RELATIONSHIPS BETWEEN HEAVY METAL CONCENTRATIONS IN SEDIMENTS AND EELGRASS AND ENVIRONMENTAL VARIABLES (ZOSTERA MARINA, THAU LAGOON, FRANCE)

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HEAVY METALS

Cu MEDITERRANEAN LAGOON EELGRASS SEDIMENTS ENVIRONMENTAL VARIABLES PLANT VARIABLES

MÉTAUX LOURDS

Cu ÉTANG MÉDITERRANÉEN HERBIER SÉDIMENTS VARIABLES ENVIRONNEMENTALES BIOMASSE ET DENSITÉ DES PLANTS

ABSTRACT. - This study focuses during one year on six heavy metals (Cu, Ni, Fe, Pb, Cr, Zn) sampled monthly in sediments, in leaves and roots of Zostera marina L. eelgrass of Thau lagoon (Mediterranean France), where the shellfish farming activity is dominant. These heavy metal concentrations were analysed in relation to twelve environmental variables measured in parallel: rain fall, temperature and salinity of water bottom, C, N and P sediment content, total inorganic nitrogen (TIN) and dissolved reactive phosphorus (DRP) of water bottom and sediment pore water, biomass and density of eelgrass. The PCA of environmental variables, with the added heavy metal concentrations of the three compartments (sediments, roots and leaves) in supplementary data, underlines especially the major relationships between specific concentrations and environmental and plant variables. The regression analysis precises the main direct correlations joining heavy metal concentrations and environmental variables: the inverse correlation between Cu content in sediments and shoot density of eelgrass (y = -0.5x + 83, with $r^2 = 0.8$); (ii) the positive correlation between Cu content in leaves and rain fall (y = 0.14x + 7, with $r^2 = 0.6$), and (iii) those between Cu content in sediments and carbon in sediments, (y = 2x + 5,with $r^2 = 0.5$). This study underlines the importance of the leaching of elements from the surrounding land and the rate of water renewal on the relatively high levels of toxic metals (copper and lead) in the sediments of a marine lagoon exploited for shellfish culture. Significantly, this study confirms the bio-accumulation of these metals in certain parts of a marine phanerogam and demonstrates that this bioaccumulation is a function of plant growth that is itself dependent upon the characteristics of the environment.

RÉSUMÉ. – Six métaux lourds (Cu, Ni, Fe, Pb, Cr, Zn) sont analysés durant un an, mensuellement, dans les sédiments, les feuilles et les racines de l'herbier à Zostera marina de l'étang de Thau (France méditerranéenne), siège d'une intense activité conchylicole. Les concentrations en métaux lourds sont mises en relation avec 12 variables environnementales mesurées en parallèle : précipitations, température et salinité de l'eau du fond, contenus en C, N et P des sédiments, azote total inorganique (TIN) et phosphore total dissous (DRP) de l'eau du fond et de l'eau interstitielle des sédiments, biomasse et densité de l'herbier. A l'ACP des variables environnementales, sont rajoutées, en données supplémentaires et successivement, les concentrations des 6 métaux lourds des 3 compartiments (sédiments, racines, feuilles) ; ceci met en évidence les relations liant chacune de ces concentrations avec les variables analysées. Les analyses de régressions précisent les corrélations majeures liant : (i) concentration en Cu des sédiments et densité des plants (y = -0.5x + 83, avec $r^2 = 0.8$; (ii) contenu en Cu des feuilles et précipitations (y = 0.14x + 7, avec $r^2 = 0.6$), (iii) concentration en Cu des sédiments et contenu en carbone des sédiments, (y = 2x + 5, avec r² = 0.5). Cette étude souligne l'impact de l'importance du lessivage des terrains entourant l'étang et du faible renouvellement des eaux du bassin, sur la concentration élevée de certains métaux (Cu et Pb). Elle confirme de façon significative la localisation préférentielle de certains métaux à l'intérieur de la phanérogame marine, cette bio-accumulation dépendant directement de la croissance du végétal, elle-même liée aux variables du milieu.

INTRODUCTION

The heavy metals resulting from human activities, concentrate in marine sediments (Tiller et al. 1989), and therefore contaminate marine macrophytes. The experiments (Bryan et al. 1980) showed that algae could be used as biomonitors of trace metals. On the other hand, seagrass have the possibility to absorb heavy metals by leaves and by root rhizomes, in contact with the interstitial water of sediment (Farely & Churchill 1979). The metal concentration in eelgrasses varies according to the vicinity or influence of the pollution source (Vzjdeman & Kovekovdova 1991, Munda & Hudnik 1991, Romeo et al. 1995), the analyzed species (Sanchiz et al. 2000), and to the specificity of the metal and of the plant or to the age of plant (Brix et al. 1983, Phillips 1994, Augier et al. 1996, Malae et al. 1994, Malae & Haritonidis 1999, Sanchiz et al. 1999), and therefore, to the season during which the species is sampled.

Heavy metal accumulation was mainly observed in leaves of seagrass, the most available part of these rooted plants. But, in spite of numerous marine local data (Lyngby & Brix 1982, Brix & Lyngby 1984, Tiller *et al.* 1989, Wasseman *et al.* 1991, Guven *et al.* 1993, Malea *et al.* 1994, Sfriso *et al.* 1995, Zwolsman, *et al.* 1996, Campanella *et al.* 2001), the relations between the heavy metal contents in eelgrass, and their physical, chemical and biotical environment were not always clearely established, particularly in lagoons submitted to important variations and where few data on the different part of vegetal were not available or reliable to the environmental variables. In consequence, we decided to focus on four common heavy metals, during one year (1994-1995) in the coastal Mediterranean Thau lagoon, the most important European lagoon for the shellfish farming activity. Research was conducted with *Zostera marina* L. eelgrass (Laugier *et al.* 1998, Rigollet *et al.* 1998), in three compartments (sediment, roots-rhizomes and leaves) with the three main following objectives: 1) Draw up, in spite of seasonal variations, the mean contamination levels in the three chosen compartments for comparison with other sites and eelgrass. 2) Analyse the outstanding data and their relations with some environmental and plant parameters.

METHODS

Study site: The Thau lagoon (Fig. 1) is located on the French Mediterranean coast. It is rather large (75 km²) and deep (average depth 4.5m) with strong marine influence (salinity ranges from 29 to 42 PSU) but a low tidal amplitude (only 2-4% of the total water volume is renewed at any tidal cycle throughout two narrow inlets). Wind induces hydrodynamism patterns (Millet 1989, De Casabianca & Kepel 1999). It is eutrophicated on account of a developed shellfish farming activity (oysters and mussels), especially intensive the north-east. Metallic shellfish farming structures cover around 20 per cent of the whole area. The annual production is about 350 000 tons of mussels and oysters (Hamon & Tournier 1981) inducing an important deposition (De Casabianca 1996). In spite of this eutrophication, an important Zostera marina bed remains in the south-western part of the lagoon, the least eutrophicated (De Casabianca et al. 1997a and b) and less polluted (Pena & Picot 1991) area.



Fig. 1. - Thau lagoon, France: study site.

The study site was located to the vicinity of small harbors and wine cultures.

Samplings and analysis: Flora and sediments were sampled on a silt-sandy substrate, by free diving, on a buoyed point, at $1.70m \pm 0.10$ depth, on a monthly basis (from February 94 to March 95). All the samplings of Z. marina (roots-rhizomes and leaves) were carried out in triplicate, with a metallic frame $(0.25m^2)$, according to permanent-quadrat method (Nienhuis 1978). After collecting biomass, samples were sieved by 3mm mesh sieve to remove sediment particles and epiphytes. Shoot density (per m^2) of Z. marina were sorted and counted at laboratory. The total biomass of Z. marina samplings was weighted before and after drying (70°C, 48 h). Dry weight (DW) corresponded to 20% wet weight (WW). Upper sediments (0-5 cm) were sampled in triplicate, with a corer, and centrifuged (5000 r min⁻¹, 10 min, 0°C) at the laboratory. Sediments were mixed together. Sediment aliquots were stored in PVC containers, frozen and lyophilized for determination of organic carbon nitrogen (CHN Analyser) and phosphorus fraction (Plasma ICP), and for heavy metal analysis.

Aeroport's Frejorgues meteorologic station provided monthly data of rain fall.

The environmental variables of bottom water (temperature in °C and salinity in PSU) were measured in parallel, at the same depth, with a multiparametric probe three times the month and averaged on monthly basis. The water bottom was collected with a Kemmerer bottle for nutrients analysis at laboratory. Surface sediments (0-5 cm) sampled in triplicate, with a corer, were centrifuged (5000 r min⁻¹, 10 min, 0°C) at the laboratory; the extracted pore water was filtered and assayed for nutrients analysis as the water bottom. The nutrients of water was filtered through a GF/C Watman glassfilter and assayed in triplicate for nutrients analysis (N-NH⁴, N-NO³, N-NO² and P-PO⁴) according to Stickland & Parsons (1972), and expressed in μ mol.l⁻¹ of total inorganic nitrogen (TIN calculated as a sum of N-NO²+ N-NO³+N-NH⁴), and of total reactive phosphorus (DRP: P-PO⁴). The environmental parameters of sediment pore water and sediments were measured three times the month and averaged on monthly basis. For Cu, Ni, Fe, Pb, and Cr analysis, the three fractions: leaves, roots-rhizomes and

sediments were treated and mineralized separately. The digestion was carried out in a microwave with 8M ultrapure nitric acid in three steps procedure. For each step, power, temperature and pressure were at a constant level. The final mixture was diluted with Milli-Q water and preserved in Teflon bombs until the analysis by Flame Atomic Absorption Spectrophotometry method (Varian Elmer, equipped with a graphite furnace and a deuterium background corrector). Fe was analysed by flame and Cr, Cu, Ni and Pb were determined by graphite furnace. The precision expressed as standard deviation percentage was less than 10%. The methodology was tested using a certified reference sample.

Data treatment: The twelve variables (TIN and DRP of water bottom; TIN and DRP of pore water sediments. C, N and P of sediments, salinity, water bottom temperature, drain fall, plant density and biomass, were analysed in Principal Componant Analysis (PCA) with ADE-4. Heavy metal concentrations in above and below vegetation and sediments were added, in supplementary data, separatly, for each metal. Regression analysis used Excel package.

RESULTS

Heavy metal contamination levels

The monthly mean concentrations (with standard deviations) of six heavy metals in the sediment, roots-rhizomes and leaves of the *Z. marina* sea-grass bed are given in table I for the study period. In decreasing order, the hierarchy of the heavy metals studied is different in the three different compartments. Copper and lead come after chromium in the sediments, but the inverse is observed in the roots-rhizomes and leaves:

 $\begin{array}{l} Fe > Cr > Cu > Pb > Ni, \mbox{ in the sediment,} \\ Fe > Cu > Pb > Cr > Ni, \mbox{ in the roots-rhizomes,} \\ Fe > Cu > Pb > Ni > Cr, \mbox{ in the leaves.} \end{array}$

		Fe	Zn	Cr	Cu	Pb	Ni
	Sédiment	6100 ± 1090	36 ± 8	21 ± 8	19 ± 4	13 ± 4	9±3
Content	Root	921 ± 87	44 ± 19	2 ± 2	9±5	2 ± 1	1 ± 1
	Leave	186 ± 82	83 ± 29	0.3 ± 0.2	10 ± 4	1 ± 1	0.6 ± 0.4
Ratio	Root / sed.	0.15	0.5	0.08	0.5	0.1	0.1
	Leave / sed.	0.03	2.3	0.01	0.5	0.1	0.1
Significan	t						
difference		root>leave	leave>root	root>leave	no	no	no

Table I. – Heavy metal contents ($\mu g.g^{-1}$ Dry Weight) in sediments, roots and leaves of Z. marina eelgrass of Thau lagoon (averages and standard deviations), ratios (Roots/sediments and Leaves/sediments), and significant differences between roots-rhizomes and leaves.

This suggests that copper and lead accumulate in the plants.

The concentration ratios between the leaves and the sediment are also given in table I, along with the significant differences observed between the two compartments of the plants.

Taken together, the results for the sea-grass bed and the sediment indicate that nickel, iron, chromium and lead are proportionately more concentrated in the sediment (80% for lead and 91% for chromium). Iron and lead, on the other hand, have distributions with 15% and 9% respectively in the leaves. Copper is equally distributed between the plants and the sediment, with 24% in the leaves and 24% in the roots. These results show a weak absorption of chromium by the plant, with accumulation in the roots, an accumulation of iron in the roots and copper, lead and nickel accumulated equally in the roots and leaves. The concentration differences between the roots and leaves are statistically significant (p<0.01).

Relations between heavy metal concentrations and environmental and plant variables

The environmental variables (Table II) show that the maxima of monthly averages of water bottom temperature (26.5°C) and salinity (42 PSU) occurred in August 1994, with the minimum of rain fall. The minimum of salinity (30 PSU) occurred in October 1994, and those of temperature (4.9°C) in January 1995. Shoot density of *Z. marina* (143 ± 23 plants. m⁻²) showed a peak in June (170 shoots. m⁻²), before the biomass peak, in August (269 g DW.m⁻²). The average of biomass was 151 ± 67 g DW.m⁻². The carbon concentration in sediments varied from 6.2 to 8.5 µg.g⁻¹ DW (De Casabianca *et al.* 1996).

The PCA analysis of 12 environmental variables analysed together showed that six environmental variables could explain the major variance, in particular, eelgrass biomass (88%), TIN of bottom water (11%), rain fall (0.1%), bottom-water temperature (0.02%), plant density (0.02%), and DRP of bottom water (0.01%).

The correlations circle of the all environmental and plant variables (Fig. 2) show among the environmental variables, different groups of variables: (1) a group of variables related to plant growth (biomass and plant density) or indirectly linked to it in the Thau lagoon (bottom temperature, TIN of bottom water and of sediment-pore water and salinity), (2) the DRP of interstitial water, (3) C, N, P contents in sediments, and (4) the rain fall.

The supplementary data of heavy metals concentrations in sediments, in roots and leaves, added separatly for each heavy metal, to the previous correlation circles, joined these described groups. Cu concentration in supplementary data which showed the most significant correlations is presented (Fig. 2).

Cu concentration in leaves was well represented and positively correlated with rain fall (4); Cu contents in sediments were in close position to C, N, P, content in sediments (3). Cu content of roots-rhizomes were well represented and positively correlated to DRP of sediment-pore water (2).

Pb and Ni content of roots-rhizomes were well represented and positively correlated to DRP of sediment-pore water (2). Fe content in sediments was well represented and positively correlated to the rain fall (4).

The regression analysis showed, some important relationships linking separatly each heavy metal concentration to each variable or each variable with another. We point out in particular the relations between Cu and environmental and plant parameters: (i) the inverse relation between Cu content in sediments and shoot density of eelgrass $(y = -0.5x + 83, \text{ with } r^2 = 0.8)$; (ii) the positive relation between Cu content in leaves and rain fall $(y = 0.14x + 7, \text{ with } r^2 = 0.6), \text{ and (iii) the positive relation between Cu content in sediments and carbon in sediments, <math>(y = 2x + 5, \text{ with } r^2 = 0.5)$.

DISCUSSION

The goal of this study was to obtain data for heavy metal concentrations, in three compartments of a sea-grass bed (sediment, roots-rhizomes and leaves) in the Thau lagoon, that are sufficiently precise to be compared with data in the litterature and to be treated statistically, in parallel with data

Table II. – Environmental and plant variables of Z. marina eelgrass of Thau lagoon, France (averages and standard deviations) (De Casabianca *et al.*1996).

36 ± 4.6	C sediment content (µg.g-1 D.W.)	7.4 ± 0.9
16 ± 7	N sediment content (µg.g-1 D.W.)	0.12 ± 0.05
62 ± 3	P sediment content (µg.g-1 D.W.)	0.03 ± 0.005
351 ± 36	Plant density (Shoot.m- ²)	143 ± 23
31 ± 4	Plant biomass (g.D.W.m ⁻²)	151 ± 67
9.3 ± 5	Rain fall (mm/month)	14 ± 16
	36 ± 4.6 16 ± 7 62 ± 3 351 ± 36 31 ± 4 9.3 ± 5	36 ± 4.6 C sediment content (µg.g-1 D.W.) 16 ± 7 N sediment content (µg.g-1 D.W.) 62 ± 3 P sediment content (µg.g-1 D.W.) 351 ± 36 Plant density (Shoot.m-2) 31 ± 4 Plant biomass (g.D.W.m-2) 9.3 ± 5 Rain fall (mm/month)



Fig. 2. – Top, PCA of twelve variables: Plant density (Pl.DENS): Biomass (BIOM); Water bottom temperature (W.B.TEMP): Salinity (SAL); Nutrients: TIN in water bottom (W.B. TIN); DRP in water bottom (W.B. DRP); TIN in pore water (P.W.TIN); DRP in pore water (P.W.DRP); rainfall (RAINFALL); carbon, nitrogen and phosphorus contents in sediments (C.SED; N.SED; P.SED). Bottom, PCA of twelve variables (see Fig. 2) with Cu in supplementary data: Cu contents in sediments (SED. Cu), roots rhizomes (ROOTS Cu) and leaves (LEAVES Cu).

for environmental variables. With this in mind, the sampling methods (sampling frequency, simultaneous sampling, choise of marking of the sampling sites and SCUBA diving sampling techniques) were chosen to take into account the large spatial and seasonal environmental variations typical of the Mediterranean lagoons that are likely to have an impact on the concentrations of heavy metals in the sediments and different compartments of the sea-grass bed.

The comparison with some other marine sediments (Table III) covered by the same Z. marina eelgrass and sampled at the same depth, showed values lower than those of the French lagoon (Lungby & Brix 1982, Fernandez et al. 1994) except Zwolsman et al. 1996. The sediments of Venice lagoon presented higher heavy metal concentrations than in Thau lagoon in general and Greece sediments (Malea & Haritonidis 1994), extreme values in copper (5.6 time higher than those of Thau sediments). Many sediments without seaweed (Guven et al. 1993, Ward & Hutching 1996, Mielke et al. 2000) presented high Cu values, but Arabic Gulf values (Al Abdali 1996) were comparable to those of Thau. The values found previously at Thau (Pena & Picot 1991) were based on the samplings carried out on the edge and in the urbanized north-east coast of the lagoon. Concerning bio-accumulation, for Cu, for example, the content in leaves was higher than the content in roots-rhizomes at Thau and in other marine or brackish water species: Lunby & Brix (1982), Brix & Lunby (1983) on Z. marina, Augier et al. 1996, on Z. noltii, Sanchiz et al. (1999) on Z. noltii and Ruppia cirrhosa, Sanchiz et al. (2000) on Cymodocea nodosa, Posidonia oceanica, Ruppia cirrhosa, and Z. noltii. At Thau lagoon, the Pb concentration in roots-rhizomes of Z.marina was higher than the content in leaves. This observation is in accordance with Lunby et al. (1982), Hoven et al. (1999) on the same species, with those of. Sanchiz (1999, 2000) for Z. noltii, and with those of Campanella et al. (2001) for Posidonia oceanica. The contents in roots-rhizomes were significantly higher in roots-rhizomes than in leaves for Fe at Thau on Z. marina. Our results showed the same trend for Ni.

The impact of seasonal environmental variations on the concentrations of heavy metals in the sediments and the sea-grass bed have been little studied before, with the only data available concerning the concentrations of heavy metals in the sediments and their concentration in the organic matter (Tiller *et al.* 1989, Cacador *et al.* 1996, Goulet & Pick 2001, Lee & Cundy 2001). Our results confirm this relationship for copper. It is of note that copper and iron in the sediments are very sensitive to rain.

We have attempted to establish the relationships existing between the concentrations of heavy metals, in the sediments and sea-grass bed, and environmental variables and variables linked to the biology of the plants *per se*. With this in mind, the results obtained have been analysed statistically by multivariate analysis (MVA) or bivariate analysis (regression analysis). In the Thau lagoon, the con-

	Thau lagoon	Venice lagoon	Arabic Gulf Kuwait	Bosphorus Strait	Greece	Mississippi Delta, U.S.A.	Scheldt estuary Netherlands	Jacarepagua lagoon, Brazil	Arcachon Gulf France	Limfjord Denmarck
	France	Italy			Antikyra Gulf					
		Sfriso et al.	Al.Abdali	Guven et al.	Malae et al.	Mielke et al.	Zwolsman	Fernandez	Wassermann	Lungby & Brix
		1995	1996	1993	1994	2000	et al.,1996	et al., 1994	et al., 1991	1982
Fe	6100 ± 1090	13200 -18300	10000-20000	10488 ± 126	6941 ± 459	2000	880 ± 60		4209	
Zn	35 ± 8	77 - 306	30 - 60	86 ± 8	28 ± 2	11	52 ± 7	127	60	4.3 -14.6
Cr	21 ± 8	41 - 106		26 ± 6		0.8	48 ± 4	7		
Cu	19 ± 4	7.8 - 30	15 - 30	25 ± 4	108 ± 18	4	8 ± 1	14	5	0.62 - 3.85
Pb	13 ± 4	17 - 40	13 - 30	44 ± 2	173 ± 25	5	14 ± 2	24		0.52 - 16.3
Ni	9 ± 3	14 - 20	70 - 80	18 ± 2		4		14		

Table III. – Heavy metal concentrations in sediments of different marine sites ($\mu g.g^{-1}$ Dry Weight).

centration of copper in the plants is linked to the dynamics of plant growth and especially to plant multiplication. The biodynamics of the sea grass are themselves a function of seasonal variations of environmental parameters dominated by the temperature of the bottom waters (Sand-Jensen 1982). The role of the phosphate concentration of the sediment interstital water is highlighted in this study, as it should influence the absorption of heavy metals by the roots.

The same relative order in heavy metal concentrations (Fe > Cr > Cu > Pb > Ni) has been found in other marine sediments (Table II). In cases of heavy pollution, the absolute concentrations of heavy metals can be 10 times (Russian arctic zone; Zhulidov et al. 1999), or even 100 times (Golf of Spensen, southern Australia; Ward & Hutching 1996) higher than those of the Thau lagoon. The concentrations of Fe, Cr and Ni are lower in the Thau lagoon than in a comparable sea-grass bed in the Lido region of the Venice lagoon (Sfriso et al. 1995), whereas the concentrations of copper and lead are similar or higher. The Thau lagoon has a limited tidal renewal of water compared to the Venice lagoon that is highly industrialised on its northern shores. The concentration of copper is also higher in the Thau lagoon with respect to the Mississipi delta (Mielke et al. 2000), the inverse being true for lead. The concentrations of copper and lead in Thau are comparable to those observed in the Persian Gulf, between Kuweit and Iran (Al-Abdali *et al.* 1996); a zone that harbours extensive oil industry installations. Similar heavy metal concentrations are also observed in sea-grass beds in Denmark (Brix et al. 1984) and the Spanish coast (Sanchiz et al. 1999): Cu and Pb are always dominant in Thau.

The role of certain factors that influence the concentrations of heavy metals in the lagoon should be underlined, especially those that are linked to sources of pollution. These factors include the leaching of elements from the surrounding land (for example, in Thau surrounding vinyards are treated regularly with copper sulphate) and the rate of renewal of marine water in the lagoon.

Taking together the concentrations of heavy metals at different sites, it is apparent that the seagrass-free sediments generally have higher concentrations than the sediments harbour in a sea-grass bed. Comparison of the Thau results with available data, both in Z. marina and in sediments, at Limfjord, Denmark (Brix & Lunby 1984), show that, despite generally higher values in sediments at Thau, the roots-rhizomes and leaf concentrations were comparable for all the metals at the two sites, except for copper, that was higher at Thau.

The accumulation of heavy metals in plants at the Thau lagoon is similar to that observed for many marine phanerogams. The work presented here confirms the bio-accumulation of heavy metals in certain parts of the phanerogam *Z. marina* (*e.g.* Pb in the roots and Cu in both roots and leaves).

This study underlines the importance of the leaching of elements from the surrounding land and the rate of water renewal on the relatively high levels of toxic metals (copper and lead) in the sediments of a marine lagoon exploited for shellfish culture. Significantly, this study confirms the bioaccumulation of these metals in certain parts of a marine phanerogam and demonstrates that this bioaccumulation is a function of plant growth that is itself dependent upon the characteristics of the environment. Close surveillance of the links in the ecosystem, in relation to environmental variables is, therefore, more than ever desirable for the preservation of our environment and health.

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