Using seagrasses to identify local and large-scale trends of metals in the Mediterranean Sea

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ABSTRACT

To manage trace metal pollution it is critical to determine how much temporal trends can be attributed to local or large-scale sources. We tracked changes in metal content in the seagrass *Posidonia oceanica*, along the NW Mediterranean from 2003 to 2010. While Cu, Cd and Ni showed a large inter-site variation, likely due to local factors, Fe, Mn and Pb showed little local variation and synchronous interannual variability across sites, most likely due to large-scale sources. Zn showed equal importance of local and large-scale sources of variation. Temporal trends of Ni, Zn, Cd, Cu remained almost stable. In contrast, Fe, Mn and Pb slightly increased in the last decade. These trends suggest that metals like Cu, Cd, Ni can be effectively managed at local scale. Whereas, elements like Fe, Mn and Pb have an important large-scale component that needs to be managed across the frontiers of national jurisdictions.

1. Introduction

Trace metals are naturally present in the environment at very low concentrations. However, centuries of intensive land use and industrialisation (agriculture, mining and industrial production) has released significant amounts of these elements from their reservoirs, making them increasingly ubiquitous, abundant and available for living organisms, dispersing through soils, the atmosphere and the hydrosphere (Callender, 2003; Sheppard, 1993). While some are essential micronutrients, critical to support plant metabolism (e.g. Fe, Mn, Co, Ni, Cu and Zn, Babula et al., 2008), others are highly toxic (e.g. Cd and Pb) or can become toxic at high concentrations (e.g. Cu and Zn). In addition, trace metals generally persist in the environment and tend to concentrate in organisms, becoming harmful for plants, animals and ecosystems, and can represent a serious threat to human health (Deboudt et al., 2004; Ikem and Egiebor, 2005; Islam et al., 2007; Stankovic and Jovic, 2012; Wang et al., 2013). There have been a host of studies examining the consequences of trace metal contamination on the environment, and there is a growing call for metal contamination to be properly monitored and controlled (Callender, 2003; Sheppard,

1993; Stankovic and Jovic, 2012). The ability to manage these contaminants is predicated on our ability to identify where they originate so they can be effectively controlled at the source itself (Chon et al., 2010). The principal difficulty with this is that metals disperse widely through the atmosphere and hydrosphere, with little respect for management units, such as countries or continents (Connan et al., 2013; Olid et al., 2010). To address this, many studies have focused on understanding dispersion patterns of trace metals in order to track them back to their sources (Ansari et al., 2004; Callender, 2003; Sañudo Wilhelmy et al., 1996; Sheppard, 1993). While these approaches are encouraging, the inherent variability associated with trace metal diffusion makes it very complex to model them accurately. In addition, these models are often impractical to employ within standard management programmes. The primary challenge for ecosystem managers is to identify the spatial scale of contamination (local, regional or even global) and to track changes through time. This will help design adequate interventions at the appropriate scales and evaluate their effectiveness.

These problems are particularly acute in marine coastal environments situated at the very end of watersheds. They are the downstream

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recipients of trace metal pollution through rivers, streams and city runoff (Migon, 2005; Oursel et al., 2013; Tiefenthaler et al., 2008). To curb their harmful effects, there have been huge investments in reducing metal concentration, especially in industrial wastewater (Chon et al., 2010). Evaluating the effectiveness of these management actions is complicated by the fact that metal can accumulate either in the water column or in sediments and their sources can be either locally or globally driven. Moreover, it is not easy to predict their dispersion patterns, since they are often controlled by the interaction of processes occurring at different spatial scales. Local sources of pollution include industrial sewage, harbours, polluted streams, storm-water drains and hydrothermal vents, among others (Nicolau et al., 2012; Sañudo Wilhelmy et al., 1996; Tovar-Sánchez et al., 2010; Vizzini et al., 2013). In addition, trace metals can travel long distances through the atmosphere and major rivers until they are finally deposited hundreds of kilometres away from their sources (Bruland et al., 2013). Once in the sea, metals accumulate in sediments that normally can act as a sink; when perturbed, however, sediments can become a significant source of historically accumulated metals. Interpreting metal contamination results are therefore complex, since it is difficult to separate recent contamination events from decades-old metal accumulation (Eggleton and Thomas, 2004; Roussiez et al., 2006). A given water body may additionally be contaminated with trace metals from both local and large-scale sources, and their concentrations will depend on how these contaminants behave in response to transport vectors operating at several different, potentially interacting scales.

The Mediterranean coastlines are among the most densely populated and developed in the world, with several large metropolises and industries concentrating along its shores. This highly transformed landscape is a rich source of contaminant trace metals which eventually find their way into the surrounding waters (Bethoux et al., 1990; Pastor et al., 1994; Turley, 1999). In the NW Mediterranean Sea, atmospheric and riverine sources (in that order) have been identified as the most important vectors of trace metal inputs from both natural and anthropogenic origins (Elbaz-Poulichet et al., 2001; Tovar-Sánchez et al., 2010). Differentiating between local and global sources for a given pollutant has major implications for how it is managed. While the inputs and distribution of persistent pollutants can be quite effectively controlled at local levels, a significant proportion of contaminants travel across the frontiers of national jurisdictions or state federations (e.g. between EU and non-EU countries). To effectively manage these remote pollution inputs requires much more trans-boundary interventions.

Bioindicators are widely used to detect variations in coastal ecosystem health (Ballesteros et al., 2007; Borja et al., 2003; Martínez-Crego et al., 2010). The millenary seagrass, *Posidonia oceanica* makes a particularly good bioindicator: is a widely distributed endemic species that has been demonstrated to be a very efficient and integrative tool both, in assessing changes in ecosystem conditions (Lopez y Royo et al., 2011; Pergent-Martini et al., 2005; Roca et al., 2015; Romero et al., 2016), as well as in tracking trace metal pollution in coastal areas (Di Leo et al., 2013; Llagostera et al., 2011; Martínez-Crego et al., 2008; Pergent-Martini, 1998; Richir et al., 2013; Roca et al., 2014). *Posidonia oceanica* tracks trace metals even better than discrete water chemical analysis (Niemi and McDonald, 2004) and is as good an indicator as filter feeders (Lafabrie et al., 2007). Several parts of the plants have been used to trace metal pollution, including leaves (Di Leo et al., 2013; Pergent-Martini, 1998; Richir et al., 2013), rhizomes (Martínez-Crego et al., 2008; Richir et al., 2013) and roots (Pergent-Martini, 1998).

In this study, we attempt to identify signals of local and large-scale sources of metal (Fe, Zn, Mn, Ni, Cu, Pb and Cd) pollution for a large coastal stretch of the NW Mediterranean (10 locations across the Catalan coast, ca. 700 km) using the seagrass *Posidonia oceanica*, over a period of seven years (2003 − 2010). We analysed the first centimetre of *P. oceanica* rhizomes (corresponding to approximately one year) as an integrative measure of trace elements in the surrounding

environment over the last year (Richir et al., 2013; Romero et al., 2007). We distinguished large-scale from local contaminant sources by analysing the variance in trace metal content levels between sites and years. Our central assumption is that large-scale and local trends should show distinct signatures in observed patterns of variation. Metals originating from large-scale trends will likely vary synchronically across sites, showing a temporal variability clearly higher than spatial variability. In contrast, metals derived largely from local sources will likely show highly site-specific temporal variability – in other words, a large part of the total variability will be due to differences *between* sites and to the interaction between sites and time. We explored these signals of large-scale and local variation using ANOVAs to partition spatial and temporal variability (and their interactions) for each metal that we monitored. In addition we used multivariate analyses to explore groups of behaviours in trace metal content from seagrass rhizomes and site specific trends of increase or decrease in contaminant values across this coastal stretch over the last decade.

2. Results

2.1. Co-variation in trace metal content

The Principal Component Analysis showed two groups of elements with significant covariation, the first included Fe, Mn and Pb, and the second Ni and Zn. The first group was associated with Principal Component 1 (PC1, 37% of the total variance explained) and the second with PC2 (29%). Cu was associated with PC3, while variability in Cd was almost evenly distributed between the three principal components (Fig. 2 and PCA weights in Table S2).

2.2. Variability patterns

All trace metals analysed showed statistically significant temporal (between years) and spatial (between site) variation, as well as a significant interaction between sites and years (Table 1). Cd, Ni and Cu showed a strong signal of inter-site variability, with the factor Site explaining 70, 49 and 59% of total variance respectively, and < 10% of variation explained by the factor Year (Table 1, Figs. 3, 4b, d, f). Cd showed the highest concentrations in sites D and E on the central coast, and Cu showed two peaks at sites B and C. The highest concentrations of Ni were found in site A. Concentrations of Zn were particularly prevalent at sites B and I, and while they appeared to derive from local sources, the factor Site accounted for considerably less of the variability (25%), and there was an important interaction between Site and Year, (Table 1, Figs. 3, 4h). The remaining trace metals showed a strong component of inter-annual variation associated with large-scale processes. In Fe and Mn the inter-annual variation represented 34 and 39% of total variance, respectively, (Fig. 3 and Table 1), varying synchronously between sites (Fig. 5a, c). Variability in the concentrations of Pb was fairly well distributed between sites, years as well as their interaction. Pb displayed a temporal trend similar to those observed for Fe and Mn (Table 1, Figs. 3, 5e).

2.3. Decadal tendency in trace metals in the Catalan coast

Fe, Mn and Pb concentrations showed an overall increase across the coast from 2003 to 2010, although this increase could not be detected in all sites (Table 2). In contrast, Zn, Cu, Cd and Ni concentrations did not show any clear temporal trend, although they decreased at some sites (Table 2).

3. Discussion

Concentrations of trace metals found in *P. oceanica* rhizomes along the Catalan coast, ranged between moderate to acute compared to levels recorded in less industrialized areas (Richir et al., 2013). For

Table 1
ANOVA results and percentage of variance explained by factors site, year, and their interaction for trace metals analysed in *Posidonia oceanica* rhizomes. DF: degrees of freedom, MS: mean squared error, σ^2 = sample variance, P = probability of error of rejecting the null hypothesis.

	Factor	% variance	DF	MS	σ^2	P
Fe	Site	23.3	9	73.75	2.205	< 0.001
	Year	33.6	5	191.45	3.677	< 0.001
	Interaction	12.0	45	7.59	0.781	< 0.001
	Error	31.1	240	3.68		
Mn	Site	18.8	9	0.09	0.003	< 0.001
	Year	38.8	5	0.35	0.007	< 0.001
	Interaction	16.2	45	0.01	0.002	< 0.001
	Error	26.2	240	0.00		
Pb	Site	16.8	9	0.27	0.000	< 0.001
	Year	19.6	5	0.58	0.000	< 0.001
	Interaction	18.7	45	0.06	0.000	< 0.001
	Error	44.9	237	0.02		
Cd	Site	70.3	9	2.41	0.002	< 0.001
	Year	6.2	5	0.38	0.000	< 0.001
	Interaction	7.8	45	0.05	0.000	< 0.001
	Error	15.6	240	0.02		
Ni	Site	49.5	9	5267.8	4.123	< 0.001
	Year	9.8	5	1844.0	0.692	0.001
	Interaction	16.9	45	361.5	1.114	< 0.001
	Error	23.7	238	96.3		
Cu	Site	59.7	9	0.33	0.010	< 0.001
	Year	7.7	5	0.07	0.001	0.003
	Interaction	16.4	45	0.01	0.003	< 0.001
	Error	16.3	240	0.00		
Zn	Site	25.3	9	3630	2.330	< 0.001
	Year	12.8	5	3290	1.137	0.005
	Interaction	29.1	45	833	2.739	< 0.001
	Error	32.8	240	176		

Table 2
Temporal trends in trace metal content in rhizomes, inferred from a linear regression over time. Regional trends ("All sites") and local trends (individual sites) are shown for each trace element. "NS" indicates a non-significant linear regression ($p > 0.05$), "+" significant slope (positive), "−" significant slope (negative).

Metal	All sites	A	B	C	D	E	F	G	H	I	J
Fe	+	+	+	NS	+	+	+	NS	NS	+	NS
Mn	+	+	+	NS	+	+	+	NS	NS	+	NS
Pb	+	+	+	NS	NS	NS	NS	NS	NS	+	NS
Cd	NS	NS	NS	−	−	NS	NS	NS	NS	−	NS
Ni	NS	NS	NS	−	NS	NS	NS	−	−	NS	NS
Cu	NS	NS	+	NS	NS	NS	NS	−	−	NS	NS
Zn	NS	NS	NS	−	NS	NS	NS	−	−	−	NS

instance, trace metal concentrations in our study are higher than those levels observed in Sicily (Bonanno and Di Martino, 2017) and in the industrialized area of Taranto (Di Leo et al., 2013). Monitoring trace metals that accumulate in seagrass rhizomes is a powerful integrative means to track change in pollution levels and to link them to local or large-scale sources. We found signals of both local and large-scale variation for all the trace metals we monitored in the rhizomes of the seagrass *Posidonia oceanica*. Elements clustered in two groups, each showing a distinct behaviour. While the variability in Cu, Cd and Ni content was mostly locally driven (> 65% of variability explained by the factors "Site" and "Site × Year"), the variability in Fe, Mn and Pb was mostly temporally driven (i.e. between-years). Zn showed an intermediate behaviour, with equal contributions of spatial and temporal variability. In addition, Fe, Mn, and Pb showed considerable spatial synchrony through time, indicating that they likely derive from a common, large-scale process. In contrast, the local variation observed in Cu, Cd and Ni was not spatially synchronised; each metal peaked at a different site. This indicates that they originate from local sources (catchment dynamics, sewage, resuspension, boating activity…) that vary for each element. The concentrations of many trace metals

remained stable through time (Ni, Zn, Cd, Cu) or decreased marginally at a few sites, which already showed some of the lowest values. In contrast Fe, Mn and Pb showed a slight increase over the last decade, mostly driven by an increase in 2010.

3.1. Site variation: detecting local contamination

The high between-sites variability we recorded in Cd, Ni, Cu and, to a lesser extent, Zn concentrations along the Catalan coast, suggests that these elements were derived from local sources. Although we cannot unambiguously identify what these sources are, there is compelling evidence linking local peaks of these elements with potential sources mainly through sewage or contact with harbours. For instance, the leaching of antifouling paints used on the hulls of boats and ships can often result in high levels of Cu in surrounding waters (Claisse and Alzieu, 1993). We recorded locally high concentrations of Cu in sites B and C, both of which were close (< 1 km) to heavily used harbours and marinas with a large number of recreational fishing and commercial moorings (Fig. 1 and Supplementary images). The vessels that used these harbours were, most likely, the source of the Cu we recorded at these sites. Zn was also likely influenced by marina activities, at least at one of the sites that showed the highest values (B), since Zn is widely used as an anticorrosive in iron alloys and antifouling paints (Marcus et al., 1988; Sørensen et al., 2009). Cd is a characteristic contaminant of the dyeing industry, combustion of urban wastes, ceramic varnishes and traffic wastes among other sources (Agarwal, 2009; Chon et al., 2010). In our study, this metal peaks in the areas north of the Barcelona province (F, E and D), which is characterised by a high industrial activity (particularly F) that pour sewage waters to coastal streams. A second area with high Cd values was observed in the southern part of the study region (I and J), very close to the industrial zone of Tarragona city. Ni peaks are often related to oil refineries, heavy fuel combustion emissions, and metallic and chemical industries (Chon et al., 2010; Pirrone et al., 1999). Surprisingly, in our study the station that had the highest concentrations of Ni, consistent in time, (A) is located within a Marine Protected Area, whose coast has low population densities and with no industrial activity within tens of km around it (ACA, 2005).

3.2. Spatial synchrony: signals of large-scale variation

Large-scale processes appeared to play a major role in influencing the variability of Fe, Mn and Pb whose concentrations varied synchronously across the coast. While we cannot unambiguously identify the origin of those large-scale variations our results suggest two potential origins: remote global contamination and large-scale environmental factors that modify bioavailability. Fe and Mn are characteristic components of Saharan dust, which is transported aerially to the Northern Mediterranean shores by North African advections. These advections are the main vectors of Fe and Mn into the NW Mediterranean sea, and are greater than riverine inputs (Guerzoni and Molinaroli, 2005; Guieu et al., 1997; Migon, 2005). In fact, previous studies using seagrass as bioindicators in the Mediterranean, also identified Saharian dust as a potential origin of observed global trends (Tovar-Sánchez et al., 2010). Although Pb is rare in Saharan dust, it also co-occurred with Fe and Mn in our observations (i.e. Pb interannual and spatial variation patterns were almost identical to those of Fe and Mn). Although we cannot claim this with any certainty, these trends can be linked to the widespread use of leaded fuel across north Africa at the time of the study (UNEP, 2008). It is more than probable that atmospheric Pb pollution could also be transported together with Fe and Mn, as has been observed in earlier studies (Guieu et al., 2010; Heimbürger et al., 2010). While these distant sources are the most likely explanation for these synchronous trends, we cannot discount the possibility of co-occurring large-scale environmental phenomena. For instance, the seagrass *P. oceanica* has been known to respond synchronically to large-scale phenomena that modify light and nutrient availability (Alcoverro
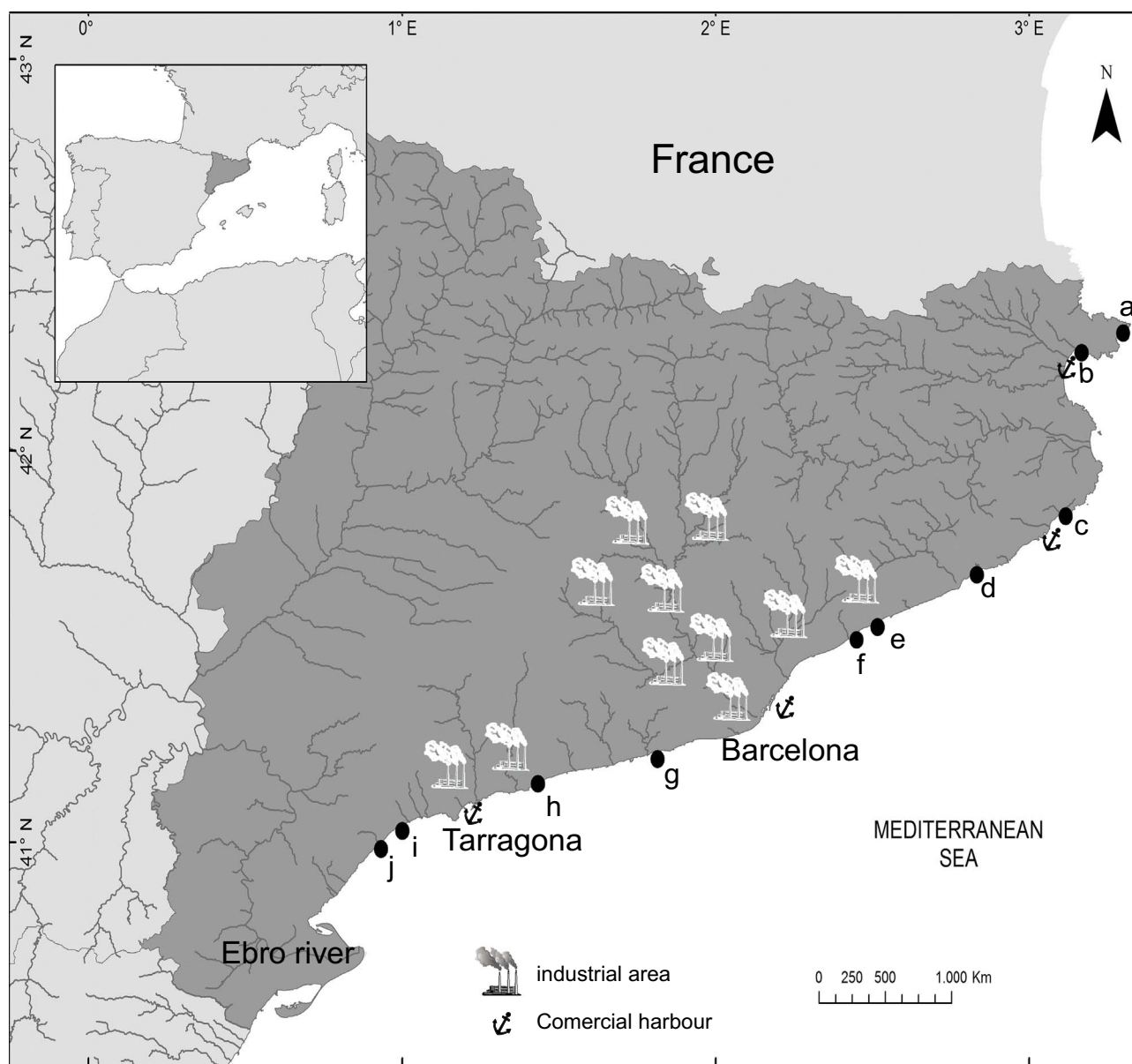
Fig. 1. Map of the study sites across the Catalan coast (NE Spain, Western Mediterranean).

et al., 1995). In addition, large regional storms that affect the entire coastline could potentially release accumulated metals from sediments, making them available for uptake (Calmano et al., 1993; Eggleton and Thomas, 2004). Both these scenarios could generate region-wide synchronicity in metals levels. However, if sediment resuspension were the unique driver of this trend, one would expect that the availability would also have an important site-specific variation depending on the local availability of the metal. The other trace elements we measured (Cd, Ni, Cu and Zn) also had a component of large-scale variation that is characteristic of European atmospheric pollution (Guerzoni et al., 1999; Guieu et al., 1991; Migon, 2005), which could eventually also be affected by large-scale processes affecting the sediment. However, the large-scale variability in the concentration of these elements, albeit significant, is largely overridden by site-level variability.

3.3. Tracking temporal trends in trace metals

Given the considerable efforts made across the region to reduce contamination, we would expect to record declining levels of toxic metals in these waters. In contrast though, we observed that

concentrations of some potentially toxic trace metals such as Ni, Zn, Cd and Cu, although decreasing at a few sites, remained, for the most part, stable over the monitoring period (2003 to 2010). This despite the implementation of a major industrial water treatment programme in the region in 2003 (ACA, 2003) that regulates industrial sewage for every industrial pollutant and has shown noticeable improvements in several other water quality parameters since its establishment (Roca et al., 2015). In addition, there was a significant downturn in industrial activity (specifically, in activities involving metal production or use) due to the economic crisis that occurred between 2008 and 2010 (IDESCAT, 2014), that has resulted in a marked reduction in atmospheric trace metal pollution across the region (Cusack et al., 2012). The fact that these factors did not reflect in large reductions in trace metals suggests that the potential reductions from these factors have either been overridden in most sites by other local processes or cannot be noticed due to a high persistence of trace metals in the coastal environment.

In contrast, we observed a significant increase in Fe, Mn, and Pb concentrations from 2007 onwards. This increase is mainly driven by the two relatively high values observed in 2008 and 2010, co-occurring
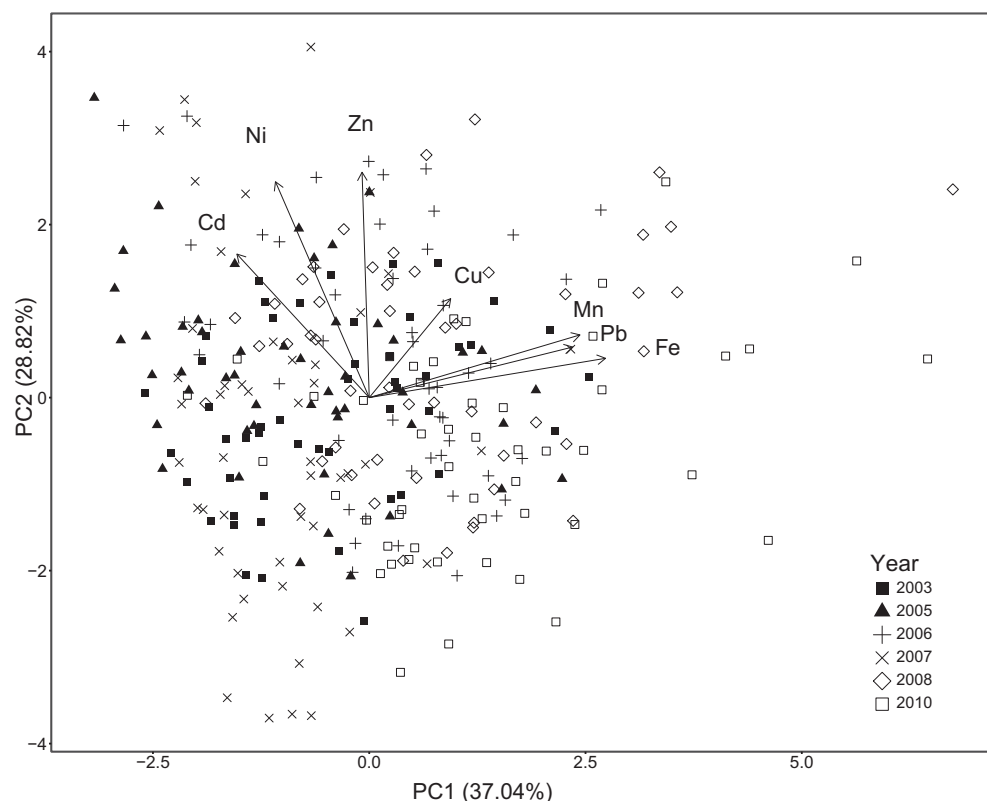
Fig. 2. Factor loadings of each trace metal in the Principal Component Analysis (components 1 and 2). Represented data is group by factor year.
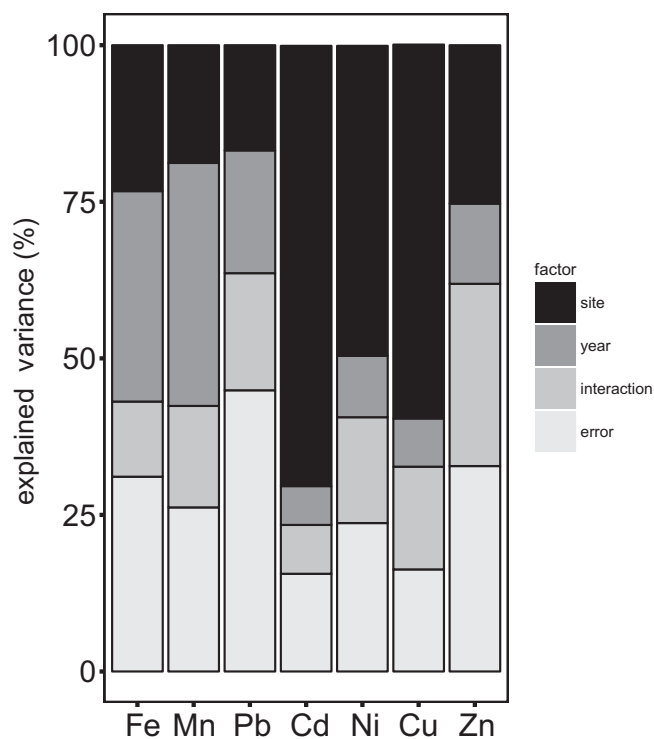

Fig. 3. Percentage of variation explained by the factors Site, Year, their interaction (Site × Year) and the error, for trace metal concentrations in *Posidonia oceanica* rhizomes.

in the three metals across most of the studied sites (6 out of 10 showed this significant increase, Table 2). As suggested above, Pb arrival has been linked to leaded fuel in African and eastern European countries (Tian and Ruiz-Pino, 1995). This large-scale influence indicates that, if we want to improve water quality effectively, controlling European

pollution, albeit commendable, is not enough, and that remedial actions should be taken at larger geographic and political scales. However, trends in Fe, Mn and Pb do not match neither the decrease in Saharan dust deposition observed in terrestrial collectors over the same period (Pey et al., 2013) nor the phasing out of leaded fuel between 2008 and 2010 in many north African countries (UNEP, 2008). This uncoupling can be attributed to the significant lag that may occur between trace metals first contaminating a water body and being finally assimilated within the benthos. This assimilation involves several physical and biological processes that could delay the integration of contaminants in the seagrass rhizome trough the sediment compartment (Migon, 2005; Schlacher-Hoenlinger and Schlacher, 1998).

4. Summary and conclusions

Our results underline the usefulness of seagrasses as indicators of metal pollution in coastal waters and the importance of maintaining large-scale, long-term monitoring networks to better understand the scale and the dynamics affecting the abundance and distribution of these pollutants. We recorded two distinct patterns of spatio-temporal variability in metal content: one in which spatial variability was prevalent (likely linked to local sources), and another dominated by high temporal variability with spatial synchrony (likely linked to large-scale processes). This highlights the need for adopting a dual strategy in controlling metal contaminants. While it is critical to implement local measures to control pollution, when the sources are overwhelmingly at larger scales, governments need to engage with trans-national strategies to control potentially harmful metals being transported from across state or continental borders (e.g. Pb from leaded fuels). Understanding the spatial scale of trace metal dispersion and accumulation in shallow coastal waters is key to taking management decisions that match that scale. The possibility of remedial actions lies in the hands of local managers only if the sources of pollution themselves are local, as observed for Ni, Zn, Cd, Cu. In contrast, local managers may be completely powerless to reduce contamination when its scale of variation is larger
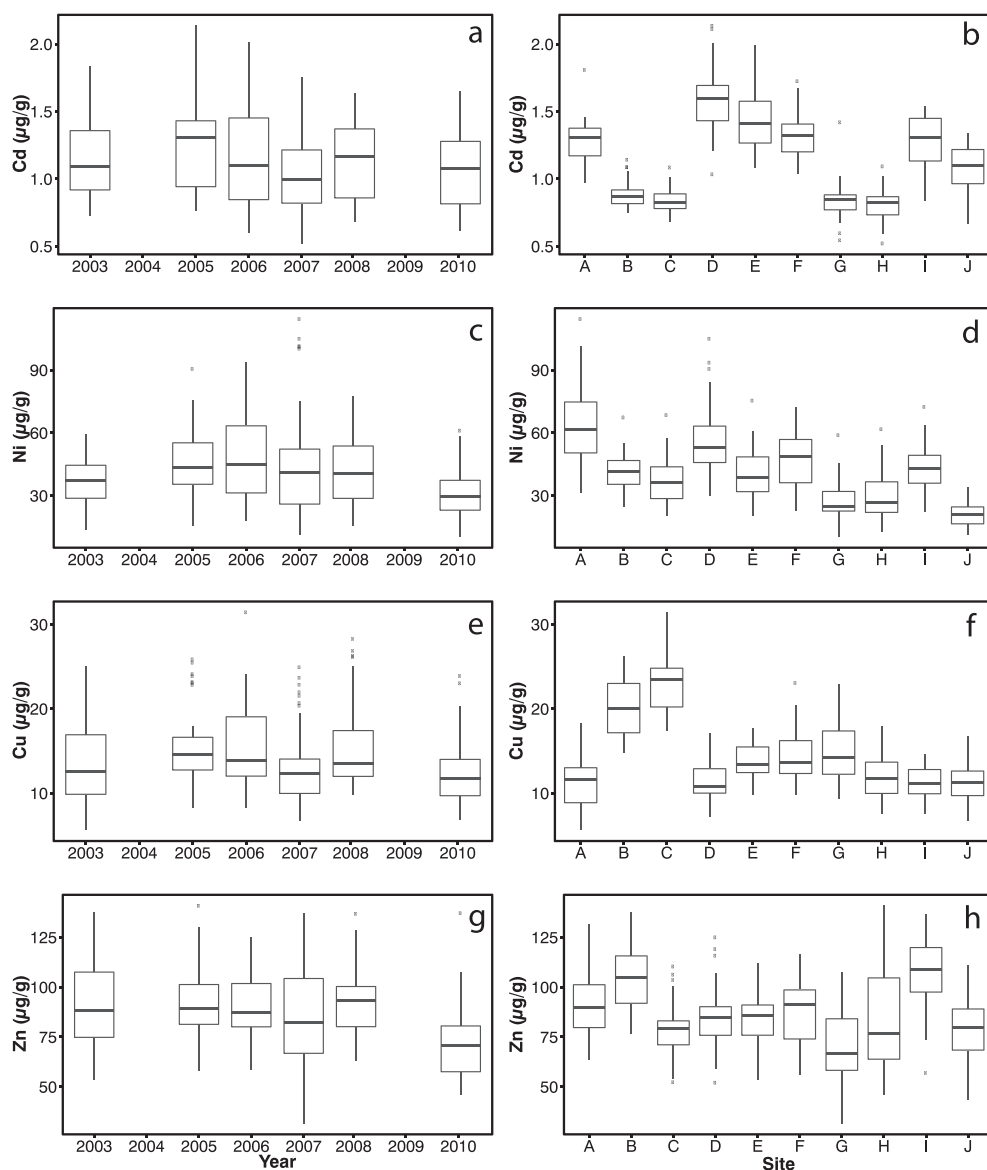
Fig. 4. Box-whisker plots showing temporal variation (left panel, a, c, e and g, all sites pooled) and spatial variation (right panel, b, d, f and h, all years pooled) of Cd, Ni, Cu and Zn in *Posidonia oceanica* rhizomes across the Catalan coast for the monitoring period (2003–2010).

and particularly if it originates outside management and political boundaries. Our results set clear limits on what regional or local management can hope to achieve with their interventions and identifies where global policy makers, politicians and governments need to take a more active role in addressing the pollution of the global commons.

5. Materials and methods

5.1. Study site and anthropogenic pressures

The study was conducted across an approximately 700 km stretch of the Catalan coast (NW Mediterranean) in NE Spain (Fig. 1). The coastal belt is densely populated with approximately 4.5 million people living in coastal municipalities, and is subject to high seasonal tourism pressure, with > 20 million tourists visiting the region per year (Gencat, 2012). Human pressures are unevenly distributed across the region; the central provinces of Barcelona and Tarragona have a dense concentration of large harbours, metropolises and industrial development. Additionally, a few commercial and many recreational harbours and marinas are distributed all along the coastline (ACA, 2005). Specifically, two of our sampling sites (B and C) are situated very close to the fishing and recreational harbours of Roses and Palamós. Other

anthropogenic pressures such as agricultural land use are common outside metropolitan areas. Climatically, the region is semi-arid, and most streams that reach the coast, have modest discharges (typically $< 20 \, \mathrm{m}^{-3} \, \mathrm{s}^{-1}$) and many of them are temporal watercourses. The one exception is the River Ebro that joins the sea at the southernmost end of the Catalan coastline, with very little influence on the study area.

5.2. Sampling design and data acquisition

The dataset used in this study was obtained from a monitoring programme from 2003 to 2010 that employed the seagrass *Posidonia oceanica* as a "biological quality element" to assess the ecological status of coastal waters within the Water Framework Directive. From the overall database, we selected sites that had been continuously monitored for the longest period to reliably detect temporal trends. These sites were evenly distributed across the coastal stretch (10 sites: A to J, Fig. 1). Sampling was conducted on SCUBA in late summer, once a year from 2003 to 2010, except 2004 and 2009. At each site 5 replicates of pooled rhizome material from 5 shoots were collected along a 50 m transect (35 shoots collected per site in total). Once in the laboratory, shoot rhizomes were separated, cleaned, and dried at 60 °C for two
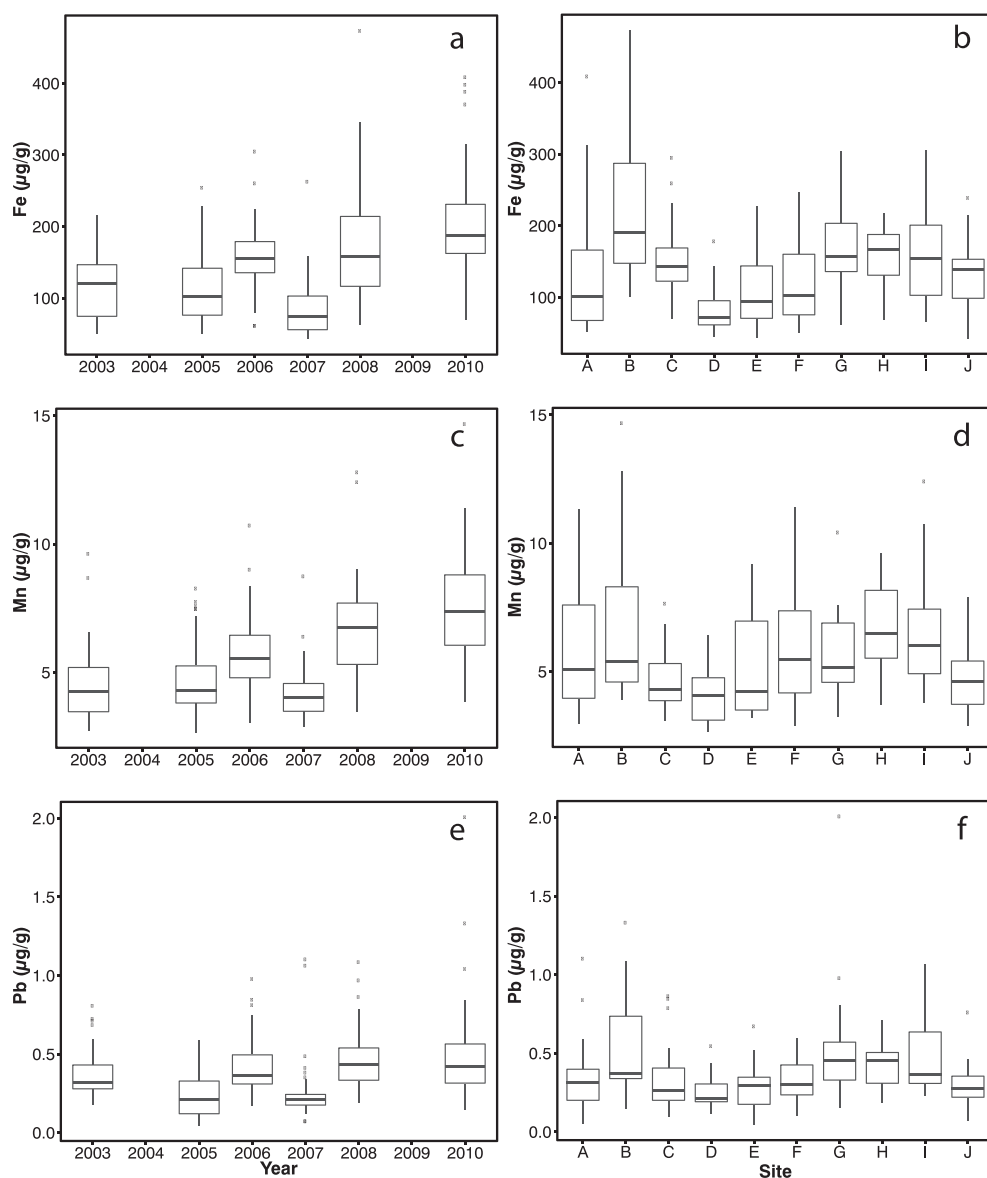
Fig. 5. Box-whisker plots showing temporal variation (left panel, a, c and e, all sites pooled) and spatial variation (right panel, b, d and f, all years pooled) of Fe, Mn and Pb in *Posidonia oceanica* rhizomes across the Catalan coast for the monitoring period (2003–2010).

days. The first 1 cm of each rhizome was cut and ground to a fine powder after having pooled together rhizomes from 5 shoots. We analysed metal concentration only in the first centimetre of newly-grown *P. oceanica* rhizomes because this portion corresponds to approximately the growth during the last year and contains trace metals accumulated during this period (Marbà and Duarte, 1998). Our data are thus an integrative measure of trace elements in the surrounding environment over the year previous to the sampling time (Richir et al., 2013; Romero et al., 2007). The concentration of seven trace metals in seagrass rhizomes was analysed using optic Inductively Coupled Plasma (ICP) (for Fe, Zn y Mn) and mass ICP (for Ni, Cu, Pb and Cd) after acid digestion (3 ml of HNO_3 65% + 2 ml H_2O_2 33%) of 0.05 g dry weight (DW) in Teflon containers for 16 h at 90 °C (modified from Romero et al., 2007). The analytical procedure was verified using standard reference material (*Ulva lactuca*, CRM 279). The processing and analysis of samples was carried out at Centre d'Estudis Avançats de Blanes (CEAB-CSIC) and at the scientific and technical services of the University of Barcelona (SCT-UB).

5.3. Data analysis

We examined different sources of variability for each trace metal using two-way ANOVAs with Year and Site as fixed factors. We employed a type (III) sums of squares using the car package (Fox and Weisberg, 2011) due to the presence of strong interactions. We calculated the percentage variance contributed by Site, Year and their interactions, dividing the variance of each factor by the total variance explained by the model and the error. Variance among sites was considered to be associated with local variability and variance between years was considered to be the result of large-scale (global patterns) variability (Alcoverro et al., 1995). We assume that high between-site variability is a signal of prevalent local sources of contamination while highly synchronous inter-annual variability signals predominant large-scale sources of contamination. This allowed us to grossly describe the temporal and spatial patterns of variability of trace metals, by comparing their relative contributions to total variability. Before analysis, we tested for assumptions of normality (Shapiro-Wilks test) and homogeneity of variances (Levene's test). Normality and homogeneity of variances was not achieved for all trace metals, even after transformation. To deal with heterogeneity of variances we examined the possibility of applying weights to our models using the gls function of nlme package (Pinheiro et al., 2017). However, due to the inability to effectively calculate type (III) sums of squares using gls models, and given that we worked with a large enough sample size ($N = 240$) we

assumed that the ANOVA *F* statistic is robust even when these assumption were violated (Underwood, 1981). We set the significance level to $p < 0.01$ to minimize the risk of type I errors. We used Principal Component Analysis to explore temporal and spatial correlations among trace metals using the PCA function of the FactoMineR package (Lê et al., 2008), and linear regressions to analyse temporal trends in trace metal. All statistics were performed in the statistical programme, R (R core team, 2012).

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.09.021.

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