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Trace metal distributions in Posidonia oceanica and sediments from Taranto Gulf (Ionian Sea, Southern Italy)

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Abstract

Distribution of metals (Hg, Pb, Sn, Cu, Cd and Zn) was determined in sediments and in different tissues of *Posidonia oceanica* collected from San Pietro Island, Taranto Gulf (Ionian Sea, Southern Italy). In seagrass, results, compared with metal concentrations in sediments, showed that the highest concentrations of Hg, Pb, Sn and Cu were found in the roots, while in the highest levels of Cd and Zn were found in the green leaves. In contrast, the lowest metal concentrations were found in the basal part of the leaf. Levels of metals in the leaves were similar to those found by other authors in uncontaminated areas of the Mediterranean Sea. Mercury levels in roots were correlated to levels in sediments. This could demonstrate that the plant memorizes sediment contamination. This study reinforces the usefulness and the relevance of *Posidonia oceanica* as an indicator of spatial metal contamination and an interesting tool for environmental quality evaluation.

Keywords: Heavy metals, Mediterranean Sea, *Posidonia oceanica,* Sediments.

Introduction

Coastal ecosystems are exposed to a wide variety of pollutants (Benoit $&$ Comeau, 2005). Of these, metals are among the most widespread and are increasingly reaching marine ecosystems, both from diffuse (including atmospheric) and point sources. Given their potential adverse effects on the environment, wildlife and human health, metals are thus of great concern.

There is currently a great interest in using living organisms as pollution biomonitors in aquatic ecosystems (Goldberg, 1986; Andersen *et al.,* 1996; Pergent-Martini & Pergent, 2000; Morillo *et al.,* 2005; Usero *et al.,* 2005; Demirezen & Aksoy, 2006), given that methods used previously, such as water chemical analysis, do not provide sufficient information on the bioavailability of metals found in the environment (Morillo *et al.,* 2005). In the Mediterranean Sea, the endemic seagrass *P. oceanica* (L.) Delile has been used as a metal bioindicator for two decades (Maserti *et al.,* 1988; Costantini *et al.,* 1991; Malea *et al.,* 1994; Capiomont *et al.,* 2000; Campanella *et al.,* 2001; Lafabrie *et al.,* 2008). The importance of this species lies in its extension, high productivity and stability, and also because its meadows function as spawning areas, hunting territory or permanent habitat for numerous plant and animal species (Ward 1987; Ballesta *et al.,* 2000; Ruiz & Romero 2003; Pergent-Martini *et al.,* 2006).

In addition, the community of organisms living in *P. oceanica* meadows is organized into numerous complex food webs, most of which begin with the consumption of *P. oceanica* leaves and the epiphytes which are associated with them (Ott, 1980). Since marketed species are among the known or potential grazers of *P. oceanica*, the study of metals in this species is not only of ecotoxicological interest, but also of public health concern.

Since the early 60s, the Northwest area of Taranto city (Apulia) has become one of the largest and most complex industrial sites in Europe, including several industrial plants, mainly consisting of an iron and steel factory, oil refinery, military base and many other industrial installations. This has led to growing concerns about the possible health effects caused by environmental exposure of residents until 1990, when the industrial area of Taranto, was declared an "area at high risk of environmental crisis", in accordance with Italian law 349 of 8/7/86.

Sampling sites are located a few kilometres southwest of this industrial area, and are examples of coastal environments with a relatively high influx of industrial effluents. The aim of our study was to evaluate the impact of these industrial discharges on the metal content of *P. oceanica*. Therefore, in this work Hg, Pb, Cd, Sn, Cu and Zn concentrations were determined in *P. oceanica* and in sediments collected at four stations along the San Pietro Island coastline (Taranto Gulf, Ionian Sea, Italy). Moreo-

ver, in order to identify bioaccumulation sites, metal distribution in different parts of the plant was determined. In addition, it was investigated whether a relationship exists between metal contamination in sediments and metal levels in plants.

Materials and Methods

Sampling

In the summer of 2009 samples of *P. oceanica* and sediment were collected at four stations in a meadow located along the San Pietro Island coastline (40° 15' N 17° 08' E) (Taranto Gulf, Ionian Sea, Southern Italy) (Fig. 1).

San Pietro Island, together with San Paolo Island, is part of the Cheradi Islands archipelago, located in the inner part of the Taranto Gulf (Ionian Sea), near the Mar Grande basin. The *P. oceanica* meadow covers a triangular shaped area, extending off the island of San Pietro, up to a maximum distance of about 2.5 km in a W and NW direction. However, it extends over 6 km from NW to SE, along the entire Southern coastline of the small archipelago. In recent decades, due to development and, in particular, the large industrial settlement, most of the seagrass meadow has regressed. In the past, the seagrass meadow stretched towards the coast, but now is limited to San Pietro Island. In the meadow, a mean density of 366 shoots /m2 was determined by Paterno *et al.* (1991). This corresponds to class III-IV, sparse or very sparse meadow according to Giraud's classification (1977) for *P. oceanica* meadows in the Mediterranean.

P. oceanica was collected at 10 ± 1 m depth by scuba divers. At each station, five plants were sampled. After collection, the plants were immediately transported, in polyethylene bags, to the laboratory. At the laboratory, epiphytes and sediment particles were carefully removed

from the samples by moderate scraping using a glass slide, according to the method described by Dauby and Poulicek (1995). Plants were divided into the following fractions: roots, rhizomes and leaves. In addition, the leaves were divided into green leaf (upper 5 cm) and basal part (bottom 5cm). Only mature leaves of comparable length were selected for seagrass analysis. For each station, all roots, rhizomes, green leaf and basal part of the leaves were pooled into four samples. Each sample was lyophilized and then manually reduced to a coarse powder.

Superficial sediments (5 cm) were collected in PVC cores by scuba divers. At each station, five samples were collected, then gathered in a single sample. Samples were stored in polyethylene bottles and frozen at -20°C until analysis. At the laboratory, sediment samples were defrosted at room temperature, dried at 30°C, up to a constant weight, ground and homogenized to a fine powder in a mortar. Particle-size analysis by dry sieving was then performed, according to the methods prescribed by the Romano & Gabellini (2001), using a sieve (Retsch AS 200).

Metal determination in sediments and plants

Mercury concentrations were determined with an Advanced Mercury Analyser (AMA-254, LECO, Stockport, Cheshire, UK), which allows a direct analysis without sample extraction procedure. The instrument consists of a nickel boat in a quartz combustion tube, containing a catalyst (cobalt oxalate and a mixture of manganese oxide, cobalt and calcium acetate) in which the solid sample (about 100mg of dried sample: sediments or plants) is initially dried prior to combustion at 750°C in an oxygen atmosphere. The mercury vapour which is produced is trapped on the surface of a gold amalgamator. After a specified time interval, the amalgamator is heated to 900°C, to release

Fig. 1: Location of the sampling stations.

the mercury which is transported to a heated cuvette (120°C), prior to analysis by AAS using a silicon diode detector at 253.6 nm (Costley *et al.,* 2000; Buccolieri *et al.,* 2006). The detection limit for mercury calculated on the basis of 10 determinations of the blanks as three times the standard deviation was 0.2 ng g^{-1} dry wt.

For the analysis of Cu, Pb, Sn, Cd and Zn about 0.5g of sample (sediments or plants) was digested with 10ml of concentrated $HNO₃$ in a closed teflon vessel using a MARSX microwave oven (CEM Corporation, Matthews, NC). For each digestion programme, a blank was prepared with the same amount of acid. After mineralization, digests were cooled and the resulting solutions were diluted to a known volume (50 mL) with Milli Q water and stored in polyethylene bottles, until analysis.

Cd, Cu, Pb and Sn were determined by graphite furnace atomic adsorption spectroscopy (GFAAS) using an Analyst 600 spectrophotometer (Perkin Elmer, USA); Zn was determined by flame atomic adsorption spectroscopy (FAAS) using a 1100B spectrophotometer (Perkin Elmer, USA). All chemicals used in sample treatments were ultrapure grade (Merck Suprapur, Daemastadt, Germany). All the glassware was cleaned prior to use, by soaking in 10% v/v $HNO₃$ for 24 h and rinsed with Milli Q water. Working standard solutions of metals were daily prepared by serial dilution of stock standard solutions of each metal, containing 1000 mg/L of metal (BDH, Poole, UK). The precision and accuracy of the analytical method was checked using standard reference materials CRM 60 (*Lagarosiphon major)* and IAEA 356 (polluted marine sediment). Results were in agreement with certified values and the standard deviations were low, proving good repeatability of the method (Table 1).

Detection limits (LOD) for various metals calculated on the basis of 10 determinations of the blanks as three times the standard deviation were: $0.06 \mu g g^{-1}$ dry wt. for Cd, 0.12μ g g⁻¹ dry wt. for Cu, 0.09μ g g⁻¹ dry wt. for Pb, 0.18 μ g g⁻¹ dry wt. for Zn and 0.20 μ g g⁻¹ dry wt. for Sn.

Geoaccumulation index

To assess the degree of sediment contamination, the

geoaccumulation index (I_{geo}) was calculated in order to evaluate if metal concentrations represent background levels for the Mediterranean Sea (Loska *et al.,* 1997; Rubio *et al.*, 2000; Ruiz 2001). I_{geo} was originally defined by Müller (1979) in order to determine metal contamination in sediments, by comparing current concentrations with pre-industrial levels. It can be calculated by the following equation:

$$
I_{\rm geo} = \log_2 C_n / 1.5 B_n
$$

where C_n is the measured concentration of the examined metal n in the sediment and B_n is the geochemical background concentration of the metal n. Factor 1.5 is used because of possible variations in background values for a given metal in the environment, as well as, very small anthropogenic influences. Müller has distinguished seven classes

Table 2. Müller's classification for the geoaccumulation index (Müller, 1981).

value geo	Class	Quality of sediment
θ	θ	Unpolluted
$0-1$	1	From unpolluted to moderately polluted
$1 - 2$	\mathfrak{D}	Moderately polluted
$2 - 3$	3	From moderately to strongly polluted
$3 - 4$	4	Strongly polluted
$4 - 5$	5	From strongly to extremely polluted
> 5	6	Extremely polluted

in the geoaccumulation index (Müller, 1981) (Table 2).

Several authors (Donazzolo *et al.,* 1981; Karageorgis *et al.,* 1998; Buccolieri *et al.,* 2006) have demonstrated that it is very difficult to establish B_n values, mainly for sediments of the Mediterranean Sea, owing to geochemical variability of various areas and different anthropogenic impact. In this work, Bn values have been estimated by Buccolieri *et al.* (2006), because they have been determined for Taranto Gulf, Ionian Sea. These B_n values are: Hg 0.07 µg g-1 d.w., Pb 59 µg g-1 d.w., Cu 47 µg g-1 d.w. and Zn 97 μ g g⁻¹ d.w.

Table 1. Metal concentrations (μ g g⁻¹ dry wt.) in certified reference materials.

Metal	BCR 60 (Lagarosiphon major)		IAEA 356 (Polluted marine sediment)	
	Certified	Found [*]	Certified	Found [*]
		$(\mu g g^{-1}$ dry wt.)	$(\mu g g^{-1}$ dry wt.)	
C _d	2.20 ± 0.10	2.31 ± 0.29	0.533 ± 0.026	0.556 ± 0.029
Cu	51.2 ± 1.9	50.2 ± 2.1	44.1 ± 1.0	43.6 ± 2.1
Hg	0.34 ± 0.04	0.35 ± 0.06	1.03 ± 0.13	1.01 ± 0.07
Pb	63.8 ± 3.2	66.4 ± 3.6	42.3 ± 1.6	44.0 ± 3.1
\mathbf{Z} n	313 ± 8	303 ± 11	142 ± 3	145 ± 5.5

 $*$ Number of replicates = 6

Statistical analysis

Two-way analysis of variance (ANOVA) and correlation analysis were used to extract information from the chemical data, in order to find the relationships among metals in plants and sediments. The correlation analysis was performed by Pearson correlation. All statistical analyses were performed using the STATISTICA® software package (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

Metal concentrations in Posidonia oceanica

Metal concentrations in the different tissues of *P. oceanica* are shown in Table 3. The results show that metal distribution in plants varies according to the considered anatomic compartment. Indeed, the highest concentrations of Hg, Pb, Sn and Cu were found in the roots, while the highest levels of Cd and Zn were found in green leaves. In contrast, the lowest metal concentrations were found in the basal part of the leaves. Similar metal distributions in *P. oceanica* and other seagrass species have been reported by other authors (Nienhuis 1986; Catsiki & Panayotidis 1993).

The relationship between metal concentrations was investigated by Pearson's correlation (Table 4). A highly significant positive correlation $(p<0.05)$ was found between Zn and Sn in the roots; a significant negative correlation ($p<0.05$) was found between Cu and Sn in the green leaves and between Pb and Cd in the basal part of the leaves.

In this study *P. oceanica* leaves showed metal contamination levels in the range of the lowest available values for Mediterranean areas (Table 5). Specifically, Cd, Cu and Pb levels determined in the leaves of *P. oceanica* are lower than those reported by other authors, while for Hg the results are comparable to those found in the literature. Mean Zn levels for *P. oceanica* leaves were similar to those of Schlacher-Hoenlinger and Schlacher (1998), but higher than those of Malea *et al.* (1994), Campanella *et al.* (2001) and Conti *et al.* (2010). As regards Sn, it is difficult to compare the results obtained in this study, due to the lack of literature data.

* mean of three analytical determinations

	Cu	Hg	Pb	Sn	Cd	Zn
Green leaf						
Cu	1.000					
Hg	-0.206	1.000				
Pb	-0.172	0.490	1.000			
Sn	-0.985	0.149	0.285	1.000		
Cd	0.845	-0.682	-0.498	-0.824	1.000	
Zn	0.789	0.135	0.469	-0.708	0.440	1.000
Basal part						
Cu	1.000					
Hg	-0.790	1.000				
Pb	-0.519	-0.112	1.000			
Sn	-0.391	0.318	0.262	1.000		
$\ensuremath{\mathrm{Cd}}$	0.597	0.000	-0.982	-0.433	1.000	
Zn	-0.110	-0.518	0.888	-0.082	-0.797	1.000
Roots						
Cu	1.000					
Hg	-0.710	1.000				
Pb	0.298	-0.413	1.000			
Sn	0.581	-0.717	-0.331	1.000		
$\ensuremath{\mathrm{Cd}}$	-0.747	0.333	0.407	-0.729	1.000	
Zn	0.702	-0.683	-0.319	0.978	-0.850	1.000
Sediments						
Cu	1.000					
Hg	-0.132	1.000				
Pb	0.538	0.764	1.000			
Sn	0.989	-0.005	0.639	1.000		
Cd	-0.464	0.433	0.064	-0.349	1.000	
Zn	0.326	0.893	0.972	0.440	0.178	1.000

Table 4. Pearson correlations between metals in the green leaf, basal part, roots and sediments.

*Correlation significant at $p \leq 0.05$

Table 5. Means and ranges of concentrations (µg g⁻¹ dry wt.) in *Posidonia oceanica* leaves reported in various studies.

Metal concentrations in sediments

Metal concentrations in sediments, expressed in μ g g-1 dry weight, are shown in Table 3. Cu, Hg, Pb, Sn, Cd and Zn levels in sediments were in the range of 8.05- 22.34, 0.10-1.79, 14.28-29.19, 0.36-4.12, 0.12-0.17 and $35-62 \mu g g^{-1}$ dry wt. respectively. Highest Hg, Pb and Zn concentrations were observed at station 3, whereas highest Cu and Sn concentrations were detected at station 1. Cd concentrations were similar at all stations. For grain size distribution, according to the AASHO (American Association of State Highway and Transportation Awards) classification system, all sediments consisted predominantly of pelitic sands. The average grain size composition was: 5.0% gravel, 49.0% sand, 46.0% silt and clay. There are no significant differences among stations as regards grain size.

The two-way ANOVA did not show significant differences in metal distribution between the stations $(p<0.05)$. Significant positive correlations in the sediments were observed between Cu-Sn and Zn-Pb concentrations ($p<0.05$) (Table 4). These correlations between metal concentrations suggest either a common or a similar geochemical behaviour or origin. Factors including source rock or soil types, weathering processes, surface adsorption phenomena, and characteristics of depositional environment affect metal distribution in sediments. Therefore, metal/metal relationships may vary significantly (NAVFAC, 2003).

A comparison with results from sediments of other marine coastal areas is shown in Table 6. In general, data show concentration levels more or less similar to those found in other areas. In particular, Hg levels were higher than those reported for the Aegean Sea and Ligurian Sea, except for the Adriatic Sea, Gulf of Trieste and the Venice Lagoon. Cd levels were always higher than those reported for the Aegean Sea and for the Adriatic Sea and Slovenia Coast, except the Ligurian Sea and Venice Lagoon. Concerning Cu, Zn and Pb, levels were lower than those reported by other authors, except for the Slovenia Coast.

Results of the I_{geo} are shown in Figure 2; for Pb, Cu, Zn, Sn and Cd, all the stations are classified in class 0 (the unpolluted class). The I_{gen} class for Hg, ranges between 1

Fig. 2: Geoaccumulation Index (Igeo) in surface sediments from San Pietro Island (Taranto Gulf, Ionian Sea, Italy).

Table 7. Pearson correlations between metal concentrations in plant and sediment.

	green leaf	hasal part	rhizome	roots	sediments
Hg					
green leaf	1.000				
basal part	0.497	1.000			
rhizome	0.084	0.884	1.000		
roots	-0.802	-0.811	-0.610	1.000	
sediments	-0.616	-0.831	-0.753	0.964	1.000
C _d					
green leaf	1.000				
basal part	0.607	1.000			
rhizome	0.474	0.826	1.000		
roots	0.953	0.763	0.499	1.000	
sediments	0.833	0.697	0.278	0.947	1.000
Ph					
green leaf	1.000				
basal part	0.968	1.000			
rhizome	0.222	0.007	1.000		
roots	0.288	0.078	0.997	1.000	
sediments	0.239	0.188	-0.292	-0.290	1.000
Zn					
green leaf	1.000				
basal part	0.185	1.000			
rhizome	0.244	0.731	1.000		
roots	-0.009	0.330	0.867	1.000	
sediments	-0.600	0.415	-0.173	-0.373	1.000
C _d					
green leaf	1.000				
basal part	-0.395	1.000			
rhizome	0.686	0.300	1.000		
roots	-0.058	-0.735	-0.340	1.000	
sediments	-0.131	-0.130	0.154	0.717	1.000
Sn					
green leaf	1.000				
basal part	0.378	1.000			
rhizome	0.278	0.899	1.000		
roots	0.204	0.909	0.901	1.000	
sediments	0.316	-0.463	-0.144	-0.226	1.000

*Correlation significant at p < 0.05

(from unpolluted to moderately polluted) and 4 (strongly polluted), indicating an Hg enrichment. Therefore, in this case the examined sediments can only be considered contaminated by Hg. This result may be related to the ILVA iron and steel factory and the ENI refinery in Taranto. In fact, industrial emissions of Hg in the Taranto area are about 1400 kg/year in air and 665 kg/year in sea water (Eper Register, 2012). In fact, several studies have qualified the Taranto Gulf as an Hg contaminated site (Cardellicchio *et al.,* 2009; Annicchiarico *et al.,* 2011).

Metal correlations

It is known that sediments can act as a source of contaminants for organisms (Villares *et al.,* 2001). In fact, marine macrophytes absorb metals in two ways: by direct absorption from water through the leaf surface, or from the sediment and interstitial water through the roots (Brinkhuis *et al*., 1980).

The foliar tissue analysis of these organisms allows us to determine the mean pollution levels over a limited time interval, whereas an analysis of the roots provides an indication of environmental contamination over a much longer period (Ledent *et al.,* 1993). This phenomenon may be due to the long lifespan of the roots and the slow regeneration levels of these structures compared with foliar structures (Ott 1980; Caye 1989; Pergent 1990).

In this work significant positive correlations ($p<0.05$) were found between Hg concentrations in roots of *P. oceanica* and in sediments (Table 7). Several authors (Ferrara & Serriti 1989; Maserti *et al.,* 1991; Sanchiz *et al*., 2001) indicated that the Hg uptake occurs primarily through the root system and only minimally through the leaves. Therefore the Hg levels found in *P. oceanica* provide an indication of sediment contamination, rather than water. Therefore, *P. oceanica* seagrass meadows could be involved in the Hg cycle at two different levels: in the mobilization of metals found in the sediment, and in the storage of Hg (sink) in the "matte", in the form of dead sheaths and rhizomes. Mattes of P. oceanica seagrass are the result of rhizome growth. These mattes can, in places, reach a thickness of several meters, which corresponds to growth over several centuries: this structure thus could represent an important store of mercury (Molinier and Picard 1952; Boudouresque *et al.,* 1980).

Moreover, significant positive correlations (p < 0.05) were found in Cd concentrations between the green leaves and roots of *P. oceanica* (Table 7). It has been reported by other authors (Brinkhuis *et al.,* 1980; Ward 1989) that the Cd uptake occurs mainly from the leaves, through passive adsorption, which depends on the leaf surface and Cd moves to rhizomes and roots at a later stage only. Therefore, the fact that no correlations were found between Cd concentrations in *P. oceanica* roots and in sediment could indicate that Cd in *P. oceanica tissues* reflects the Cd in the water column.

Significant positive correlations $(p<0.05)$ were observed in Pb concentrations between green leaves and the basal part of *P. oceanica* and in Pb concentrations between roots and rhizomes of *P. oceanica* (Table 7). The fact that no correlation was found between Pb concentrations in roots of *P. oceanica* and in sediment could indicate that Pb in *P. oceanica tissues* reflects the Pb in the water column. The uptake of Pb, like Cd, seems to occur mainly through the water column with a passive process dependent on the leaf surface exposed (Ward 1989). In fact, it has been demonstrated that aquatic plants can remove Pb from the surrounding water (Axtell *et al.,* 2003).

Finally, no correlations were observed in Cu and Zn concentrations among various parts of the plant and the various parts of the plant and sediments, indicating that absorption can take place, both through the roots and the leaves (Lyngby & Brix 1982). However, Cu as well as Zn are essential for the growth and metabolic processes of plants and their accumulation is affected by processes of metabolic regulation.

A number of laboratory experiments have shown that there are significant correlations between metal levels in the tissues and toxicological consequences for the physiological, biochemical and tissue levels (Cristiani *et al*., 1980; Augier *et al.,* 1984). These elements can cause various effects, which can even lead to growth interruption. Other effects include a gradual inhibition of juvenile leaf formation, foliar deformations, tissue and cell necrosis and a loss of pigmentation (Cristiani *et al.,* 1980; Lyngby & Brix 1983; Lyngby & Brix 1984; Augier 1986; Ward 1989; Malea 1994; Malea and Haritonidis 1996).

Conclusions

Research has allowed us to determine the levels of trace metals in different parts of *P. oceanica* and the ability of seagrass to accumulate metals. This shows that the study of the distribution of metals in *P. oceanica* is very important for the monitoring of coastal marine areas. *P. oceanica* is located at the basis of the food web in the Mediterranean and is probably the main source of metals for many animals grazing on its leaves. Therefore, the investigation of trace metal concentrations in the tissues of this species may provide useful information on the transfer of potentially toxic elements from abiotic compartments (water, sediments) to higher consumers. However, even if the use of this species as a biomonitor for trace metals looks attractive for many reasons, further studies are needed in order to evaluate the robustness of the methodology for routine use in marine biomonitoring.

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