rhizomes, roots and epiphytes) in six bimonthly samplings in a non-polluted meadow representative of the Mediterranean. An annual budget was calculated from a mass balance analysis.

2 Materials and methods

2.1 Study area

The study was conducted in a *P. oceanica* meadow in Sounion (37° 39.617′ N, 23° 58.276′ E), Aegean Sea (Greece), which served as a reference area in other studies dealing with anthropogenic impact (Apostolaki et al., 2009a, b, 2011a). The meadow was situated in a shallow strait (14.5 m depth) with 5.5 cm s^{-1} bottom current speed. The site was characterized by coarse sand (0.90 mm diameter pore size), low percentage of silt/clay (4.83 %), and oxic conditions (353 mV redox potential). Concentration of dissolved inorganic nitrogen and phosphorus in the water column was 1.43 and 0.21 µM, respectively. Shoot density was 312 shoots m^{-2}, shoot biomass was 518 g dry wt m^{-2}, and shoot production was 377 g dry wt m^{-2} yr^{-1} (Apostolaki et al., 2009a).

2.2 Sampling procedure

Six bimonthly sampling events from June 2006 to April 2007 were done to integrate the natural variability during the year. During each sampling event and two months before the first one, 24 to 45 *P. oceanica* shoots were punched with a hypodermic needle just above the leaf sheath according to a modified Zieman method (Alcoverro et al., 2000) to measure growth. At each sampling event, punched shoots (including the below-ground parts) were collected in triplicates using cores to measure trace element concentration in plant tissues and in epiphytes growing on the leaves of the plant. Divers inserted cores approximately 20 cm in the sediment, to enclose adequate number of punched shoots. Then, they gently dag the sediment around the core (from the exterior part), inserted a knife to cut the roots, and retrieved the enclosed shoots.

To estimate trace element loss rate through decomposition of *P. oceanica* detritus, a litter bag experiment was conducted. In June 2006, the oldest alive leaf blades from different seagrass shoots were collected by hand. Then, 15 bags containing 10 g fresh weight of the senescent leaf blades with its epiphytes were collected and enclosed in a 1 mm pore size mesh bag. The bags were anchored under the canopy of the seagrass meadow. The bags were retrieved in triplicates one, two, three, four, and six months after deployment, (i.e. from July until December 2006).

2.3 Laboratory analyses

In the laboratory, epiphytes were gently scraped from leaves (i.e. 8 to 15 leaves per replicate). Epiphyte biomass obtained was variable but always sufficient to do a proper sample homogenization. As a reference, the average epiphyte biomass on the studied seagrass meadow was 12.6 g dry wt m^{-2} (Apostolaki et al., 2011b). *P. oceanica* tissues were separated into new (i.e. unmarked tissue produced between sampling events) and old leaves, rhizomes and roots. *P. oceanica* tissues, epiphytes, and detritus collected during each retrieval point of the litter bag experiment were dried at 70 °C for 48 h, ground to powder and stored in a moisture-free atmosphere.

For the analysis of Ag, As, Ba, Bi, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mn, Ni, Pb, Rb, Sr, Tl, V and Zn, ∼0.1 g of sample was weighted and placed in a Teflon reactor. Then, 3 ml ultrapure water (18.2 $M\Omega$cm), 5 ml of concentrated HNO_3

Table 1. Analysis of the reference materials of the Community Bureau of Reference *Ulva lactuca* CRM 279 and *Lagarosiphon major* CRM 060: certified values, measured values and recovery (mean ± SD). Certified values are given when they are available from the Community Bureau of Reference. Values in parentheses are indicative values of the reference materials.

Element	*Lagarosiphon major* (CRM-60)			*Ulva lactuca* (CRM-279)		
	Measured	Certified	Recovery	Measured	Certified	Recovery
	($\mu g\,g^{-1}$ dry wt)	($\mu g\,g^{-1}$ dry wt)	(%)	($\mu g\,g^{-1}$ dry wt)	($\mu g\,g^{-1}$ dry wt)	(%)
Ag	0.24 ± 0.01	(0.2)		0.036 ± 0.003		
As	5.1 ± 0.2	(8)		3.95 ± 0.2	3.09 ± 0.2	128
Ba	97 ± 4.3			10 ± 0.6		
Bi	0.42 ± 0.03			0.07 ± 0.001		
Cd	2.11 ± 0.04	2.2 ± 0.1	96	0.27 ± 0.004	0.27 ± 0.02	98
Co	3.5 ± 0.1	(4)		1.96 ± 0.1		
Cr	20 ± 5.3	(26)		9 ± 1.6		
Cs	0.22 ± 0.009	(0.4)		0.38 ± 0.02		
Cu	40 ± 1.3	51.2 ± 1.9	77	10 ± 0.2	13.1 ± 0.4	76
Fe	1668 ± 66			1802 ± 65	(2400)	
Ga	0.45 ± 0.06			0.45 ± 0.02		
Li	0.96 ± 0.1			2.2 ± 0.2		
Mn	1424 ± 25	1760 ± 60	81	1660 ± 1.2	(2090)	
Ni	35 ± 4.4	(40)		12 ± 0.1		
Pb	64 ± 4.2	64 ± 4	101	11 ± 1.3	13.5 ± 0.4	81
Rb	12 ± 0.2	(23)		8 ± 0.03		
Sr	460 ± 6			476 ± 12		
Tl	0.18 ± 0.01	(0.24)		0.026 ± 0.003		
V	3.8 ± 0.3	(6)		4.8 ± 0.4		
Zn	265 ± 9.1	313 ± 8	85	42 ± 2.2	51.3 ± 1.2	81

(Promochem, high purity for trace analysis), and 2 ml of 30 % H_2O_2 (Fluka, TraceSelectUltra for trace analysis) were added. The reactor was maintained in a microwave digester for 40 min with a top temperature of 200 °C. For each batch of samples there was a blank (another reactor with all the reagents used in the digestion but without sample of *P. oceanica*), which was used as a control of the reagents used, but also to check sample contamination and differences among the sample digestions on each batch. Following digestion, the content of each vessel was poured into volumetric flasks and ultrapure water was added to make up 25 ml, the final volume. Then, samples were stored at 4 °C. Trace element concentrations were done with an X-series inductively coupled plasma-mass spectrometer (ICP-MS; Thermo Fischer Scientific, Winsford, United Kingdom).

The limits of detection of the ICP-MS were calculated as three times the standard deviation of the blanks and were: 0.009 (Ag), 0.368 (As), 0.117 (Ba), 0.002 (Bi), 0.005 (Cd), 0.034 (Co), 1.766 (Cr), 0.001 (Cs), 0.524 (Cu), 42.51 (Fe), 0.005 (Ga), 0.204 (Li), 0.164 (Mn), 1.372 (Ni), 0.043 (Pb), 0.010 (Rb), 0.519 (Sr), 0.006 (Tl), 0.428 (V) and 6.009 (Zn) $\mu g\,g^{-1}$. Yttrium and Indium were used as internal standards. The accuracy of the technique was checked with the analysis of standard reference materials (*Ulva lactuca* CRM **279**, *Lagarosiphon major* CRM **060**, Community Bureau of Reference). For most of the trace elements for which we had the certified value, recoveries were within the limits of required performance (between 80 and 120 %), nevertheless, in the case of As and Cu, values were close but did not fall within this range (Table 1).

2.4 Calculations

Data on shoot biomass, shoot production rate, leaf shedding rate and leaf residual loss rate (biomass consumed by herbivores or torn off by waves and currents) were obtained from Apostolaki et al. (2009a). Biomass (g dry wt m^{-2}) of leaves, rhizomes and roots was estimated as the product of dry weight per shoot and shoot density (shoots dry wt m^{-2}) at each sampling event. Leaf production rate (g dry wt m^{-2} d^{-1}) at each sampling event was estimated as the product of dry weight of "new" leaf tissue per shoot and the mean shoot density at the beginning and end of sampling interval, divided by the duration of sampling interval in days.

For rhizome and root production, we used the annual production rate (g dry wt m^{-2} yr^{-1}), since more detailed estimates were not possible. This extrapolation was reasonable because belowground biomass growth of *P. oceanica* shows low variation among seasons (Wittmann, 1984). In the case of rhizome, it was estimated as the sum of annual horizontal and vertical production rate. Annual horizontal rhizome production rate (27.51 g dry wt m^{-2} yr^{-1}) was estimated as the product of annual rhizome elongation rate per apex, the

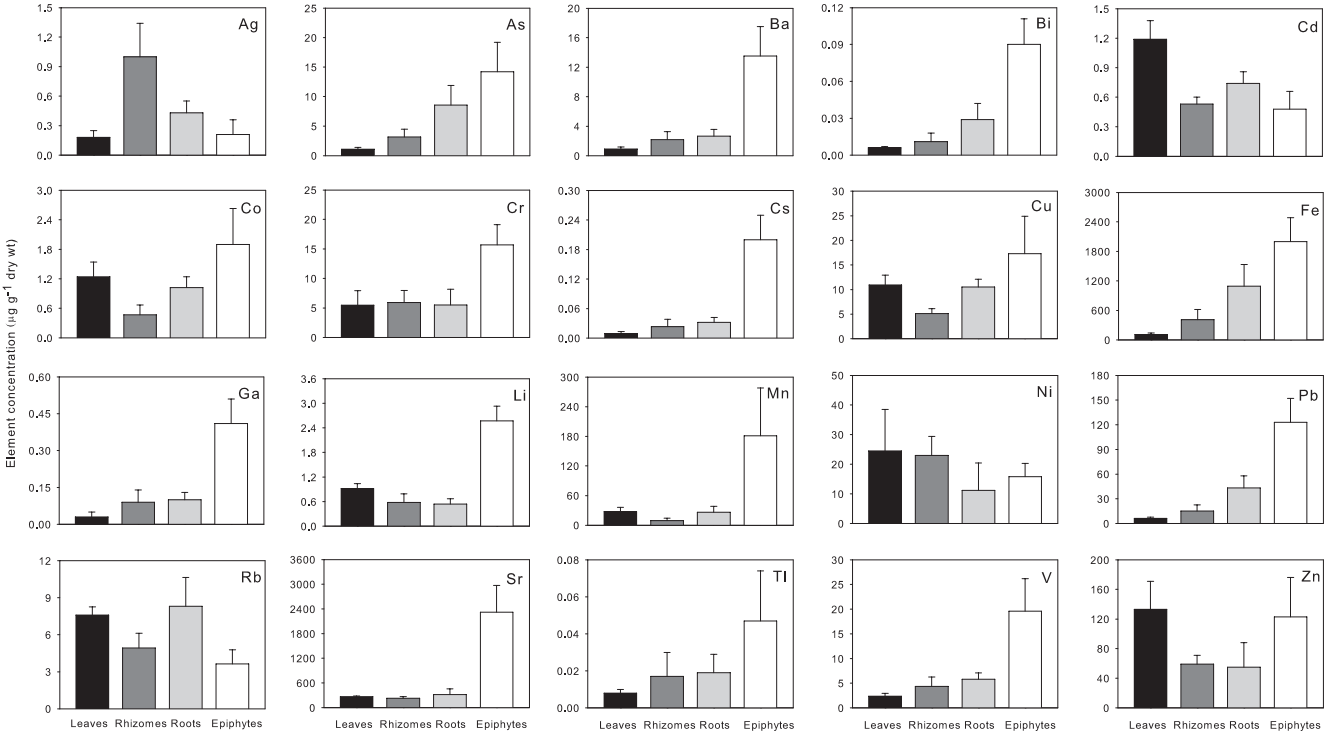

Fig. 1. Annual trace element concentration ($\mu g\,g^{-1}$ dry wt; mean $\pm$ SD) in the compartments of *Posidonia oceanica* (leaves, rhizomes, roots and epiphytes). The letters on the right top of each graph indicate the trace element.

horizontal rhizome biomass per cm of rhizome and apex density at the study area. Data on apex density were obtained from measurements at the same *P. oceanica* meadow in June 2003 (Apostolaki et al., 2009a). Similarly, annual vertical rhizome production rate (27.96 g dry wt m^{-2} yr^{-1}), obtained from Marbà et al. (2006), was calculated as annual vertical rhizome elongation multiplied by the vertical rhizome biomass per cm of rhizome and mean shoot density during the study.

Annual root production rate (25.81 g dry wt m^{-2} yr^{-1}) was estimated by multiplying the maximum root biomass measured during this study with the mean root turnover (0.13 yr^{-1}) estimated for *P. oceanica* Duarte et al., 1998).

Leaf shedding rate (g dry wt m^{-2} d^{-1}) was calculated at each sampling event as the product of the number of leaf blades per shoot shed by senescence, the dry weight of the oldest leaf per shoot and the mean shoot density at the beginning and end of sampling interval, divided by the duration of sampling interval in days.

Leaf residual loss rate (g dry wt m^{-2} d^{-1}) at each sampling event was calculated from leaf production rate minus leaf shedding rate and minus the difference in standing leaf biomass during the sampling interval, divided by time in days (Cebrian et al., 1997).

Trace element incorporation rate (EIL_i; μg dry wt m^{-2} d^{-1}) in leaf tissue at each sampling event was estimated as

$$EIL_i = LP_i \times EC_i,$$

where LP_i is the leaf production rate (μg dry wt m^{-2} d^{-1}) at the corresponding sampling event and EC_i is the mean trace element concentration ($\mu g\,g^{-1}$ dry wt) in "new" leaf tissue at the corresponding sampling event. Then, the annual mean trace element incorporation rate (g dry wt m^{-2} yr^{-1}) is calculated as the mean of the trace element incorporation rates calculated at each sampling event.

Trace element incorporation rate for rhizomes and roots (EIR_i; g dry wt m^{-2} yr^{-1}) was estimated as

$$ERI_i = RP_i \times ECo_i,$$

where RP_i is either, rhizome and root production rate (g dry wt m^{-2} yr^{-1}), and EC_i is the corresponding annual mean trace element concentration ($\mu g\,g^{-1}$ dry wt). In the case of rhizomes, the trace element incorporation rate was calculated separately for the horizontal vertical rhizome production. Then, both are summed up to obtain the trace element incorporation rate in rhizomes.

Trace element loss rate through leaf shedding (ELS_i; μg dry wt m^{-2} d^{-1}) at each sampling event was estimated as

$$ELS_i = LS_i \times EC_i,$$

where LS_i is the leaf shedding rate (g dry wt m^{-2} d^{-1}) at the corresponding sampling event and EC_i is the corresponding trace element concentration (µg g^{-1} dry wt) of "old" leaf tissue at the corresponding sampling event. Then, the annual mean trace element loss rate through leaf shedding (g dry wt m^{-2} yr^{-1}) was calculated as the mean of the trace element loss rates at each sampling event.

Trace element residual loss rate (ERL_i; μg dry wt m^{-2} d^{-1}) at each sampling event was estimated as

$$ERL_i = RL_i \times EC_i,$$

where RL_i is the leaf residual loss rate (g dry wt m^{-2} yr^{-1}) at the corresponding sampling event and EC_i is the corresponding annual mean trace element concentration (μg g^{-1} dry wt) of leaf tissue at the corresponding sampling event. Then, the annual mean trace element residual loss rate (g dry wt m^{-2} yr^{-1}) was calculated as the mean of the trace element residual loss rates at each sampling event.

Trace element release rate through decomposition of *P. oceanica* detritus was estimated as the fraction of element released $k(d^{-1})$ according to this formula:

$$E_t = E_o e^{-kt},$$

where E_t (μg dry wt trace element bag^{-1}) is the element content at each retrieval event of the litter bag experiment, and was calculated by multiplying the weight of litter mass at that time with the corresponding trace element concentration of leaf litter. E_o (µg trace element bag^{-1}) is the initial element content and was calculated by multiplying the initial weight of litter mass with the corresponding trace element concentration of leaf litter. t is the time of retrieval (d) from the beginning of the experiment. k was calculated as the slope of the regression analysis between $\ln(E_t\ E_o^{-1})$ at each retrieval event and time elapsed since the start of the experiment. Estimates of detritus decomposition were obtained from Apostolaki et al. (2009b). We estimated annual trace element release rate through decomposition of *P. oceanica* detritus k (yr^{-1}) by multiplying the mean daily rate over a year with 365 days.

Annual trace element incorporation rate per shoot was calculated as the sum of annual incorporation rates in leaves, rhizomes and roots. The trace element accumulation excess was calculated as the element incorporation minus the sum of both element loss sources (shedding, as well as grazing and mechanical breakage).

The annual trace element budget at basin level was calculated by extrapolating the total coverage of *P. oceanica* in the Mediterranean, 50 000 km^2 estimated by Pasqualini et al. (1998).

2.5 Data analyses

A two-way factorial ANOVA was used to analyze the variability in trace element concentrations among plant compartments and time. The factors considered were compartment of the plant (four levels, fixed) and time (six levels, random). The independence of data among samples was checked by plotting the mean versus the standard deviation. Homogeneity of variances was tested by Cochran's C-test (Underwood, 1997). When variances were not homogeneous, data was ln(x+1) transformed. After transformation, some data was still not showing homogeneity of variances. In these cases, we analyzed the data untransformed, since ANOVA is considered robust to lack of homogeneity of variances with balanced designs and a considerable large amount of treatments (Underwood, 1997). ANOVA main effects may be difficult to interpret in the presence of statistically significant interactions (Underwood, 1997), but in mixed effect ANOVAs, the test of the fixed main effect is potentially interpretable even in the presence of an interaction (Quinn and Keough, 2002). Student–Newman–Keuls test was performed to check for *a posteriori* comparisons among levels after significant main effects in ANOVA.

Two principal component analysis (PCA) were performed to identify patterns in element concentrations among *P. oceanica* compartments through time. Both PCAs were based on a matrix with the concentrations of the elements (samples) on the plant compartment for each sampling event (variables). Data was previously normalized (for each variable, values had their mean subtracted and were divided by their standard deviation) since element concentrations had different scales (Clarke and Gorley, 2006). One PCA was performed with all plant compartments to investigate the general element concentration patterns among all the compartments of *P. oceanica* through time. The other PCA comprised only the compartments of the plant that are physiologically connected, i.e. leaves, rhizomes and roots. This second PCA was intended to investigate element translocation and discerned which elements showed similar accumulation patterns among plant compartments that are physiologically connected.

PERMANOVA was applied to compare the concentration patterns of the different trace elements in the compartments of the plant and along time using the complementary package PERMANOVA + (v. 1) of the Primer software. The PERMANOVA design was the same as the ANOVA and was performed based on resemblance matrices calculated using Euclidean distances. Prior to the PERMANOVA routine, a PERMDISP (Distance-based test for homogeneity of multivariate dispersions) analysis was used to measure the dispersion of the data for each factor independently, which is equivalent to an analysis of the homogeneity of variances in the univariate analyses. After checking that the results of the PERMDISP indicated that the dispersion of the data was homogeneous, then the PERMANOVA analysis was performed. Both analyses comprised 9999 permutations. Multivariate analyses were done with Primer (v6) software package. All statistical tests were conducted with a significance level of α=0.05 and data was reported as mean $\pm$ standard deviation (SD).

Table 2. Two-way factorial ANOVA on differences in trace element concentration of *Posidonia oceanica* among the factors, *compartment of the plant* (df = 3) and *time* (df = 5) and the interaction between them (df = 15). Le: leaves; Rh: rhizomes; Ro: roots; Ep: epibiota.

	Compartment of the plant		Time		Interaction		SNK test
	MS	*F*	MS	*F*	MS	*F*	
Ag	2.6	35.8***			0.07	2.6**	Rh > Ro > Ep = Le
As #	12.8	56.1***			0.2	7.2***	Ep > Ro > Rh > Le
Ba #	12.2	113***			0.109	2.1*	Ep > Ro > Rh > Le
Bi	0.02	89.8***			0.0003	2.2*	Ep > Ro > Rh = Le
Cd	1.8	56.6***	0.01	0.4	0.03	1.7	Le > Ro > Rh = Ep
Co	6.2	12.0***			0.5	12.6***	Ep > Le > Ro > Rh
Cr	412	43.7***	5.6	0.6	9.4	1.5	Ep > Rh = Ro = Le
Cs	0.1	110***			0.001	2.1*	Ep > Ro = Rh > Rh = Le
Cu	430	18.1***			24	2.8**	Ep > Le = Ro > Rh
Fe #	28.4	106***			0.3	3.9***	Ep > Ro > Rh > Le
Ga	0.5	188***	0.004	1.4	0.003	0.7	Ep > Ro = Rh > Le
Li #	2.5	162***			0.02	2.9**	Ep > Le > Rh = Ro
Mn #	24.5	33.4***			0.7	16.5***	Ep > Le = Ro > Rh
Ni	686	6.3**	110	1.0	109	1.4	Le = Rh > Ep = Ro
Pb #	26.0	72.1***			0.4	7.8***	Ep > Ro > Rh > Le
Rb	82.8	35.8***	3.0	1.3	2.3	1.1	Ro = Le > Rh > Ep
Sr	17 166 117	65.8***			262 279	7.4***	Ep > Ro = Le = Rh
Tl	0.005	21.5***	0.0004	1.6	0.0002	1.1	Ep > Ro = Rh = Le
V #	9.7	80.7***			0.1	2.4*	Ep > Ro > Rh > Le
Zn	30 925	19.3***	3017	1.9	1608	1.5	Le = Ep > Rh = Ro

= ln(x+1) transformation
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

3 Results

Annual concentrations showed very different scales among trace elements, varying from thousands (Sr and Fe) to thousandths (Bi, Cs and Tl) of µg g^{-1} dry wt (Fig. 1). Different trace elements exhibited contrasting accumulation patterns among *P. oceanica* compartments (Fig. 1; Table 2). All trace elements showed significant ($p < 0.01$) differences on trace element concentrations among plant compartments, while there were no significant (p>0.15) temporal variations for Cd, Cr, Ga, Ni, Rb, Tl and Zn. For Ag, As, Ba, Bi, Co, Cs, Cu, Fe, Li, Mn, Pb, Sr, and V; the main effect *time* could not be tested because there was an interaction between the two factors (*compartment of the plant* and *time*; Table 2).

The PCA plot based on trace element concentrations in all *P. oceanica* compartments grouped samples according to plant compartments. PC1 and PC2 explained, respectively, 68 % and 14 % of the variation. Epiphyte samples stood out from the rest of the plant compartments along PC1. Leave samples were at the opposite position from rhizome and root samples for PC2. Epiphytes were the plant compartment that showed the greatest temporal variation (Fig. 2).

The main test of the PERMANOVA showed significant differences for the factor *compartment of the plant* ($p = 0.0001$). Since the interaction between both factors was significant ($p = 0.0002$), the other factor, *time*, could not be tested. The pair-wise test of the factor *compartment of the plant* was significantly ($p = 0.0001$) different for all plant compartments, showing that trace element concentration among plant compartments was highly dependent on the trace element.

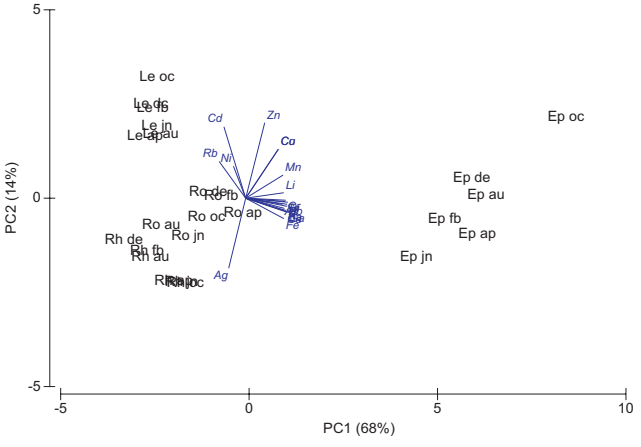

Fig. 2. Principal component analysis (PCA) of *P. oceanica* compartments for each sampling event based on element concentrations. Le: leaves; Rh: rhizomes; Ro: roots; Ep: epiphytes; jn: June 2006; au: August 2006; oc: October 2006; de: December 2006; fb: February 2007; ap: April 2007. Note that the names of some trace elements are plotted very close to each other, this is the case of Co and Cu; and As, Ba, Bi, Cr, Cs, Fe, Ga, Pb, Sr, Tl, and V.

The epiphyte samples showed significantly ($p < 0.05$) highest concentrations in most elements (As, Ba, Bi, Co, Cr,

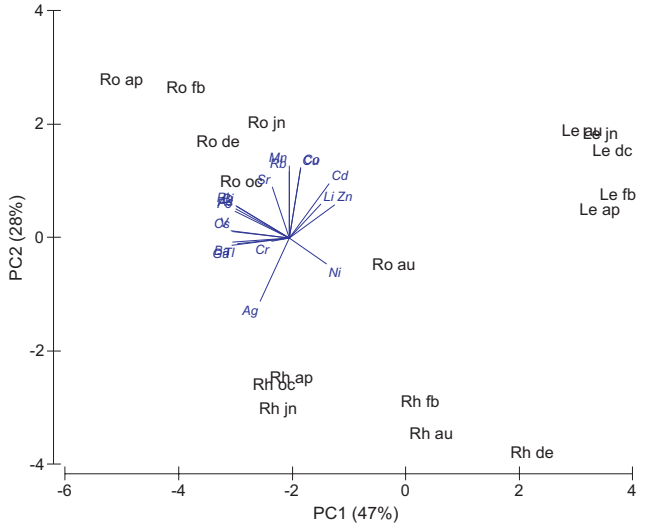

Fig. 3. Principal component analysis (PCA) of *P. oceanica* compartments that are physiologically connected (leaves, rhizomes and roots) for each sampling event based on element concentrations. Acronyms are explained in the caption of Fig. 2. Note that the names of some trace elements are plotted very close to each other, this is the case of (from top to bottom in a counterclockwise direction) Co and Cu; Mn and Rb; As, Bi, Fe and Pb; Cs and V; Ba, Ga and Tl.

Cs, Cu, Fe, Ga, Li, Mn, Pb, Sr, Tl and V; Figs. 1 and 2; Table 2). Out of these trace elements, As, Ba, Bi, Cr, Cs, Fe, Ga, Pb, Sr, Tl and V showed the lowest concentrations on leaves, while Co, Cu, Li, Mn and Zn showed, to some extent, high concentrations on leaves. Cd, Ni and Rb were the elements which had the highest concentrations in leaves. Ag had significantly ($p < 0.05$) greatest concentrations in rhizome samples, although the concentration of this element in roots was relatively high (Figs. 1 and 2).

The PCA that only comprised leaves, rhizomes and roots grouped samples mainly according to plant compartments. Roots and rhizomes showed higher temporal variation in trace element concentration compared to leaves (Fig. 3). PC1 explained 47 % of the variation and gathered, on the one hand, all leave samples and rhizome samples from February, August and December, and, on the other had, root and the remaining rhizome samples. PC2 explained 28 % of the variation and grouped, on the one hand, leave and most root samples, and, on the other had, rhizome samples and the root sample from August (Fig. 3). The PCA of *P. oceanica* compartments that were physiologically connected plotted same elements very close together, indicating very similar accumulation patterns among these plant compartments. This was the case for: Co and Cu; Mn and Rb; As, Bi, Fe and Pb; Cs and V; Ba, Ga and Tl. In contrast, Ag and Ni showed particular accumulation patterns that markedly differed from the rest of the elements (Fig. 3).

Zn, Cd, Sr and Rb showed the highest release rates through decomposition of *P. oceanica* detritus, while Cs, Tl and Bi showed the lowest (Table 1S, see Supplement). According to the obtained budget, Fe was the trace element with the greatest incorporation rate (37 815µg dry wt m^{-2} yr^{-1}), followed by Ni, Cr, As, Ag and Cs. Sr was the trace element with the highest release rate (35081 µg dry wt m^{-2} yr^{-1}) followed by Zn, Mn, Cu, V, Co, Rb, Cd, Pb, Li, Ba, Ga, Bi and Tl (Table 1S, see Supplement). The estimates of *P. oceanica* annual trace element release and incorporation for the Mediterranean basin were considerably high for some elements such as Sr (1 754 053 kg yr^{-1}) and Fe (1 890 708 kg yr^{-1}), respectively (Table 3).

Table 3. Annual amount of trace element accumulation excess (kg yr^{-1}) by *P. oceanica* for the Mediterranean basin according to the estimates of the total cover of *P. oceanica* (50 000 km^2) (Pasqualini et al., 1998). Positive or negative values indicate either incorporation or release by *P. oceanica*, respectively.

Trace element	Annual accumulation excess
Fe	1 890 708
Ni	174 804
Cr	30 000
As	4591
Ag	3605
Cs	73.92
Tl	−29.59
Bi	−63.93
Ga	−225.0
Ba	−3074
Li	−5636
Pb	−7895
Cd	−11 239
Rb	−19 262
Co	−21 067
V	−38 489
Cu	−45 097
Mn	−587 261
Zn	−1 459 340
Sr	−1 754 053

4 Discussion

Trace elements are toxic above certain concentrations on marine life (Alloway 1995). We choose the studied trace elements since all of them are potentially toxic above natural concentrations and because some of them are essential for organisms.

Trace element concentrations in this study were mainly within the range of values reported for *P. oceanica* in previous studies (Table 4). Cr and Pb were the only elements whose concentrations were generally high compared to previously reported data (Table 4). The only source of pollution that, to the best of our knowledge, is close to the studied site is a fish farm (1 km away). The studied site has been used in many studies as a reference site and widely proved not

to be affected by this facility (e.g. Apostolaki et al., 2011a). Besides, concentrations of Cr and Pb in *P. oceanica* compartments at 20 m from the fish farm were similar or moderately lower than in the studied site (unpubl. data.). Furthermore, Cr and Pb are not pollutants derived from fish farming (Dean et al., 2007; Sanz-Lázaro et al., 2011). Thus, the relatively high concentrations found in this experiment may be due to an unknown diffuse source of these trace elements or because the basal level of the area has naturally high Cr and Pb levels.

Some trace elements for which we did not find previously reported concentrations in *P. oceanica* (As, Ba, Li and Sr), the values reported here were within the same range of other seagrasses, such as *Thalassia testudinum* (Whelan et al., 2005) and *Zostera capricorni*, (Sanchez-Jerez et al., 2002). For Bi, Cs, Ga, Rb, Tl and V, we did not find previous reported concentrations in seagrasses. The compartments which showed the greatest lack of data on trace element concentrations were the roots and epiphytes (Table 4). Thus, this study fills this gap, providing novel data on trace element concentrations in *P. oceanica* compartments, which helps to understand their cycling dynamics in seagrass meadows.

Marine macrophytes, i.e. seagrasses and macroalgae, accumulate trace elements, but seagrasses, opposed to macroalgae, have a well developed belowground system. On one hand, this detritus is very recalcitrant and can form mattes where roots and rhizomes *P. oceanica* can persist for thousand of years (Mateo et al., 1997). Because of that, a fraction of the trace metals accumulated by *P. oceanica* is sequestered, reducing the total amount that is available to other organisms (Pergent et al., 1997). On the other hand, seagrasses, since they have roots, can also mobilize metals that are buried in the sediment (Amado et al., 2004). Depending on the plant compartment where the trace elements are mainly accumulated and on their incorporation and loss dynamics, *P. oceanica* can act as a sink or source of these elements.

Trace element concentrations were mainly dependent on plant compartment rather than on time, and the accumulation trends among plant compartments varied depending on each trace element (Figs. 1 and 2; Table 2). This differential accumulation patterns within plant compartments has been reported for some metals in *P. oceanica* (Catsiki and Panayotidis, 1993; Schlacher-Hoenlinger and Schlacher, 1998; Sanchiz et al., 2000) and other seagrasses (Lyngby and Brix, 1984; Llagostera et al., 2011). We found preferential accumulation of Cd and Zn in the above-ground plant parts compared to roots (Pergent-Martini and Pergent, 2000 and references therein).

Cd, Cu and Zn had a higher accumulation in the leaves than in the rhizomes (Sanchiz et al., 2000; Campanella et al., 2001; Tranchina et al., 2005), while Cr, Fe and Pb showed an opposed trend (Lewis and Devereux, 2009). The accumulation dynamics in *P. oceanica* compartments (leaves, rhizomes, roots and epiphytes) agreed to some extent with Schlacher-Hoenlinger and Schlacher (1998) for Cd, Pb and Zn, while for Cu it was totally different. Differences in trace element accumulation among compartments within studies could be due to differences in the relative bioavailability of trace elements in the sediments and the water column (Malea et al., 2008).

Trace element uptake and translocation in seagrasses differs depending on the trace element and plant tissue. This specificity depends on the chemical properties of each trace element (Pulich, 1985). We found that some groups of trace elements have similar accumulation patterns. This was the case for Co and Cu; Mn and Rb; As, Bi, Fe and Pb; Cs and V; Ba, Ga and Tl (Fig. 3). Thus, trace elements within each of these groups are expected to have very similar uptake and translocation pathways in *P. oceanica*. On the other hand, Ag and Ni showed very different accumulation dynamics with the rest of the trace elements, indicating unique uptake and translocation dynamics.

Element translocation dynamics in seagrasses are hard to elucidate since seagrasses take up trace elements by leaves and roots (Schroeder and Thorhaug, 1980). Thus, trace elements that mainly accumulate in the rhizomes are expected to have a high translocation rate either from leaves and/or roots. In this experiment, this was only the case for Ag (Fig. 1; Table 2). Also, trace elements that have similar concentrations in all the compartments of the plant that are physiologically connected are also expected to have a considerable translocation rate. This was the case for Cr and Sr.

For some trace elements, accumulation trend followed the order: roots > rhizomes > leaves. These were: As, Ba, Bi, Cs, Fe, Ga, Pb, Tl and V. While other trace elements accumulated in the order: leaves > roots > rhizomes. These were: Cd, Co, Cu and Mn (Fig. 1; Table 2). These observations seem to indicate that, for most analyzed trace elements, translocation was low and acropetal. In fact, under oligotrophic scenarios such as the Mediterranean sea, seagrass root uptake of nutrients may notably exceed leaf uptake (Stapel et al., 1996). Thus, acropetal translocation is expected to be the main direction of element translocation.

Among all *P. oceanic*a compartments, epiphytes showed the greatest, while leaves showed the lowest temporal variation on element concentrations (Figs. 2 and 3). Temporal variation of trace element concentrations in plant compartments among seagrasses is common, even though variations are not necessarily significant (Malea and Haritonidis, 1999; Pergent-Martini and Pergent, 2000). In this study, there were no significant differences on trace element concentrations among sampling events for all the trace elements for which the main effect *time* could be tested. In the case of trace element variation among plant compartments, there were significant differences among plant compartments for all trace elements (Table 2). Furthermore, PCA plot showed that samples were mainly grouped according to plant compartment than to sampling events (Fig. 2). So, although the concentrations of trace elements showed temporal variations to some

Table 4. Trace element concentration ($\mu g\,g^{-1}$ dry wt) in *P. oceanica* compartments. Data is given as range of the means, mean ± SD or just the mean, depending on the data availability. The symbol "∼" indicates that the value is approximated because it was estimated from a graph.

References[a]	Compartment	Location	Trace element									
			Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
1	Leaves	Sounion, Greece	0.18 ± 0.07	1.19 ± 0.19	1.24 ± 0.3	5.46 ± 2.47	10.9 ± 2	105 ± 35	27.5 ± 8.7	24.5 ± 14	6.12 ± 1.6	133 ± 38
2	Leaves	France & Italy	–[b]	2.1–5.38	1.7–12.1	0.2–1.27	–	–	–	27.47–60.3	1.4–1.8	–
3	Leaves	Corsica, France	–	1.47–3.97	1.83–7.73	0.15–1.07	–	–	–	14.6–48.73	1.30–3.37	–
4	Leaves	Corsica, France	–	2.8 ± 0.9	–	1.6 ± 1.5	11.1 ± 6.5	–	–	22.9 ± 10.2	5.2 ± 3.8	109.3 ± 41.1
5	Leaves	Ischia, Italy	–	∼ 1.05	–	–	14	–	–	–	3	167
6	Leaves	Antikyra Gulf, Greece	–	2.7–44.0	–	–	2.8–148	164–815	–	–	10.5–123	27.1–97.7
7	Leaves	Sicily, Italy	–	5.98 ± 1.64	–	0.35 ± 0.11	31.88 ± 15.8	–	–	–	2.29 ± 1.56	213 ± 47
8	Leaves	Sicily, Italy	–	2.42 ± 1.17	–	0.11 ± 0.03	11.7 ± 4.58	–	–	–	1.94 ± 1.67	70.9 ± 31.2
9	Leaves	Sicily, Italy	–	1.13–3.03	–	0.31–0.94	5.7–20.2	–	–	–	0.7–10	105–155
10	Leaves	Sicily, Italy	–	1.2–3.4	–	–	8.4–15.3	–	–	–	5.8–12.5	213–676
11	Leaves	Aegean Sea, Greece	–	–	–	1.75–5.73	7.67–13.7	–	–	19.1–30.7	–	–
12	Leaves	Spain	–	∼ 2.2–∼ 25	–	–	–	–	–	–	∼ 1–∼ 31	∼ 100–∼ 700
1	Rhizomes	Sounion, Greece	1 ± 0.34	0.53 ± 0.07	0.47 ± 0.2	5.93 ± 2.04	5.1 ± 1	411 ± 209	9.1 ± 5	23 ± 6.4	15.2 ± 7.5	59 ± 12
5	Rhizomes	Ischia, Italy	–	0.63	–	–	17	–	–	–	∼ 12	60
9	Rhizomes	Sicily, Italy	–	0.40–1.16	–	0.91–1.29	6.6–15.3	–	–	–	2.81–16.86	41–140
10	Rhizomes	Sicily, Italy	–	0.45–2.44	–	–	7.6–14.6	–	–	–	4–6.1	135–421
11	Rhizomes	Aegean Sea, Greece	–	–	–	1.05–5.93	3.44–10.1	–	–	9.24–17.7	–	–
12	Rhizomes	Spain	–	∼ 0.6–∼ 2.0	–	–	–	–	–	–	∼ 1.5–∼ 7.5	∼ 20–∼ 75
13	Rhizomes	Balearic Islands, Spain	4.66–16.08	0.72–1.13	0.22–0.86	0.24–1.06	9.41–15.22	48.5–190.9	4.22–16.16	3.66–17.05	0.45–8.89	23.4–49.3
14	Rhizomes	Gulf of Naples, Italy	–	0.25–1.6	–	–	6.0–62	100–600	4–23	–	0.15–1.25	20–220
1	Roots	Sounion, Greece	0.43 ± 0.12	0.74 ± 0.12	1.02 ± 0.22	5.52 ± 2.66	10.5 ± 1.6	1092 ± 444	26.4 ± 11.6	11.2 ± 9.2	43.1 ± 14.7	55 ± 33
5	Roots	Ischia, Italy	–	1.23	–	–	27	–	–	–	∼ 4	∼ 75
11	Roots	Aegean Sea, Greece	–	–	–	2.66–4.84	6.23–10.6	–	–	7.96–13.4	–	–
12	Roots	Spain	–	∼ 0.6–∼ 2.1	–	–	–	–	–	–	∼ 4–∼ 23	∼ 25–∼ 90
1	Epiphytes	Sounion, Greece	0.21 ± 0.15	0.48 ± 0.18	1.9 ± 0.73	15.7 ± 3.4	17.3 ± 7.6	2000 ± 484	181 ± 97	15.8 ± 4.5	123 ± 29	123 ± 53
5	Epiphytes	Ischia, Italy	–	0.25	–	–	16	–	–	–	30	109

[a] References = 1, present study; 2, Lafabrie et al. (2007); 3, Lafabrie et al. (2008); 4, Gosselin et al. (2006); 5, Schlacher-Hoenlinger and Schlacher (1998); 6, Malea et al. (1994); 7, Conti et al. (2007); 8, Conti et al. (2010); 9, Campanella et al. (2001); 10, Tranchina et al. (2005); 11, Catsiki and Payanotidis (1993); 12, Sanchiz et al. (2000); 13, Tovar-Sánchez et al. (2010); 14, Ancora et al. (2004)
[b] = no data

extent, plant compartment was the main driver of trace element concentrations.

Accumulation of trace elements was significantly higher in epiphytes for most trace elements (Table 2). High concentrations in epiphytes may be due to its great accumulation capacity of trace elements (Sanz-Lázaro et al., 2011), but also to seagrass leaching of elements through leaves, which is a pathway to transfer elements from sediments to epiphytes (Mcroy and Goering, 1974) . Thus, epiphytes are expected to play a relevant role in the accumulation and transfer of trace elements in *P. oceanica* meadows. Epiphytes should be taken into consideration when studying trace element cycling in *P. oceanica* as well as in other seagrass meadows, since it is ubiquitous on the leaves of seagrass species.

Zn, Cd, Sr and Rb were the trace elements that showed the highest release rate through decomposition of *P. oceanica* detritus. Therefore, they are expected to be released in the *P. oceanica* meadow. In contrast to, Cs, Tl and Bi, which had the lowest release rate through decomposition. So, Cs, Tl and Bi are more likely to be exported to adjacent ecosystems.

Based on the mass balance analysis, this study shows that *P. oceanica* acts as a sink for Fe, Ni, Cr, As, Ag and Cs. Out of these elements, Fe had the highest incorporation rate. This is maybe because Fe is a micronutrient which normally limits primary production, specially in the Mediterranean (Marbá et al., 2007). The rest of the elements for which *P. oceanica* acts as a sink (Ni, Cr, As, Ag and Cs) are common pollutants at relatively low concentrations (Lewis and Devereux, 2009). According to our calculations, *P. oceanica* in the whole Mediterranean can sequester 174.8 t Ni in a year (Table 3). The high incorporation rate of some trace elements by *P. oceanica* in the Mediterranean basin (Table 3) points to the major role that *P. oceanica* may have in sequestering some potentially toxic trace elements, and reducing their bioavailability.

Even though, we acknowledge the limitations of doing an estimate for the whole Mediterranean based on just one meadow, this estimation can be a good starting point, since, to the best of our knowledge, it has never been done before for a wide set of trace elements. Furthermore, the studied *P. oceanica* meadow can be taken as representative of the Mediterranean for the following reasons. Firstly, the primary production rate of the *P. oceanica* meadow sampled ($377\,g\,dry\,wt\,m^{-2}\,yr^{-1}$) is close to the mean production rate of *P. oceanica* meadows in the Mediterranean ($352\,g\,dry\,wt\,m^{-2}\,yr^{-1}$) (Pergent et al., 1997). Secondly, the characteristics of the studied *P. oceanica* meadow, such as depth (14.5 m), mean shoot density ($312\,shoots\,m^{-2}$), biomass ($518\,g\,dry\,wt\,m^{-2}$), and mean rate of decomposition ($0.0033\,d^{-1}$ reported in Apostolaki et al. (2009b)) are similar to other *P. oceanica* meadows (Pergent et al., 1994, 1997).

Apart from the many important ecosystem functions that have been reported on *P. oceanica* (Hemminga and Duarte, 2000), the present study demonstrates that *P. oceanica* acts as a sink of potentially toxic trace elements. Further studies

should be done in other seagrass species, since they may also sequester trace elements, which can be potentially toxic.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/9/2497/2012/bg-9-2497-2012-supplement.pdf.

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