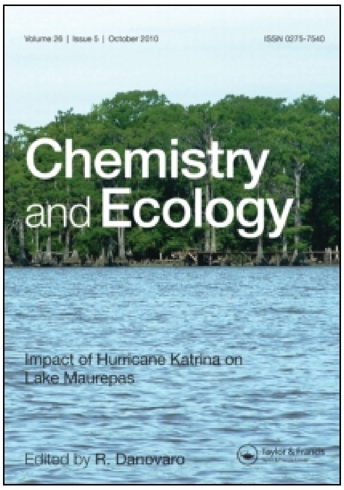

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Trace metals in "*Posidonia oceanica*" seagrass from south-eastern Sicily

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Heavy-metal concentrations were measured in sediments and tissues of *Posidonia oceanica* seagrass from south-eastern Sicily (Italy) in order to assess the degree of metal pollution in the coastal area. Seagrasses and sediments were collected at four sites along the south-eastern coast of Sicily. Flame atomic absorption spectrophotometry (FAAS) was used to measure concentrations of Cu, Zn, Cd and Pb. Standard statistical analyses were used to assess significant differences among the levels of the elements measured in different tissues and sediment and spatial distribution. The greatest values of potentially toxic metal concentrations were observed at the station near the industrial sites of Augusta and Priolo. Comparisons with the concentrations of the same metals in other sites of north and western Sicily and with data from surveys in other areas of the Mediterranean Sea are shown.

Keywords: *Posidonia oceanica*; Marine sediments; Metals; Mediterranean Sea

1. Introduction

Posidonia oceanica, a marine phanerogam, forms dense infra-littoral communities, called meadow ecosystems [1], which are widely distributed throughout the Mediterranean Sea and are a biological indicator of the coastal water quality [2]. They are the most important contributors to coastal primary production. Because of its bathymetric range (0–40 m depth), this ecosystem is directly exposed to various anthropogenic inputs derived from traffic, industrial and domestic sources [1, 3, 4]. In particular, heavy metals, of natural and anthropogenic origin, were shown to contaminate the *Posidonia oceanica* meadows due to both terrestrial and atmospheric contributions [5–11]. Some authors [12, 13] have suggested that high levels of

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heavy metals could perturb the community structure of similar ecosystems. The status of the pollution in the Mediterranean Sea has, until now, been debatable, and an impact study is important to determine the degree of metal pollution. Extrapolation of concentration values in tissues of biota under similar pollution conditions from one site to another may be confounded by several factors: physiological status of the organisms, environmental variability, and the geochemistry of the habitat.

The aim of our work was to conduct a survey of metal concentrations in *Posidonia oceanica* at four sites located along the south-eastern coast of Sicily. Four metals, Cu, Cd, Pb and Zn, were analysed. The analyses of the relative concentration of pollutants in each of these tissues of plant and in adjacent sediment could provide a mechanism for focusing the scientific attention on the management of these delicate ecosystems against anthropogenic impact. Moreover, the survey can give an indication of the correlation between the status of the marine environment and the activities present along the south-eastern coast of the Sicily.

2. Materials and methods

Four sites were selected: Capo Passero, Marzamemi belonging to the marine reserve of Vendicari, Capo Negro and Ognina Bay, very near to an industrial area, Augusta, and Priolo (figure 1). The sampling of *Posidonia oceanica* was conducted by colleagues from the Dipartimento di Scienze Botaniche of the University of Palermo (DSB) [14].

In March 2002, 20 orthotropic shoots of *Posidonia oceanica* samples were collected by scuba dives in every meadow at a water depth of 6 m, and sediments were collected near the plants. The DSB researchers [14] also carried out sample fractioning into scales, rhizomes and leaves.

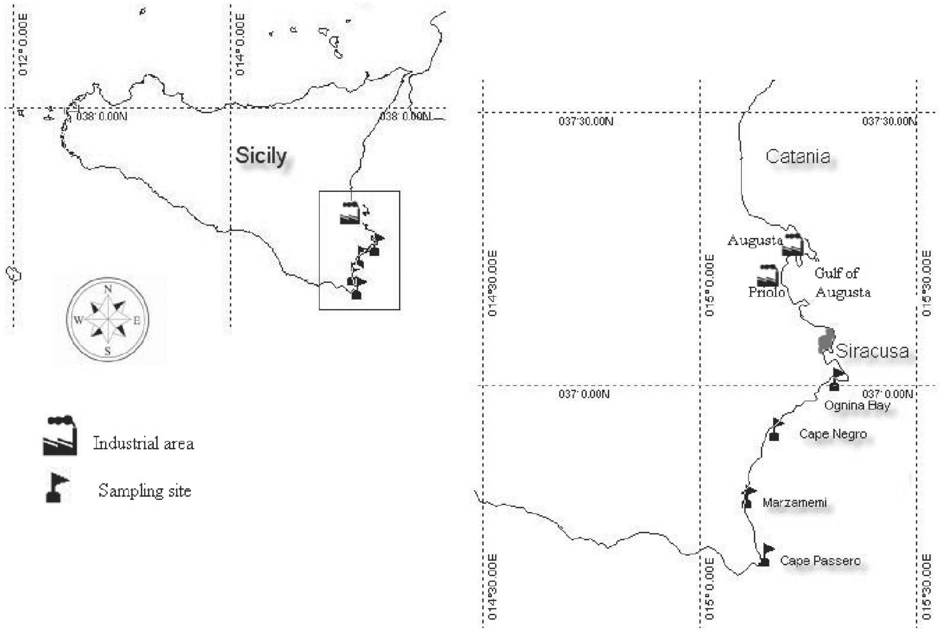

Figure 1. Map of Sicily with sampling sites and macro areas (see main text for details).

Every plant was fractioned into three parts: scales, rhizomes and leaves. In particular, leaves were pooled into juvenile, adult and intermediate. The scales and rhizomes were dissected in lepidochronological years for a maximum range of 20 years (1982–2001). Fractions belonging to the same tissue, same lepidochronological year and same site were grouped to form a pool sample representative of the lepidochronologic year. Tissues dated lepidochronologically were placed into polyethylene bags (PE) and frozen. Only the 1989–2001 period was covered from the age of shoots, and then only the last 13 lepidochronological years are reported in the results. However, the data on leaves are referred only to the sampling year.

The tissue samples were carefully removed from the PE bag; scrubbed to remove epiphytes and fine sediments with a glass slide; rinsed rapidly with distilled water to minimize loss of metals [15]; dried at 60 °C for 12 h then 105 °C for 24 h; and then cooled in a drying tube. Scales, rhizomes and leaves were milled separately using a Fritsch Pulverisette 2, sieved to obtain a grain size of approximately 100 μm, and then stored in hermetically sealed PE vials. From each aliquot, 500 mg was taken and digested in a CEM Star 2 open-cavity microwave using a mixture of concentrated $HNO_3/HClO_4$ 1:1 v:v in a multistep temperature process. The digested samples were then cooled and diluted to 25 ml with distilled and deionized water. To prevent any contamination, all glassware used in the procedure was cleaned with a strong detergent, soaked in 10% HNO_3 solution for 24 h, and then rinsed three times with distilled water. All the chemical reagents used were of Merck suprapure grade.

The sediment samples, collected from the same station as *Posidonia oceanica*, after sieving on 2 mm meshes, were dried at 105 °C for 48 h and then milled to obtain grains of about 100 μm size using a Fritsch Pulverisette 2. Milled samples were then digested using an open-cavity Star 2 microwave (CEM Corporation).

Five millilitres of HCl (37%) was added to a 500 mg aliquot of the sample, and the mixture kept at 75 °C for 5 min. Afterwards, 7.5 ml of HNO_3 65% was added and the mixture heated to 95 °C; after 15 min, the temperature was raised to 110 °C and maintained for 10 min. Fifteen millilitres of H_2O_2 was then added, and the temperature was kept at 110 °C for a further 10 min. After cooling, the solution was filtered through a 0.45 μm Millipore filter and, to allow for evaporation during the digestion process, distilled and deionized water was added to make up the final volume to 25 ml.

The metals were determined using a Varian AA20Plus (updated to AA100) atomic absorption spectrophotometer in the air/C_2H_2 flame, equipped with an atom concentrator tube (ACT-80) to improve the sensitivity. A linear calibration curve was produced for each element, and for each set of measurements using opportune dilution of CertiPur Merck standard solutions. Each metal was detected using a single-element hollow cathode lamp using a deuterium background corrector. The detection limits of Zn, Cu, Cd, and Pb are 0.6, 0.6, 0.25, and 0.9 $\mu g\,g^{-1}$, respectively.

A National Research Council of Canada PACS-2 marine sediment was used as a certified reference material for sediment measurements. The Community Bureau of Reference (European Union) BCR RM 60 (*Lagarosiphon major*) was used as a certified reference material for *Posidonia oceanica* measurements.

Table 1 lists the values of metal concentrations obtained using the aforementioned procedure and equipment with certified reference material BCR RM 60 *Lagarosiphon major*. The method gave values repeatable with $r \leq DS_W$ where D is 3.3 for three repeated measurements, and S_W is the within-laboratory standard deviation of the certification campaign [16]. Our data are in agreement with the certified values at the 95% confidence level.

A standard statistical variance test coupled with a multiple-range test (MRT) was used to determine the significance of the difference of metal levels in the three tissues. The P-values of the Fischer test (F-test) were used to evaluate statistically significant differences in metal

Table 1. Analysis of certified reference materials BCR 60 *Lagarosiphon major*: certified values and found values (mean ± S.D.) on three measures.

	BCR RM 60 (*Lagarosiphon major*)		
	Certified	Found	Range
Metal	$\mu g\, g^{-1}$ (dry weight)		
Cd	2.20 ± 0.10	2.08 ± 0.10	0.19
Cu	51.2 ± 1.9	50.0 ± 2.7	5.0
Pb	63.8 ± 3.2	60.1 ± 2.1	4.16
Zn	313 ± 8	327 ± 30	15.0

concentrations in the different tissues. The same tests were used to evaluate the site dependence for each element.

3. Results

Figure 1 shows the four sites of our survey and shows the industrial areas of Augusta and Priolo and the city of Siracusa (126,000 inhabitants). Ognina Bay and Cape Negro are located very near the Siracusa–Augusta–Priolo area (about 20 km). Marzamemi and Cape Passero are far from the industrial areas and urbanized sites.

In the industrial areas of Augusta and Priolo, there are essentially several petrochemical plants. Table 2 lists the mean metal concentrations measured in scales and rhizomes from *Posidonia oceanica* collected from the various sites. The data reported in table 2 are the mean concentrations determined from 12 samples obtained from each shoot after lepidochronological division [14]. In this way, the sample corresponding to a lepidochronological year is obtained, mixing the 20 parts extracted from each shoot and belonging to the same lepidochronological year. Twelve years were considered, from 1989 to 2000. Table 2 also shows the standard deviations in each case. Table 3 lists the metal concentrations obtained for the last lepidochronological year (2001) in scales, rhizomes, leaves and sediment samples collected from the various sites. The metal concentrations in scales, rhizomes, leaves and sediment are expressed in table 3 as the means from three measurements on three aliquots of the same sample ± standard deviation and are referred to the 2001 lepidochronological

Table 2. Mean concentration ± S.D. of trace metals ($\mu g\, g^{-1}$ dry weight) in *Posidonia oceanica* collected in the four sites of the survey (see main text for sampling sites details). In parentheses are reported the number of lepidochronological year considered.

	Cd	Cu	Pb	Zn
Cape Passero				
Rhizomes (12)	0.97 ± 0.12	9.3 ± 5.9	6.7 ± 1.9	65 ± 26
Scales (12)	1.07 ± 0.12	6.3 ± 1.3	8.0 ± 0.7	159 ± 26
Marzamemi				
Rhizomes (12)	1.15 ± 0.39	4.1 ± 0.7	3.5 ± 0.7	63 ± 18
Scales (12)	0.92 ± 0.27	6.4 ± 0.8	6.6 ± 1.4	206 ± 53
Cape Negro				
Rhizomes (12)	0.73 ± 0.38	2.6 ± 1.1	4.5 ± 1.2	150 ± 110
Scales (12)	0.80 ± 0.19	5.1 ± 1.6	8.2 ± 2.6	229 ± 50
Ognina Bay				
Rhizomes (12)	2.25 ± 0.18	7.9 ± 1.1	6.0 ± 0.6	172 ± 117
Scales (12)	1.16 ± 0.14	9.0 ± 1.6	14.8 ± 2.3	271 ± 51

Table 3. Mean concentration ± S.D. of trace metals ($\mu g\,g^{-1}$ dry weight) in *Posidonia oceanica* scales and rhizomes (last lepidochronological year), leaves, and in sediments collected in the four sites of the survey (number of samples analysed is shown in parentheses).

	Cd	Cu	Pb	Zn
Cape Passero				
Leaves (3)	2.10 ± 0.15	11.5 ± 0.9	5.8 ± 0.6	255 ± 25
Rhizomes (3)	0.90 ± 0.10	14.6 ± 1.7	5.9 ± 0.6	208 ± 22
Scales (3)	1.05 ± 0.10	3.4 ± 0.5	4.5 ± 0.5	143 ± 15
Sediment (3)	<0.25	3.2 ± 0.1	1.0 ± 0.2	80 ± 8
Marzamemi				
Leaves (3)	3.03 ± 0.18	8.4 ± 0.75	9.6 ± 0.8	213 ± 19
Rhizomes (3)	1.29 ± 0.12	7.6 ± 0.6	4.4 ± 0.5	135 ± 16
Scales (3)	0.81 ± 0.09	4.5 ± 0.5	4.5 ± 0.5	130 ± 16
Sediment (3)	<0.25	1.1 ± 0.08	1.5 ± 0.2	60 ± 7
Cape Negro				
Leaves (3)	1.20 ± 0.18	9.0 ± 0.8	7.0 ± 0.7	676 ± 57
Rhizomes (3)	0.45 ± 0.07	7.8 ± 0.6	4.0 ± 0.5	407 ± 50
Scales (3)	0.85 ± 0.09	3.3 ± 0.3	5.5 ± 0.5	444 ± 50
Sediment (3)	<0.25	1.7 ± 0.1	4.0 ± 0.4	55 ± 6
Ognina Bay				
Leaves (3)	3.40 ± 0.24	15.3 ± 1.2	12.5 ± 1.1	277 ± 30
Rhizomes (3)	2.44 ± 0.15	13.7 ± 1.0	6.1 ± 0.5	421 ± 50
Scales (3)	2.00 ± 0.18	7.1 ± 0.7	8.5 ± 0.7	445 ± 50
Sediment (3)	<0.25	1.5 ± 0.1	4.5 ± 0.4	38 ± 4

year for scales and rhizomes and 2002, the sampling year, for leaves and sediment. Data for Cd in sediments are below the low detection limit of our equipment for cadmium ($0.25\,\mu g\,g^{-1}$).

Metal distribution in different tissues of plant scales and rhizomes group was evaluated from a multiple sample comparison test (MSCT) and multiple range test (MRT). The same tests were used to compare the metal concentrations from the different sites.

From data presented in tables 2 and 3 some observations on the metal distribution can be done. Cd is distributed homogeneously in the scales and in the rhizomes and only in Ognina Bay site a mean concentration value in rhizomes twice higher than in the scales is present. This difference is statistically significant at the 95% confidence level because the P value of the F-test is less than 0.05. The Cd concentrations measured in leaves are about twice as high as those measured in scales and rhizomes from the last lepidochronological year (2001).

The mean values of copper are greater in scales than in rhizomes. Only in the case of Cape Passero is the ratio inverted (see table 2, where the historical mean is reported). Data for the last lepidochronological year confirm that Cu is oddly distributed in the different plant tissues. Pb is more present in leaves than in scales and rhizomes, and this indicates that absorption comes mainly from the water column. The distribution in scales and rhizomes reported in table 2 shows that there is accumulation in the scales. Data for Zn in tables 2 and 3 indicate a wide variability in rhizomes. In leaves, this element is always highly concentrated. Table 3 also lists the concentrations in the sediment; these values are generally low for all the elements analysed. The lead concentrations in Ognina Bay and Cape Negro are four times higher than the mean values in the other two sites. This is related to the emission of lead into the air from the industrial areas of Augusta and Priolo.

In order to analyse the dependence of the metal concentrations from the site features, the data shown in table 2 have been grouped according to automotive traffic, industrialization and urbanization conditions of the coastal areas close to the sampling sites. We have considered two

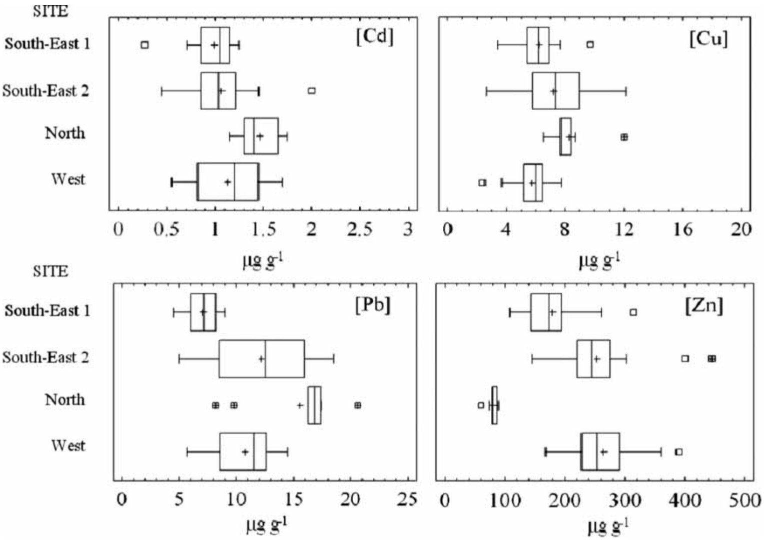

Figure 2. Box-Whisker plots for heavy-metal concentration (μg g^{-1} dry weight) distributions in *Posidonia oceanica* scales. For each graph, results for South-East 1, South-East 2, North and West macro-areas, from top to bottom, are shown (see figure 1 and main text).

macro-sites. The South-East-1 (SE1) macro-site includes the Marzamemi and Cape Passero sites, characterized by low levels of urbanization and automotive traffic conditions (*e.g.* Cape Passero is a natural reserve). Finally, the South-East-2 (SE2) macro-site includes the highly urbanized site of Ognina Bay and Cape Negro. Figures 2 and 3 show a series of Box-Whisker plots, thus allowing a comparison of heavy-metal levels in scales and rhizomes among the macro-areas defined above. Also in figures 2 and 3, the SE1 and SE2 macro-areas are compared with the North (N) and West (W) Sicilian macro-areas reported in table 4 and relative to data

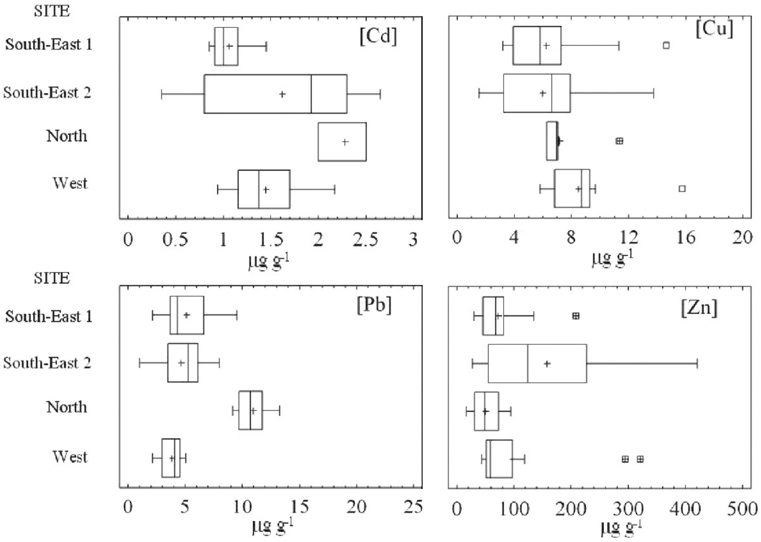

Figure 3. Box-Whisker plots for heavy-metal concentration (μg g^{-1} dry weight) distributions in *Posidonia oceanica* rhizomes. For each graph, results for South-East 1, South-East 2, North and West macro-areas, from top to bottom, are shown (see figure 1 and main text).

Table 4. Mean values ± S.D. or ranges (in parentheses), of metal concentrations ($\mu g\,g^{-1}$ dry weight) in the tissues of *Posidonia oceanica* and in sediments measured in the coastal areas of the Mediterranean Sea.

	Cd	Cu	Pb	Zn	Notes
Rhizomes	(1–2)[a]	–	(2.5–8)[a]	(40–80)[a]	Seventeen sampling stations located on the Spanish Mediterranean coast
	(0.4–1)	(3.1–8.7)[b]	(0.4–1.1)	–	Liscia Bay, Sardinia (I)
		2.84 ± 0.46[c]	–	60.41 ± 11.21[c]	Tissue formed between 1987 and 1992. Figari, Corsica (F)
	–	4.15 ± 0.77[c]	–	21.90 ± 4.35[c]	Tissue formed between 1987 and 1992. S. Amanza, Corsica (F)
	0.77 ± 0.28[e]	7.87 ± 3.39[e]	14.0 ± 14.0[e]	94.9 ± 16.2[e]	Mean annual concentrations from Calvi Bay, Corsica (F)
	0.72 ± 0.34[e]	12.4 ± 3.42[e]	14.5 ± 9.4[e]	103 ± 31[e]	Mean annual concentrations from Ischia Island, Campania (I)
	1.48 ± 3.58[e]	11.94 ± 3.53[e]	10.2 ± 4.8[e]	107 ± 43[e]	Mean annual concentrations from Marseille (F)
	(1.5–2.5)	(6.3–13.7)	(4.4–13.3)	(17–123)	Three sampling stations located on the North coast of Sicily (Carini Bay)[g]
	(0.9–2.2)	(5.8–10)	(3.1–5.1)	(40–320)	Six sampling stations located on the West coast of Sicily (Trapani and Marsala)[g]
	(0.3–1.7)	(6.4–14.8)	(1.8–5.9)	(36–153)	Nine sampling station located on the Egadi Islands[g]
Scales	(0.3–0.8)[b]	(3.2–13.1)[b]	(1.7–36.6)[b]	–	Liscia Bay, Sardinia (I)
	–	14.34 ± 2.69[c]	–	59.47 ± 7.47[c]	Tissue formed between 1987 and 1992. Figari, Corsica (F)
	–	8.29 ± 1.67[c]	–	39.14 ± 5.65[c]	Tissue formed between 1987 and 1992. S. Amanza, Corsica (F)
	(1.2–2.5)	(6.2–12.0)	(8.2–20.7)	(60–103)	Three sampling stations located on the North coast of Sicily (Carini Bay)[g]
	(0.5–1.7)	(2.3–8.0)	(5.3–14.9)	(160–391)	Six sampling stations located on the West coast of Sicily (Trapani and Marsala)[g]
	(1.1–2.1)	(3.9–11.4)	(6.5–13.5)	(74–336)	Nine sampling station located on the Egadi Islands[g]
Leaves	(1–8.6)[a]	–	(0.7–10)[a]	(105–180)[a]	Seventeen sampling stations located on the Spanish Mediterranean coast
	(1.13–2.78)[d]	(5.7–20.2)[d]	(0.7–1.18)[d]	(105–118)[d]	Four clean stations in Favignana Island, Sicily (I)
	2.30 ± 0.85[e]	10.2 ± 4.3[e]	5.96 ± 3.02[e]	154 ± 30[e]	Mean annual concentrations of leaf–epifite complexes from Calvi Bay, Corsica (F)
	2.1 ± 0.6[e]	16.2 ± 4.6[e]	8.35 ± 3.61[e]	144 ± 41[e]	Mean annual concentrations of leaf–epifite complexes from Ischia Island, Campania (I)
	2.4 ± 0.5[e]	12.1 ± 2.6[e]	7.76 ± 2.74[e]	179 ± 69[e]	Mean annual concentrations of leaf–epifite complexes from Marseille (F)
	3.6 ± 0.4	11.3 ± 2.2	10.8 ± 1.7	182 ± 20	Three sampling stations located on the North coast of Sicily (Carini Bay)[g]
	2.6 ± 0.3	6.8 ± 0.8	9.36 ± 1.2	320 ± 50	Six sampling stations located on the West coast of Sicily (Trapani and Marsala)[g]
	2.7 ± 0.5	9.0 ± 1.0	14 ± 2.0	190 ± 40	Nine sampling station located on the Egadi Islands[g]
Sediment	(0.04–0.65)[a]	–	(10–90)[a]	(30–130)[a]	Seventeen sampling stations located on the Spanish Mediterranean coast
	–	13.3 ± 2.7[c]	–	433.0 ± 95.9[c]	Mean values between Figari and S. Amanza sites
	0.1 ± 0.002[f]	13.2 ± 0.7[f]	–	152.5 ± 6.8[f]	Mean concentrations in a polluted site from Larymna, Northern Evoikos Gulf (GR)
	0.1 ± 0.002[f]	16.6 ± 1.7[f]	–	44.3 ± 3.9[f]	Mean concentrations in a clean site from Vravrona, South Evoikos Gulf (GR)
	<0.25	2.2 ± 0.3	8.5 ± 1.0	15 ± 2	Three sampling stations located on the North coast of Sicily (Carini Bay)[g]
	<0.25	4.1 ± 0.5	7.5 ± 0.9	23 ± 3	Six sampling stations located on the West coast of Sicily (Trapani and Marsala)[g]
	<0.25	4.3 ± 0.4	6.0 ± 1.0	43 ± 7	Nine sampling station located on the Egadi Islands[g]

[a] [4]. [b] [18]. [c] [1]. [d] [19]. [e] [20]. [f] [21]. [g] [17].

from a previous study by our group [17]. The North macro-site includes only the Carini site, which is located very close to the city of Palermo and a heavily used highway connecting Palermo and Trapani. The West macro-site includes Trapani and Marsala sites, characterized by intermediate levels of urbanization and automotive traffic conditions. It is worth noting that for Cd and Pb, the values in the Se1 macro-area are statistically lower ($P < 0.05$) than in the other urbanized macro-areas.

4. Discussion and conclusions

Metal distributions in the different parts of the plant depend on many factors, such as the absorption mechanism, the metabolic processes linked to plant physiology and the physicochemical conditions of the environment, as obtained from the literature [12, 22, 23].

The results obtained indicate that levels of heavy metals in *Posidonia oceanica* tissues depend on both element and tissue. Our results are in agreement with surveys carried out in other Mediterranean Sea areas (table 4), showing Zn concentrations that are higher than Pb and Cu, which are all higher than the Cd values.

All the metal concentrations found in *Posidonia oceanica* tissues are higher than in the sediment (table 3), thus indicating that the seagrass absorbs these metal contaminants directly from the water column. However, no significant correlations were found between metal concentrations in *Posidonia oceanica* tissues and metal concentrations in sediments, owing to insufficient data.

Cadmium concentrations are comparable at all sites of the present survey, except the higher levels in rhizomes from Ognina Bay and in agreement with concentration values reported in table 4 for other natural areas of the Mediterranean Sea.

Data from this paper confirm the influence of environmental metal pollution on the concentration in scales [18, 24], indicating that *Posidonia oceanica* can be considered a good candidate for monitoring metal concentrations (*e.g.* Pb) in marine environments [25]. Pb values found in scale samples from Ognina Bay are generally higher than in samples from other sites of this survey and comparable with those obtained in samples from Carini Bay, an area with similar urban and industrial conditions [17]. The lead concentrations in Cape Passero, Marzamemi and Cape Negro are lower than those measured in samples from other areas in Sicily (table 4) [17].

Before the introduction of unleaded fuel, lead in petrol used in vehicle engines was the main anthropogenic source of this metal in the environment. For instance, report n. 31 of the GKSS Forschungszentrum Geesthacht GmbH Institut für Küstenforschung [26] estimates that the percentage of lead emission in the air from transportation sources in Europe was 76.1% in 1985, 72.1% in 1990 and 68.7% in 1995 with respect to the total lead emission in air. The Environmental Criteria and Assessment Office [27] showed that a positive correlation does exist between Pb in consumed gasoline and Pb concentration in the air.

The values of Pb concentrations that we find in sediments are generally lower than those reported in the literature and are compatible with sites free from direct pollution sources, i.e. heavy-metal pollution is essentially due to phenomena occurring at the air–sea interface, and this is particularly true for lead deposition in water and sediment [28].

In order to further test the well-known reliability of *Posidonia oceanica* as a bio-indicator of heavy-metal pollution [5–8, 10, 24] macro-areas are compared. The macro-areas clearly show Cd and Pb concentrations correlated with their features, as outlined above. In fact, SE1, a natural reserve area, shows the lowest level of these elements in scales. Analysis of rhizome data reveals that sites nearest industrial areas have higher concentrations of this element, and this difference is statistically significant at the 95% confidence level.

The enrichment of Pb in scales is correlated with anthropogenic sources [17], whereas the behaviour and distribution of other metals are still debatable [29]. Cu and Zn are micronutrients involved in the plant metabolism, and their values are not strongly site-dependent.

To allow an easier comparison of results, table 4 lists the heavy-metal concentrations in *Posidonia oceanica* tissues and sediments taken from the literature. Our data for the Cd, Cu, and Pb range between the minimum and maximum values are very similar to data obtained from other authors from various sites of the Mediterranean Sea. Zn shows a poor agreement with literature data.

This work underlines the importance of *Posidonia oceanica* in determining the quality of the marine coast and provides data on heavy-metal concentrations for several coastal areas of Sicily, which had not been described to any great extent until now. Measurement of other elements and a more accurate elaboration of lepidochronological results is under way. A study on annual emission in air of contaminant metals will be performed to find a correlation with the temporal trend in *Posidonia oceanica* tissue concentration to test the ability of the plant to act as an historical register of pollution.

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