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Bioaccumulation of Metals in Sediment Elutriates and Their Effects on Growth, Condition Index, and Metallothionein Contents in Oyster Larvae

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Abstract. The bioavailability of Cd, Cu, Zn, and Pb from two metal-contaminated sediments (Bidassoa and Dunkerque) was studied using *Crassostrea gigas* larvae exposed to sediment elutriates. The metal contents within the sediments, the larvae and larval growth, the condition index, and the induction of metallothionein in the larvae were measured. The larval growth and condition index were only affected after exposure to the highest elutriates concentration (5 to 25%) from the most contaminated sediment (Dunkerque). Bioaccumulation of all metals was observed in larvae exposed to Dunkerque elutriatre; only Cu bioaccumulation was observed in the Bidassoa elutriate. The results from larvae exposed to both sediment elutriates show a strong correlation between bioaccumulated metal considered individually or in combination and the metallothionein level in larvae presenting no detrimental effect. On the other hand, in the case of larvae exposed to the highest Dunkerque elutriate concentration and showing the highest metal body burden, we observed a drop in the metallothionein level. These results indicate that metallothionein is a more sensitive indicator of heavy metal pollution than physiological endpoints taken into account in bioassays and could be proposed as an early biomarker of metal exposure in larvae. However, care must be taken with ''fault control'' due to the toxicological effect on larvae metabolism in the case of substantial contaminant exposure.

Key words: Sediment elutriates—*Crassostrea gigas* larvae—Larval growth—Metal bioaccumulation—Metallothionein

Marine and coastal areas are constantly subjected to the introduction of natural and anthropogenic pollutants, which are mostly adsorbed by suspended particles and subsequently accumulated in the sediments. Sediments can accumulate metals at concentrations 10,000 times higher than in the overlying water column (Förstner 1979), constituting an important source of contamination and risk for living organisms. Coastal and estuarine areas serve as reproductive and nursery grounds for many invertebrate and fish species and should be preserved.

Benthic and epibenthic species are the most heavily exposed to contaminants originating in sediments, to those which are adsorbed on sedimentary particles, as well as to those that are dissolved in interstitial water. Pelagic organisms are also exposed to sediment-bound contaminants either via the food web and/or after the remobilization of contaminants into the sea water (Chapman and Long 1983, Fichet *et al.* 1998, Miller *et al.* 2000). The contamination of the water column occurs by diffusion and when the sediments are resuspended by natural factors such as bioturbation (Burgess *et al.* 1993, Peterson *et al.* 1996, Ciarelli *et al.* 1999, 2000), storms, wave and tide action, and by human activities such as dredging operations (Chapman *et al.* 1998). When sediments are resuspended, the amounts of heavy metals released into the sea water are relatively low (Kwan and Dutka 1996, Van den berg *et al.* 2001) and do not generally induce short-term toxicity. Long-term toxicity assessment of these low contamination levels requires that sensitive and early-warning biological tools be developed. Elutriate tests can assess the toxicity of sedimentary metals released in the water column when sediments are resuspended (Thompson *et al.* 1999).

Chemical analyses characterize the contamination level of the medium (water or sediment), but they are inadequate to assess the biological quality of a zone being studied. Only living systems are able to integrate the various complex effects of contaminants that are really bioavailable (Chapman and Long 1983). Numerous bioassays, standardized to varying degrees, have been developed over the two past decades (see review by Burton 1992; His *et al.* 1999). Bivalve embryos are among the most sensitive test organisms and have been used in numerous environmental toxicology studies (Taylor 1978, Carr *et al.* 1996, McPherson and Chapman 2000). Instead of the endpoints considered in bioassays, a larval growth test is most

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difficult to carry out, but it is more sensitive (Geffard *et al.* 2002a) and assesses the bioavailability and toxicity of both the soluble contaminant fraction and contaminants adsorbed on suspended particles. Determining the condition index in veligers of *Crassostrea gigas* was also shown to be a sensitive endpoint (A. Geffard *et al.*, 2002).

Bioassays can evaluate the toxicity of contaminants that are really bioavailable and, therefore, are a valuable tool to study the biological quality of estuaries and coastal areas. However, the physiological endpoints of these tests are not early-warning biological responses. When harmful effects are observed, the biological quality of the area studied is poor and the contamination levels are already high, with consequent irreversible effects on living organisms. Moreover, bioassays do not identify the compounds inducing the observed biological effects.

The bioavailability of contaminants depends on several factors such as the physico-chemical properties of the contaminant itself, the characteristics of the environment, and the characteristics of the organism used in the bioassay (Borgmann 2000). One of the recognized methods to assess the bioavailability of contaminants is to observe their accumulation in test organisms. This method takes into account all the factors that control their bioavailability (Connell *et al.* 1999, Borgmann 2000). Recently, for several compounds, various authors have determined critical body burden concentrations above which biological effects could be observed. However, this approach cannot be used with essential metals (Cu, Zn) in species that regulate efficiently these elements (Rainbow, 1998) and with PAHs which may be biodegradable.

Biomarkers could also be used to study the bioavailability of contaminants, such as metallothionein for metal exposures. Many studies have shown that metallothionein, a low-molecular-weight cytosolic protein, is induced by metal contamination in numerous taxa, including mammals, fish (Hamza-Chaffai *et al.* 1997), and marine invertebrates (Bebianno and Serafim 1998, Bebianno *et al.* 2000, Mouneyrac *et al.* 2000, 2002; see review by Amiard *et al.*, 2006). It is generally admitted that the primary role of metallothionein is the homeostasis of essential metals such as Cu and Zn, but this protein is also involved in the detoxification of non-essential metals such as Ag, Cd, and Hg (Roesijadi 1992, Amiard *et al.*, 2006). Metallothionein-like protein has also been detected in bivalve larvae of *Mytilus galloprovincialis* (Pavicic *et al.* 1994) and *Crassostrea virginica* (Ringwood and Brouwer 1993, 1995, Roesijadi *et al.* 1997). However, the inductions are observed for studies using metallic salts and experimental doses that are unrealistic compared to environmental concentrations present even in metal-rich areas. More recently, metallothionein-like protein induction was detected in veligers of *M. galloprovincialis* exposed to natural contaminated sediment (A. Geffard *et al.* 2002). However, these authors used freeze-dried sediment known to be more toxic than the fresh sediment (Geffard *et al.* 2002b, 2004).

The aim of this study was to use several endpoints at different levels of biological organization, namely the larval growth test, the condition index determination, and the metallothionein concentration as sensitive and early-warning tools to assess the bioavailability and the toxicity of metals released into the water column when sediments are re-suspended. For this, fertilized eggs and larvae of *Crassostrea gigas* were exposed to elutriate obtained from natural sediments. Two metal-rich sediments were selected, one from a coastal zone (Bidassoa Estuary, on the French-Spanish border) and the second from the port of Dunkerque (North of France). The final aim of this study was to establish links between biochemical response (metallothionein), metal accumulation, larval growth, and the condition index of larvae.

Materials and Methods

Sampling and Preservation of Sediment

Sediments were sampled in July 1999. At the Bidassoa estuary, only the top 2 cm of the surface were scraped using a plastic blade. Sediments from the port of Dunkerque were collected with a stainless steel Van Veen grab. All sediments were wet-sieved at 2 mm to eliminate debris, homogenized, and stored in glass bottles at 4°C in darkness for less than 1 week prior to bioassays.

Elutriate Preparation

Elutriates were prepared using a modification of Melzian's method (1990). Sediments were shaken mechanically (multi-wrist shaker, 500 rpm) in filtered seawater (FSW) at a ratio of 1:4 (sediment: water) for 8 h and allowed to settle for another 8 h before recovery of the supernatant (elutriate). Elutriate was diluted with FSW to concentrations of 0 (control), 5, 10, 25, and 50% for Bidassoa sediment and 0 (control), 1, 5, 10, and 25% for Dunkerque sediment. These concentrations were selected because they did not result in abnormal effects on embryonic development of *Crassostrea gigas* in previous experiments. The maximum concentrations tested correspond to the no-observed-effect concentrations (NOEC; Geffard *et al.* 2002a). An aliquot of each raw elutriate and of the FSW used were used for metal analysis.

Larval Rearing

Mature oysters were collected in Arcachon Bay (France), which is extensively used for oyster farming based on Pacific oysters *Crassostrea gigas*, and was, therefore, assumed to have a good biological quality (Geffard *et al.* 2002b). The procedure to obtain embryos was described in detail by Geffard *et al.* (2001a) and A. Geffard (2002). Fertilized eggs were counted and placed in 2-l beakers (60,000 l^{-1}) filled with the different media to be tested (three replications per treatment). After the first 24 h, veligers maintained in rearing status were placed in 2-l beakers (10,000 larvae l^{-1}, three replications) and fed with *Isochrysis galbana* (150 algae μl^{-1}). All experimental solutions were renewed at 48-h intervals, using elutriates prepared a few hours before. At this time, larvae were photographed with a Canon camera fitted to an inverted microscope and larval height of 50 individuals per replicate (distance between the umbo and the ventral valve margin; Galtsoff 1964) was measured. The growth test was stopped after 10 days for the Bidassoa elutriate. On the contrary, the Dunkerque elutriate had severe effects on *C. gigas* growth, so much so that experiments were stopped on day 5 for the highest concentration and on day 7 for another. In the end, larvae were recovered through a sieve (32 μm), rinsed with 0.9% aqueous ammonium formiate (to eliminate the salt), freeze-dried, weighed, and stored at 4°C in a hermetic bag

Table 1. Heavy metal contents in Dunkerque and Bidassoa sediments ($\mu g \cdot g^{-1}$ dry weight, mean and SD in parantheses), raw elutriates obtained from these sediments and control seawater ($\mu g \cdot g^{-1}$) used to extract and dilute these raw elutriates to obtain the different exposure media.

	Site	Cd	Cu	Zn	Pb
Sediment	Dunkerque	2.2 (0.05)	158 (10)	542 (36)	391 (24)
	Bidassoa	0.8 (0.1)	70 (11)	268 (31)	74 (9)
Filtered seawater		0.009 (0.001)	0.7 (0.2)	2 (0.5)	0.7 (0.2)
Raw elutriate	Dunkerque	0.05 (0.01)	5 (1.1)	21.5 (4)	12.3 (2)
	Bidassoa	0.10 (0.03)	10.6 (3.3)	25.3 (5.3)	8.3 (2.7)

before being used for analysis. The condition index (CI) of larvae from each replicate was determined using the following equation:

$$\mathrm{CI} = \frac{\text{mean weight of a lyophilised larvae } (\mu g)}{\text{mean shell height of a larvae } (\mu m)} * 100$$

Pretreatment of Sediments for Metal Analysis

Aliquots (three 0.5-g replicates) of each fresh sediment were taken from the well-homogenized total sample and placed in acid-washed glass tubes. These samples were then dried and weighed to determine metal concentration as a function of dry weight. Hot mineralization (95°C) was performed by adding 5 ml of HNO_3 and 3 ml of HCl. This process was conducted until dryness, and the residues were then suspended again in 10 ml of 1N HCl for metal analysis.

Extraction of Metals and Metallothionein from Larvae

A. Geffard *et al.* (2002) described the process for tissular compartmentalization of metals and partial purification of metallothionein. Each replication of lyophilized larvae was homogenized in Tris-NaCl buffer and the cytosolic (S1) and insoluble (P1) fractions were separated by initial centrifugation (25,000*g*, 55 min at 4°C). The insoluble fraction was made up of all cellular and tissular debris plus larva shells. Metallothionein was isolated from an aliquot (50 µl) of the S1 fraction by a second centrifugation (15,000*g*, 10 min at 4°C) after being subjected to heat (75°C, 15 min). This second supernatant (S2) containing metallothionein was frozen at –80°C prior to being used for metallothionein analysis. Before metal analysis, an acid digestion step was required for the soluble (S1) and insoluble (P1) fractions, using a procedure described in A. Geffard *et al.* (2002). After acid digestion, the solutions obtained were supplemented to a known volume (2 ml) with deionized water. The three replications of each exposure condition were treated separately with the exception of the larvae exposed to 5, 10, and 25% concentrations of the Dunkerque elutriate, where larval growth and weight were low. The three replicate samples were mixed together to prevent problems of sensitivity related to the analytical tools.

Metal Assays

Following the acid digestion phase (for sediment and cytosolic (S1) and insoluble (P1) fraction of larvae), metals were analysed by flame atomic absorption spectrophotometry (AAS) for Cu in sediment and Zn in sediment and larva fraction. We used electrothermal AAS with the Zeeman effect (Hitachi Z8200) for Cu in larvae fractions and for Cd and Pb in larva fractions and sediment. The analytical method was previously described by Amiard *et al.* (1987). To eliminate the matrix effect, standard addition analysis was performed in an iso-medium, and the concentration of each element was +125, 250, and 500 ng ml^{-1} for Zn and Cu in flame AAS, +12.5, 25, and 50 ng ml^{-1} for Cu, +0.25, 0.50, 1 ng ml^{-1} for Cd, and 6.25, 12.5, and 25 for Pb in electrothermal AAS. Metal concentrations were determined in the elutriates according to the method described by Danielsson *et al.* (1982). The assays were validated using certified samples of sediments (SD-M-2/TM IAEA) and mussel tissues (BCR, 278R). The total bioaccumulation of metals (Cd, Cu, Zn, and Pb) in larvae was calculated by summing the amounts that were measured in the soluble (S1) and insoluble (P1) fractions.

Larval Metallothionein Assay

The metallothionein assay was performed in the S2 fraction by differential pulse polarography. The thiol groups (SH) were determined using Brdicka reagent (1933) according to the method of Thompson and Cosson (1984). Measurements were performed at a constant temperature (4°C) on a polarograph using a PAR Model 174 analyser, a PAR/EG&G Model 303 electrode in SMDE mode, and a RE0089 type X-Y recorder. The metallothionein amounts were measured by a standard addition using the metallothionein rabbit liver metallothionein standard (Sigma Chemical Co., St Louis, MO, USA) (no metallothionein standard exists for oysters). The validity of this method was confirmed by Olafson and Olsson (1991).

Statistical Analysis

For each series of results, values were compared by one-way ANOVA (Statistica software). Significant differences (at the 95% level) were then determined by Tukey's test, except for larvae exposed to 5, 10, and 25% of Dunkerque elutriate because there was only one value per elutriate concentration tested.

Results

Sediment and Elutriate Contamination

Metal concentrations in each sample of studied sediments are indicated in Table 1. For comparison, Table 2 gives the French Géode classification system, which defines sediment quality according to the degree of contamination with PCBs and eight metals (Lamy Environnement 1996). ''Géode background'' denotes the natural concentrations and ''Géode median'' is calculated from the samples taken at various ports in France.

Table 2. French ''géode classification of sediment quality

	PCB	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Géode background	0	4.4	0.5	45	35	0.2	20	47	115
Géode median	0.00025	12.5	0.6	45	22.5	0.2	18.5	50	138

PCB = polychlorinated biphenyl. Géode background denotes mean PCB and metal contamination level ($\mu g \cdot g^{-1}$ dry weight) of sediments collected from reference and uncontaminated areas. Géode median (Md) is calculated on the basis of sediments taken in various harbours in France.

Table 3. Condition index (CI, mean; ± SD between bracket) of larvae recovered after 10 (Bidassoa) or 7 (Dunkerque) days of rearing in medium containing different percentage of elutriate

Site	0%	1%	5%	10%	25%	50%
Bidassoa	0.52 (0.10)	nd	0.68 (0.09)	0.44 (0.31)	0.50	0.46 (0.08)
Dunkerque	0.39 (0.006)	0.24* (0.02)	0.16* (0.0008)	0.23* (0.006)	ns	nd

No CI was calculate in the case of 25% of Dunkerque elutriate because rearing was stopped after 5 days.
* Significant difference at 95% level of the CI compare to the control (0%) for each sediment tested.
nd: no data.

Sediments having values lower than double the median (termed ''level 1'') are authorized for offshore dumping. Sediments with values higher than four times the median (termed ''level 2'') cannot be dumped offshore. Sediments with intermediate values require further analyses, including bioassays, before a decision is taken. The Dunkerque sediments were more highly contaminated than the Bidassoa sediments. In the sediments from Dunkerque, Cd and Zn levels and Cu and Pb levels surpassed level 1 and level 2, respectively. Bidassoa sediments were characterized by a Cu contamination with a value higher than level 1. Metal concentrations measured in the raw elutriates and control filtered seawater are also presented in Table 1. Inversely to values for sediments, higher metal concentrations were generally observed in elutriate with Bidassoa sediment, except in the case of Pb.

Larval Biometry

The condition index (CI) of larvae exposed to the Bidassoa elutriate was not significantly different depending on the exposure ($p = 0.274$; Table 3). For the Dunkerque elutriate, the CI significantly ($p < 0.001$) decreased between the control and the lowest concentration (1% elutriate). The CI of larvae exposed to 5 and 10% elutriate was lower than of the CI of control larvae. The CI of larvae exposed to the 25% Dunkerque elutriate was not indicated because the recovered larvae were only 5 days old.

Bidassoa elutriate induced a weak inhibition of larval growth at the highest concentrations and on day 10 (Fig. 1), but the difference with the control value was not significant ($p = 0.07$). On the contrary, Dunkerque elutriate had harmful effects on *C. gigas* growth (Fig. 1). The growth of larvae reared in 25% elutriate was significantly reduced ($p < 0.001$) from day 3 to the end of the experiment. The same phenomenon occurred at all concentrations on day 5 ($p < 0.001$).

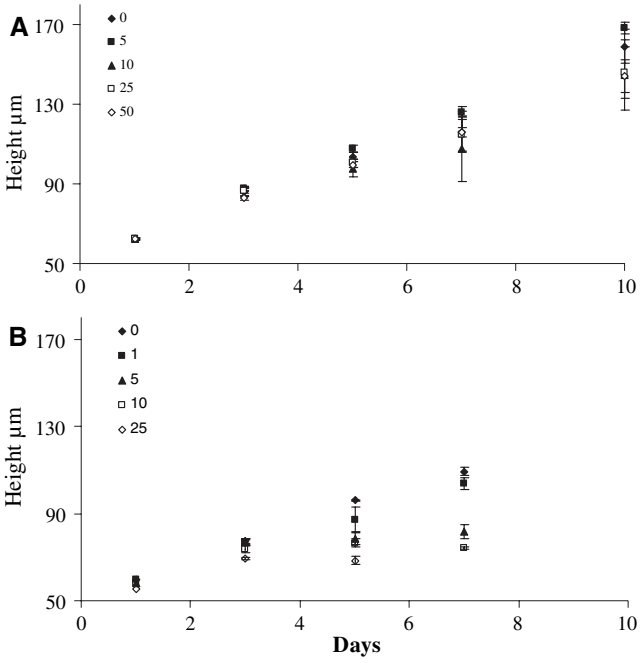

Fig. 1. Growth (µm; mean ± SD) of *Crassostrea gigas* larvae exposed to Bidassoa (**A**) and Dunkerque elutriates (**B**) for 10 and 7 days, respectively. Statistical comparison is presented in the text

Metallothionein Concentrations

For the Bidassoa elutriate, metallothionein contents in larvae significantly increased ($p < 0.001$) for a concentration higher than 5%. Metallothionein values ranged from 864 mg kg^{-1} for controls to 1,430 mg kg^{-1} in larvae exposed to 50% elutriate (Fig. 2). Metallothionein values were higher by a factor of between 1.2 to 1.7, depending on the degree of contamination. For the Dunkerque elutriate (Fig. 2), the metallothionein

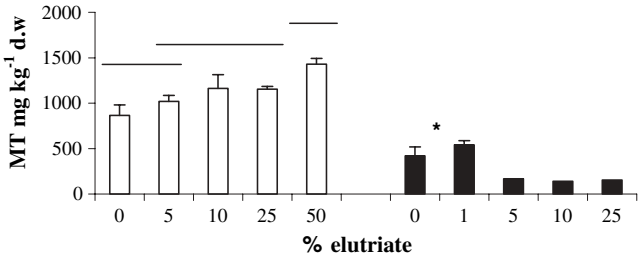

Fig. 2. Metallothionein contents (mean ± SD, n = 3) in larvae after 10 days (Bidassoa, white bars) or 7 days (Dunkerque, black bars, except at 25% only 5 days) of exposure to different elutriate concentrations. For Bidassoa, values not significantly different from each other are grouped under a common overhead line (ANOVA, $p < 0.05$; Scheffé's test). For Dunkerque only, control larvae and larvae exposed to 1% of elutriate could be compared (see text). *Significant difference at the 95% level of significance

concentration significantly increased between the control and the 1% concentration elutriate ($p < 0.05$), while the concentration rapidly decreased for the highest concentrations. The significance decrease of the metallothionein between the control and the 5, 10, and 25% concentrations could not be tested because of the lack of replicates. However, metallothionein concentrations in larvae exposed to 5, 10, and 25% elutriate were three or four times lower than those observed in larvae reared in control sea water and 1% elutriate. The metallothionein concentration in 7-day-old control larvae (Dunkerque experiment) were significantly lower ($p < 0.001$) than this in 10-day-old control larvae (Bidassoa experiment).

Metal Bioaccumulation

Metal levels of larvae reared in the presence of the different elutriates are showed in Figure 3. For the Bidassoa elutriate, no significant ($0.108 < p < 0.9938$) bioaccumulations of Cd, Zn and Pb were observed in larvae. Only Cu levels significantly ($p < 0.001$) increased for the elutriate concentration higher than 5%. The Cu values were higher by a factor of between 1.1 to 1.8, depending on the degree of contamination. Larvae exposed to Dunkerque elutriate showed contamination levels higher than those exposed to Bidassoa (except in Zn). Cd and Cu concentrations increased as the elutriate concentration rose. However, the lack of replicates meant that the increase of this significance could not be tested. With Zn and Pb, significant ($p < 0.0108$) accumulations were observed for the lowest tested concentration (1%). The metal values were higher by a factor ranging from 1.6 to 2 and 2 to 20 for Zn and Pb, respectively, depending on the degree of contamination.

Metal concentrations in the cytosolic fractions (S1) (Fig. 4) showed patterns similar to those previously observed in the whole organism (S1 + C1) (Fig. 3), except for the Cd at the highest concentration of the Bidassoa elutriate and Pb for the Dunkerque elutriate. Contrary to what was observed in the whole larvae, Pb concentrations in the cytosolic fraction did not increased consistently according to the degree of exposure.

Metallothionein is a cytosolic heat-stable protein. Consequently, it should be preferable to examine the relationship between metal and metallothionein levels, taking into account metal concentrations in the S2 supernatant obtained after heat denaturation of the cytosol. (However, the fate of metals during heating is questioned, since metal analysis of chromatographic fractions obtained from raw cytosol (S1) and heat-denaturated cytosol (S2) revealed differences in metal binding to cytosol ligands) (Bragigand and Berthet, 2003). Consequently, relationships between metallothionein and S1 metal concentrations were studied. As all studied elements could bind to metallothionein and might, therefore, contribute concomitantly to metallothionein induction, the relationship between metallothionein and metal levels (S1) was examined, taking into account the metals individually or combined. For relationship studies, all data were taken into account except the highest elutriate concentrations from Dunkerque (5, 10, and 25%) (Fig. 5), where harmful effects were noted (growth inhibition). Positive and significant relationships were observed for Cu, Zn, and for all combined elements.

From a biomonitoring point of view and to use metallothionein as a biomarker of metal contamination, metallothionein must reflect the gross metallic concentration. The relationship between total metal (S1+P1) and metallothionein levels was also examined. Results were similar to those previously observed with cytosolic metal concentrations. Relationships between metal and metallothionein concentrations were significant for Cu and Zn and in the case of all elements combined (not shown).

Discussion

Metals analysis showed that Bidassoa and Dunkerque sediments are heavily contaminated by metals. In comparison with the French Géode classification system, Dunkerque sediment is characterized by Cu and Pb contamination and is more contaminated than the Bidassoa sediment, which is only Cu-enriched. Dunkerque sediments are also highly contaminated by polycyclic aromatic hydrocarbons (Geffard *et al.* 2002b).

The metal contamination of the control sea water was in the same range of magnitude as the metal levels observed in waters from uncontaminated areas (Campanella *et al.* 2001, Prego and Cobelo-Garcia 2004) and lower than those of the Gulf of Gaeta, Tyrrhenian Sea (central Italy), known to be slowly contaminated (Conti and Cecchetti 2003). The metal contents of the Bidassoa and Dunkerque elutriates were approximately 10 times more concentrated than the control seawater, with values in the same range of magnitude as metal contaminations currently observed in impacted coastal and estuary areas (RNO 1995, Martino *et al.* 2002). Cu levels in Bidassoa and Dunkerque elutriates exceeded the environmental quality standard (Matthiessen *et al.* 1999). According to Slotten and Reuter (1995) and Van den Berg *et al.* (2001), these results showed that re-suspending sediments induce an increase in the total seawater metal contamination.

Bioavailability of contaminants can be determined using a bioaccumulation test. With Bidassoa elutriates, only the Cu was bioavailable and accumulated by the larvae. For Dunkerque, all studied metals were bioavailable and accumulated by larvae. Even if highest metal contaminations (the difference between control and exposed larvae) were observed in larvae exposed to the Dunkerque elutriates, the contamination level

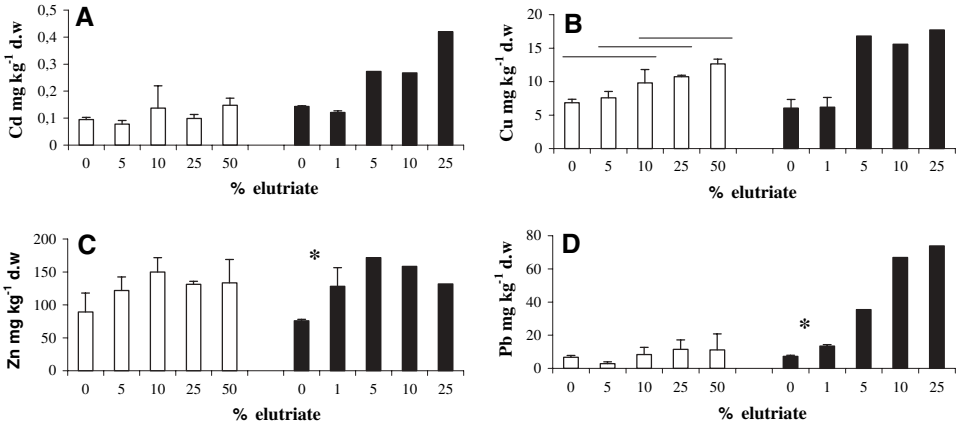

Fig. 3. Total metal concentrations (mean ± SD, three replications) in larvae after 10 days (Bidassoa, white bars) or 7 days (Dunkerque, black bars; except at 25% only 5 days) of exposure to different elutriate concentrations. **A:** Cd. **B:** Cu. **C,** Zn. **D:** Pb. For statistical comparison presentation, see Figure 2 legend

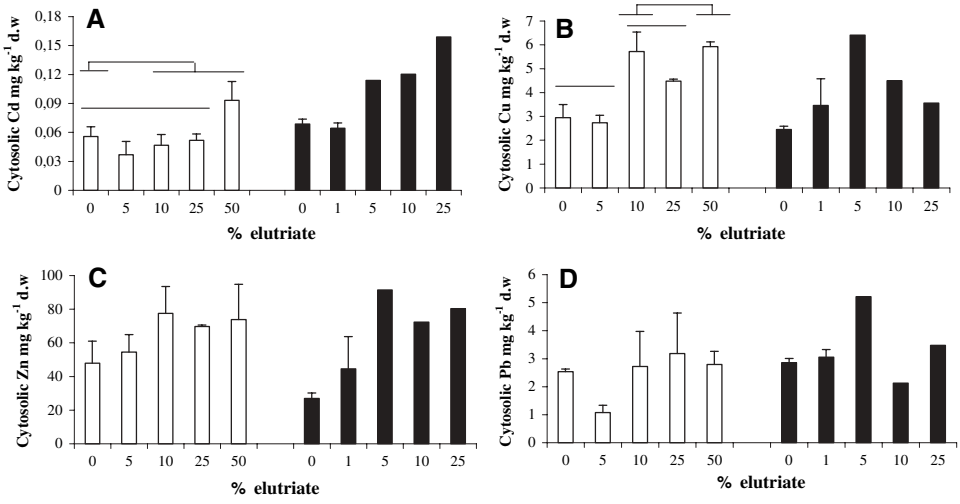

Fig. 4. Cytosolic metal concentrations (mean ± SD, three replications) in larvae after 10 days (Bidassoa, white bars) or 7 days (Dunkerque, black bars; except at 25% only 5 days) of exposure to different elutriate concentrations. **A:** Cd. **B:** Cu. **C:** Zn. **D:** Pb. For statistical comparison presentation, see Figure 2 legend

of exposure for the highest tested concentration (25%, i.e., 0.019 µg $Cd.l^{-1}$, 1.78 µg $Cu.l^{-1}$, 6.88 µg $Zn.l^{-1}$, and 3.6 µg $Pb.l^{-1}$, estimated according to metal concentrations in raw elutriate and control seawater) was lower than the level of Bidassoa elutriates (50%, 0.054 µg $Cd.l^{-1}$, 5.65 µg $Cu.l^{-1}$, 13.65 µg $Zn.l^{-1}$, and 4.5 µg $Pb.l^{-1}$). These observations show that the bioavailability of metals from the Dunkerque elutriates was higher than those from the Bidassoa elutriates. This difference could be due to several parameters influencing metal bioavailability such as dissolved organic carbon or total suspended solids. These parameters were not available for this study, but a previous study using sediments sampled at the same sites indicated differences in the size of particles constituting the major part of sediment, dissolved organic carbon and suspended particulate matter in the elutriates (Geffard *et al.*, 2002b; Geffard *et al.*, 2004). According to Fichet *et al.* (1998) and Geffard *et al.* (2002a), parts of the heavy metals released in the water, when sediments are re-suspended, and/or are bioavailable, could be accumulated and toxic. According to Borgmann (2000), the contamination level in larvae is a better indicator of a potential biological impact than the contamination levels of the medium.

In our previous study (Geffard *et al.* 2002a), the Bidassoa and Dunkerque elutriates had no effect on *C. gigas* embryogenesis at 50% and 25% concentrations, respectively. The 50% Bidassoa elutriate concentration did not induce deleterious effects on the growth and condition index of larvae, after a 10-day exposure. On the contrary, with the Dunkerque elutriate, the larval growth test was much more sensitive than the embryotoxicity test. Growth and CI inhibition were observed at the lowest elutriate concentration (1%). These results are in agreement with those of His *et al.* (1999) who found that larval growth is the most sensitive life stage in this organism. The decrease in the CI indicates that in exposed larvae, the inhibition of weight was greater than the inhibition of height. The CI is an interesting and early warning marker of contaminant effects on larvae. These observations show that released sediment-bound contaminants may affect the physiology and metabolism of oyster larvae. The resuspension of sediments constitutes a great risk for pelagic species.

The larval growth test in *C. gigas* is a sensitive bioassay and can assess the toxicity of bioavailable contaminants, a useful tool to study the biological quality of sediments. However, they cannot identify the compounds inducing the observed biological effects.

The metallothionein level in larvae exposed to the Bidassoa elutriates increased as the degree of contamination rose. Metallothionein is thought to be produced in response to increased intracellular levels of free metal to prevent or reverse potentially detrimental, nonspecific binding of metals to other

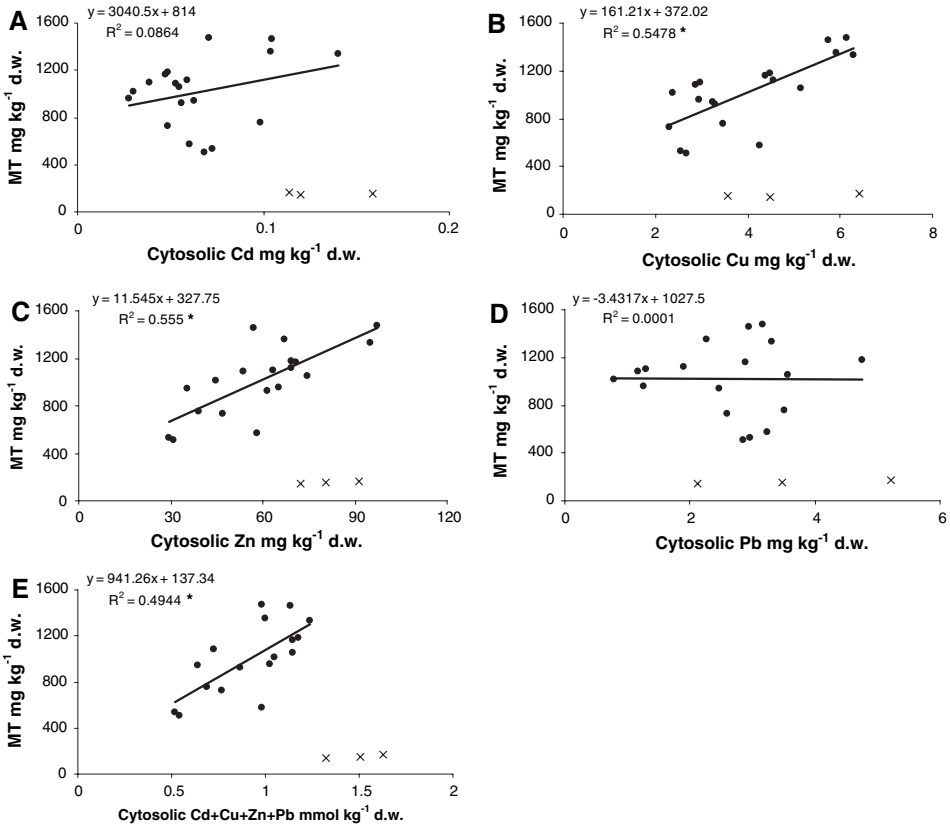

Fig. 5. Relationships between cytosolic (S1) metal (individual or combined) and MT concentrations in whole larva categories (**A:** Cd; **B:** Cu; **C**: Zn; **D:** Pb; **E:** Cd+Cu+Zn+Pb). The linear regression takes into account all the larvae except those showing dramatic biological effects (larvae exposed to 5, 10, or 25% Dunkerque elutriate as indicated by an x). Significant correlations (at 95% level) are indicated with an asterisk

ligands (Roesijadi *et al.* 1996). These observations confirm that released metals from the Bidassoa sediment were really bioavailable. Ringwood and Brower (1993, 1995) and Roesijadi *et al.* (1996) have also showed metallothionein inductions in *C. gigas* larvae exposed to metallic salts under laboratory conditions. However, they found that the first induction occurred at a Cd concentration of 0.6 $\mu g.l^{-1}$, which is 30-fold higher than the 10% Bidassoa elutriate concentrations (0.018 $\mu g\ Cd.l^{-1}$) that resulted in a significant metallothionein induction in this study. The early timing of the metallothionein response to metal bioaccumulation, according to the degree of exposure, compared to the responses of other physiological endpoints taken into account in this study (growth and CI), make it possible to define a scale of sensitivity of biological and biochemical responses in *C. gigas* larvae:

$$\text{Metallothionein} > \text{larval growth} = CI > \text{abnormal embryogenesis}$$

Similar observations were made by several authors in another bivalve species, *Mytilus galloprovincialis* (Pavicic *et al.* 1994, A. Geffard *et al.* 2002).

For the Dunkerque elutriates, metallothionein levels slowly increased between the control and the 1% elutriate concentration, and then sharply decreased. These observations could be explained by the poor physiological state of larvae (the condition index and growth lower than controls). These results are good examples of a situation that has been referred to as spillover (Brown and Parsons 1978), the saturation of detoxification mechanisms, producing the first harmful effects. This phenomenon has been described in several species in laboratory: fish *Pleuronecta platessa* (George and Olsson 1994), copepods, *Tigriopus brevicornis* (Barka *et al.* 2001), and bivalve larvae, *Mytilus galloprovincialis* (A. Geffard *et al.* 2002).

According to earlier results and the good relationships observed between the most highly bioaccumulated metals (Cu and Zn) and metallothionein, the concentrations indicated that the metallothionein level reflects: (1) the cytosolic metal load was the highest in toxicological terms (Wallace *et al.* 2003) and (2) the gross metallic concentrations (S1+P1) suggested its possible use as a metal exposure biomarker. The absence of a relationship between metallothionein and cadmium concentrations could be explained by the very low bioaccumulation of this metal by larvae. Concerning lead, the total bioaccumulated metal due to elutriate exposure was found in the insoluble fraction (P1), suggesting a low involvement of metallothionein in the detoxification of this element as well as a low induction of metallothionein synthesis by lead. As shown with other species (*Mytilus edulis* larvae, A. Geffard *et al.* 2002), oyster larvae could be used as a biological matrix to determine metallothionein as a biomarker of metallic pollution. The greatest advantage of using the larva stage of the bivalve as a biological matrix compared to the adult stage stems from the higher sensitivity of this stage to different contaminant families (His *et al.* 1999). The potential use of metallothionein as a biomarker in bivalve larvae in biomonitoring programs could be determined during in situ tests as developed by Geffard *et al.* (2001b).

Larvae exposed to the highest Dunkerque elutriate concentrations (5, 10, and 25%) had the highest metal body burden

and corresponded to the lowest metallothionein level. These individuals constitute ''fault controls'' not due to low levels of contaminant exposure, but inversely to very high levels of contaminant exposure, which induce detrimental effects and the spillover phenomenon, as described above. In biomonitoring programs, in order to avoid these fault controls, it is important to use several biochemical biomarkers that are more or less specific to a contaminant family, as proposed by several authors (Narbonne *et al.* 1999, de Lafontaine *et al.* 2000, Cajaraville *et al.* 2000), including easily measured global physiological markers such as the condition index.

These results showed that Dunkerque sediments are a biological hazard when they are re-suspended (e.g., dredging). Moreover, the present study, using metal concentrations similar to those found in natural environments, showed an early (low-dose) metallothionein response as compared to the time that abnormalities appeared. Thus, the determination of metallothionein in oyster larvae could be used as a biomarker of metal exposure, an important ecological factor because successful breeding is necessary to maintain the population.

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