
Trace metals assessment in water, sediment, mussel and seagrass species - Validation of the the use of *Posidonia oceanica* as a metal biomonitor

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Abstract

The accumulation of trace metals (Cd, Co, Cr, Hg, Ni and Pb) was measured in water, sediment, the mussel *Mytilus galloprovincialis* and the seagrass *Posidonia oceanica*. Samples were collected in three locations of the north-western Mediterranean (Canari, Livorno and Porto-Torres) which present different levels and sources of human impact. Analyses in the different compartments (water, sediment, *Mytilus galloprovincialis* and *Posidonia oceanica*) have allowed to identify Canari as the most Cd, Co, Cr and Ni contaminated site; Livorno as the most Hg contaminated and Porto-Torres as the most Pb contaminated. Furthermore, for the first time metal concentrations found in *Posidonia oceanica* have been compared with those found in the water column, in the sediment and in the recognized metal bio-indicator species *Mytilus galloprovincialis* and the results obtained have led to the same conclusions. Thus, this study allows to validate the use of *Posidonia oceanica* as metal biomonitor of coastal waters.

Keywords: metal contamination, biomonitoring, seawater, sediment, marine organisms, Mediterranean Sea

1. Introduction

The continental shelf constitutes the most important zone of the marine environment as far as human activities are concerned, as it contains the main source of living marine resources (Castro et al., 1999; Usero et al., 2005) and as it is the area that is most concerned by human-induced changes (Zoller, 2006). The coastal part of this continental shelf is the most sensitive, as it receives large amounts of contaminants introduced by domestic, industrial and agricultural activities, directly or via rivers or through atmospheric deposition (Zoller and Hushan, 2000; Usero et al., 2005). In most cases, the impact and synergistic effects of contaminants on marine ecosystem are poorly known (Zoller, 2006). The early identification of inorganic contaminants such as trace metals, which are all toxic above a specific threshold of bioavailable level (Kucuksezgin et al., 2006), is essential to avoid damage to marine biocenoses (Girotti et al., 2006).

The comparison of metal contamination in different aquatic environments is possible by analysis of water (Davison and Zhang, 1994; Manfra and Accornero, 2005; Stark et al., 2006), sediment (Calmano et al., 1996; Acevedo-Figueroa et al., 2005; Tankere-Muller et al., 2006) and indigenous biota (Rainbow and Phillips, 1993; Goldberg and Bertine, 2000; Pergent-Martini and Pergent, 2000; Ferrat et al., 2003b). Bioaccumulation studies led to the adoption of the bio-indicator concept (Langston and Spence, 1995). Monitoring networks, mostly based on bivalves (O'Connor, 1996; Goldberg and Bertine, 2000; Andral et al., 2004; Morillo et al., 2005; Usero et al., 2005), have been developed in order to evaluate the marine environment quality. Seagrasses are increasingly used as indicators of chemical contaminations of coastal regions (see synthesis in Ferrat et al., 2003b). In the Mediterranean Sea, the endemic species *Posidonia oceanica* (L.) Delile is often considered a useful metal bio-indicator (Capiomont et al., 2000; see synthesis in Pergent-Martini and Pergent, 2000; Campanella et al., 2001; Ferrat et al., 2003a).

The aim of this study is three fold: (i) first, to determine trace metals concentrations in water, sediment, the mussel *Mytilus galloprovincialis* (L) Lamarck, 1819 and the seagrass *Posidonia oceanica* in three Mediterranean locations; (ii) second, to compare, for the first time, the metal concentrations in these different compartments (water, sediment, *Mytilus galloprovincialis*, *Posidonia oceanica*); and (iii) third, to evaluate the relevance of *Posidonia oceanica* as a metal bio-indicator.

2. Materials and methods

2.1. Sampling locations

Samples were collected in three stations, selected randomly, in three north-western Mediterranean locations (Canari, Livorno and Porto-Torres; Figure 1) situated in proximity of different human-induced impact activities.

Canari (Corsica-France), on the west coast of the Cap Corse, is located next to a previous asbestos mine which discharged, between 1948 and 1965, more than 11 million tons of serpentinite rubble directly into the sea (Bernier et al., 1997). Livorno (Toscana-Italy) is situated in proximity of the chemical plant of Rosignano Solvay which discharged, until the last decade, about 8000 m³.h⁻¹ of industrial waste into the sea (Petrelli et al. 1979; Balestri et al., 2004). Porto-Torres (Sardinia-Italy) is situated close to the Porto-Torres industrial harbour which is one of the most important in Sardinia in terms of hydrocarbons and petrochemical products traffic (De Luca et al., 2004). In January 2004, a hazardous materials accident occurred in this harbour (tanker unloading benzene, loss of ship; CEDRE, 2004; Ronza et al., 2006). Samples were collected in summer 2004 in Canari and Porto-Torres, and in summer 2005 in Livorno.

2.2. Sampling method, sample preparation and trace metals analyses (cadmium: Cd; cobalt: Co; chromium: Cr; mercury: Hg; nickel: Ni and lead: Pb)

Diffusive gradients in thin films (DGT) sampling technique, based on diffusion of metals through a diffusive layer until a binding phase where they are concentrated (Davison and Zhang, 1994; Zhang and Davison, 2000), was used to determine seawater metal

concentrations. DGT units consist of polyacrylamide hydrogel diffusion layers (0.76 mm thick), Chelex 100 impregnated binding phases (0.40 mm thick) and nylon DGT holders (DGT Research Ltd. UK). They were assembled with 0.45 µm pore-size cellulose acetate filters, used as covering membranes. DGT units were positioned at approximately 2 m above the bottom of the water column and were deployed for about 24h. Elution of metals, from the Chelex 100 binding phase, was carried out by immersion in 1.0 M HNO₃ (Suprapure). Metal analysis was carried out by inductively coupled plasma mass spectrometry (ICP-MS).

Superficial sediment (80 to 500 g dry wt.) was collected in PVC cores by scuba divers, in the vicinity of the *Posidonia oceanica* meadow. The fraction of the sediment inferior to 2 mm was frozen, lyophilised and analysed by atomic absorption spectrometry, with quality assurance procedures, at the Laboratory of Rouen / ETSA (France).

Transplanted mussels were stored in conchylicultural pouches and immersed 6 m below the surface, above *Posidonia oceanica* meadow. Immersion period lasted three months and occurred during the mussel sexual dormancy period in order to improve immersion conditions and to minimize tissue-growth variations (Andral et al., 2004). Mussels were opened raw, the flesh scraped out of the shells, which were then frozen, lyophilised, reduced to powder and analysed by atomic absorption spectrometry with quality assurance procedures at the Laboratory of Rouen / ETSA (France).

Posidonia oceanica shoots were collected at 10 ± 1 m depth by scuba divers. Only the blades (Giraud, 1979) were analysed, as it has been previously reported that most trace metals accumulate preferentially in blades (Lafabrie et al., 2007). Epiphytes and sediment were removed from the blades using a glass strip. Samples were rinsed (ultrapure water), frozen, lyophilised and reduced to powder. For Hg analyses, 50 mg of each sample was weighed in a Teflon digestion vessel CEM[®] ACV of 100 ml (CEM Corporation, USA). 5 ml of 69% HNO₃ (Normapur) and 1 ml of H₂O₂ 30% (Normapur) were added. The vessels were sealed and placed into the CEM[®] MARS 5 chamber (20 minutes at 200°C and 20 minutes of cooling). The content of each vessel was poured into 25 ml volumetric flasks and diluted to volume with ultrapure water and then transferred to 60 ml polypropylene flasks. Mineralized samples were analysed with a cold vapour atomic absorption spectrometer (CV-AAS - Perkin Elmer[®]). The standard addition method was applied for calibration. Calibration standards were prepared from a mercury standard solution 1 000 mg.L⁻¹. Cd, Co, Cr, Ni and Pb were analysed by atomic absorption spectrometry with quality assurance procedures at the Laboratory of Rouen / ETSA (France).

Limit of detection was defined as three times the standard deviation from the mean blank (US Environmental Protection Agency, 2000). The instrumentation limits of detection were between 0.3 and 2 µg.L⁻¹. The standard addition method was applied for calibrations and calibration standards were prepared from standard solutions of 1 000 mg.L⁻¹ (Merck). The analytic procedure was verified using certified reference material (*Lagarosiphon major*, CRM 60; Community Bureau of Reference - Commission of the European Communities; Table 1).

2.3. Statistical analysis

For metal concentrations in sediment, differences between locations were evaluated by Kruskal-Wallis test. For metal concentrations in *Mytilus galloprovincialis* and *Posidonia oceanica*, differences between organisms and between locations were determined by a two-way analysis of variance (ANOVA) followed by the post-hoc test of Tukey. Correlations between metal concentrations in *Posidonia oceanica* and sediment were performed by analysis of Pearson's correlations.

3. Results

3.1. DGT units (DGTs)

The highest Cd, Co and Ni concentrations are recorded in Canari whereas the highest Cr concentration found in Livorno and the highest Pb concentration in Porto-Torres (Table 2).

3.2. Sediment

The highest Co, Cr and Ni concentrations are recorded in Canari ($P < 0.05$) whereas the highest Cd, Hg and Pb concentrations are recorded in Livorno ($P < 0.05$; Table 3).

3.3. Organisms

Metal concentrations in *Mytilus galloprovincialis* and *Posidonia oceanica* in the different locations are shown in Table 4.

Cd, Co, Ni and Pb concentrations are significantly higher ($P < 0.05$) in *Posidonia oceanica* than in *Mytilus galloprovincialis* whereas Cr and Hg concentrations are significantly higher ($P < 0.05$) in *Mytilus galloprovincialis* than in *Posidonia oceanica* (Table 4).

Canari presents the highest Cd, Co, Cr and Ni concentrations ($P < 0.05$) whereas Livorno shows the highest Hg concentration ($P < 0.05$) and Porto-Torres the highest Pb concentration ($P < 0.05$; Table 4).

3.4. Metal bioconcentrations

To evaluate the efficiency of metal bioaccumulation by *Posidonia oceanica*, the biosediment factor (BSAF), defined as the ratio between the metal concentration in the organism and that in the sediment (Lau et al., 1998; Szefer et al., 1999), has been calculated for both species (Figure 2). Cd is the metal which presents the highest BSAF mean and Cr is the metal with the lowest one (Figure 2). *Posidonia oceanica* has the highest BSAF mean for all metals except Hg (Figure 2).

3.5. Relationship between metals in *Posidonia oceanica* and in sediment

There are significant positive relations for Co, Cr, Hg and Ni concentrations in *Posidonia oceanica* relative to their concentrations in sediment ($r_{Co} = 0.81$; $P < 0.05$ - $r_{Cr} = 0.93$; $r_{Hg} = 0.95$; $r_{Ni} = 0.98$; $P < 0.01$). Non significant relations are found in the case of Cd ($r_{Cd} = -0.22$) and Pb ($r_{Pb} = -0.20$).

Linear regression between concentrations in *Posidonia oceanica* (y values) and those in sediment (x values) is calculated for metals with positive significant correlations. Trace metals can be ordered as follows in terms of the value of their slope a : Hg ($a_{Hg} = 0.136$) > Co ($a_{Co} = 0.134$) > Ni ($a_{Ni} = 0.025$) > Cr ($a_{Cr} = 0.001$).

4. Discussion

Canari is identified as the most Cd, Co, Cr and Ni contaminated location by all the compartments investigated, except sediment analysis of Cd and DGTs analysis of Cr which reveal a maximum value for Livorno (as a reminder: there are no replicates for DGTs). Concerning Cd, high values of Cd in the north-west of Corsica have already been revealed (Roméo et al., 1995; Andral et al., 2004) and may be explained by a potential source of Cd contamination in the south-west of Corsica (Lafabrie et al., in press) or by a natural phenomenon such as the upwelling of deep waters rich in Cd (Roméo et al., 1995). The fact that no correlation has been found between Cd concentrations in *Posidonia oceanica* and in sediment could indicate that Cd in *Posidonia oceanica* tissues reflect the Cd in water column. This hypothesis seems plausible as Lyngby and Brix (1982) reported that Cd concentrations in the eelgrass *Zostera marina* L. would reflect Cd concentrations in the ambient water. The fact that, in sediment, the maximum value of Cd is found in Livorno may be related to the presence of a high amount of calcium carbonate grains in the sediment (Petrilli et al., 1979; Ferrara et al., 1989; Balestri et al., 2004) as, in terrestrial environment, it has been suggested that the addition of calcium carbonate to soil may reduce the uptake of some trace metals by plants (Kabata-Pendias and Pendias, 1984; Sanchiz et al., 2001). Concerning Co, Cr and Ni, our results are consistent with literature as previous studies mentioned the presence of high levels of these metals near Canari (Andral et al., 2004) and demonstrated the link with the asbestos mine (see in Lafabrie et al., in press). Furthermore, the fact that positive correlations have been found

between Co, Cr and Ni concentrations in *Posidonia oceanica* and in sediment would suggest that Co, Cr and Ni in *Posidonia oceanica* tissues reflect Co, Cr and Ni in sediment.

Livorno is identified as the most Hg contaminated location by all the compartments investigated. This result may be related to the chemical plant of Rosignano. Indeed, previous studies revealed the presence of a high level of Hg and of high levels of suspended particulate matter (200-300 mg/l) in the effluents of this plant (Petrilli et al., 1979; Ferrara et al., 1989; Balestri et al., 2004), and it has been reported that Hg would be mainly associated to particulate matter (Maserti and Ferrara, 1991; Cossa et al., 1997). Moreover, several studies qualified Livorno as an Hg contaminated site (Maserti et al., 1988; Ferrara et al., 1989; Capiomont et al., 2000; Ferrat et al., 2003a). Furthermore, the fact that a positive correlation has been found between Hg concentrations in *Posidonia oceanica* and in sediment, as in the studies of Ferrara et al. (1989) and Sanchiz et al. (1999, 2001), would suggest that Hg in *Posidonia oceanica* tissues reflects Hg in sediment.

Porto-Torres is identified as the most Pb contaminated location by all the compartments investigated, except sediment which shows a maximum Pb value for Livorno. This result may indicate an influence of the Porto-Torres industrial harbour on Pb contamination as, moreover, recent studies (Baumard et al., 1998, 1999; Escartin and Porte, 1999; De Luca et al., 2004) revealed a general chemical contamination of the Porto-Torres coast. The fact that, as in the study of Malea et al. (1994) and Sanchiz et al. (1999, 2001), no correlation has been found between Pb concentrations in *Posidonia oceanica* and in the sediment, would indicate that Pb in *Posidonia oceanica* tissues reflects Pb in the water column. This hypothesis is reinforced when considering laboratory studies which showed that aquatic plants can remove Pb from the surrounding water (Bond et al., 1988; Axtell et al., 2003). As for Cd, the Pb maximum value in sediment, found in Livorno, may be related to the presence of high amounts of calcium carbonate grains in the effluents of the Rosignano plant (Petrilli et al., 1979; Ferrara et al., 1989; Balestri et al., 2004) which would reduce the uptake of Pb by *Posidonia oceanica* (Kabata-Pendias and Pendias, 1984; Sanchiz et al., 2001).

The DGT technique is considered a useful tool for the evaluation of the marine environment quality (Zhang and Davison, 2001; Manfra and Accornero, 2005; Larner et al., 2006; Stark et al., 2006). Therefore, even if caution must be used in the interpretation of DGTs results (no replicate), it is worth noting that *Posidonia oceanica* results globally lead to the same conclusions as DGT ones. In the same way, sediment is considered a sensitive human-induced impact indicator (Calmano et al., 1996; Acevedo-Figueroa et al., 2005; Tankere-Muller et al., 2006) and it is thus interesting to notice that *Posidonia oceanica* results equally lead to the same overall conclusions as sediment results. Finally, *Mytilus galloprovincialis*, considered a good indicator of trace metals level in the environment (Ostapczuk et al., 1997a, b; Goldberg and Bertine, 2000; Claisse et al., 2001; Webb and Keough, 2002; Andral et al., 2004; Chiffolleau et al., 2005; Funes et al., 2006), presents the same trends as *Posidonia oceanica*. Therefore, the endemic Mediterranean seagrass *Posidonia oceanica*, leading to the same conclusions than the water column, the sediment and the recognized biological indicator *Mytilus galloprovincialis* in terms of metal contamination, can be described as accurate and reliable indicator.

5. Conclusions

Posidonia oceanica is often considered as a useful metal bio-indicator (see Capiomont et al., 2000; Pergent-Martini and Pergent, 2000; Campanella et al., 2001; Ferrat et al., 2003a). This assessment is validated in this study by comparing for the first time metal concentrations found in *Posidonia oceanica* with those found in the water column, in the sediment and in the recognized metal bio-indicator species *Mytilus galloprovincialis*. Furthermore, this study shows that *Posidonia oceanica* may have a greater bioaccumulation capacity than *Mytilus galloprovincialis* for all the metals considered except Hg and may reflect both contaminations in the water column and in sediment. Several studies suggest employing seagrasses as bio-indicators of coastal waters metal contamination (see synthesis in Ferrat et al., 2003b). Therefore, in future, seagrasses, which are widely distributed in the entire biosphere, could be integrated in the setting-up of a general monitoring network as initiated by the European Commission in the Water Framework Directive (2000/60/EC; EC, 2000).

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Table 1 : Analysis of trace metals in the certified reference material *Lagarosiphon major*. Results are expressed in $\mu\text{g.g}^{-1}$ dry wt. (mean value \pm 95% confidence interval).

	Cd	Co	Cr	Hg	Ni	Pb
Certified values	2.20 \pm 0.10			0.34 \pm 0.04		63.80 \pm 3.20
Uncertified values		4.00	26.00		40.00	
Our values	2.03 \pm 0.01	3.70 \pm 0.20	24.00 \pm 1.00	0.35 \pm 0.01	37.00 \pm 1.00	62.40 \pm 0.40

Table 2: Metal concentrations in seawater (in ng.L^{-1} ; the location presenting the maximum value is in bold).

	Cd	Co	Cr	Ni	Pb
Canari	16	17	152	1380	48
Livorno	6	8	616	197	38
Porto-Torres	9	16	282	378	75

Table 3: Metal concentrations in sediment (mean \pm S.E. in $\mu\text{g.g}^{-1}$ dry wt. with S.E.: Standard Error; Standard Error = Standard Deviation / \sqrt{n} ; n = 3 replicates - the location presenting the maximum value is in bold).

	Cd	Co	Cr	Hg	Ni	Pb
Canari	0.03 \pm 0.00	55.33 \pm 14.19	1194 \pm 282	0.02 \pm 0.01	1325 \pm 5	4.67 \pm 0.67
Livorno	0.40 \pm 0.10	7.00 \pm 1.00	85 \pm 17	0.56 \pm 0.14	40 \pm 6	44.50 \pm 4.50
Porto-Torres	0.07 \pm 0.03	2.50 \pm 0.00	9 \pm 4	0.07 \pm 0.04	4 \pm 1	18.67 \pm 2.19

Table 4: Metal concentrations in *Mytilus galloprovincialis* (*M.g.*) and *Posidonia oceanica* (*P.o.*; mean \pm S.E. in $\mu\text{g.g}^{-1}$ dry wt.; n = 3 replicates - the location presenting the maximum value is in bold).

	Cd	Co	Cr	Hg	Ni	Pb
<i>M.g.</i>						
Canari	1.82 \pm 0.05	1.43 \pm 0.09	3.00 \pm 0.67	0.09 \pm 0.00	3.67 \pm 0.86	1.17 \pm 0.09
Livorno	1.13 \pm 0.05	0.06	0.47 \pm 0.07	0.12 \pm 0.01	1.10 \pm 0.10	1.07 \pm 0.09
Porto-Torres	1.77 \pm 0.12	0.83 \pm 0.13	0.43 \pm 0.09	0.10 \pm 0.01	1.37 \pm 0.12	1.43 \pm 0.19
<i>P.o.</i>						
Canari	5.38 \pm 0.14	12.07 \pm 0.52	1.27 \pm 0.23	0.05 \pm 0.00	60.30 \pm 3.67	1.47 \pm 0.03
Livorno	3.39 \pm 0.12	5.73 \pm 0.03	0.27 \pm 0.07	0.13 \pm 0.00	28.90 \pm 0.65	1.40 \pm 0.25
Porto-Torres	2.10 \pm 0.10	1.70 \pm 0.06	0.20 \pm 0.06	0.06 \pm 0.00	27.47 \pm 1.10	1.80 \pm 0.00



Figure 1: Sampling locations in the north-western Mediterranean. 1: Canari (Corsica, France) -2: Livorno (Toscana, Italy) – 3: Porto-Torres (Sardinia, Italy).

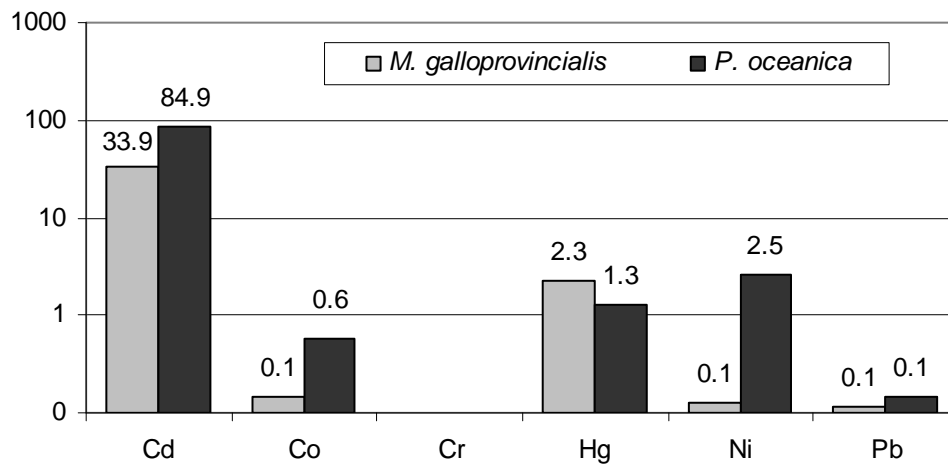


Figure 2: Mean bio-sediment accumulation factor values (BSAF) in *Mytilus galloprovincialis* and *Posidonia oceanica*.