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Assessment of the bioavailability and toxicity of sediment-associated polycyclic aromatic hydrocarbons and heavy metals applied to *Crassostrea gigas* embryos and larvae

O. Geffard [a,*], A. Geffard [b], E. His [c], H. Budzinski [a]

[a] *Laboratoire de Physico-Toxicochimie des Systèmes Naturels, LPTC, Université de Bordeaux 1, 351 cours de la Libération, 33405 Talence Cedex, France*
[b] *Service d'Ecotoxicologie, SMAB, ISOMer, GDR CNRS 1117, Université de Nantes, 2 rue de la Houssinière, BP 92208, 44322 Nantes cedex 3, France*
[c] *IFREMER, DEL/PC, Station d'Arcachon, Quai Silhouette, 33120 Arcachon, France*

Abstract

Sediments represent a vast sink for contaminants in aquatic systems, and may pose a threat to pelagic and benthic organisms. The objective of this research was to determine the bioavailability and toxicity of sediment-associated PAHs and heavy metals, using embryos and larvae of the oyster *Crassostrea gigas*, exposed to two sediment fractions: the whole sediment and the elutriate. The percentages of abnormal larvae, the contaminant accumulation and, (in the case of metal contamination), the induction of metallothionein in the larvae, were investigated. Sediment-associated PAHs and heavy metals were available for exposure, as indicated by their accumulation in *C. gigas* larvae and by the abnormalities induced during larval development. The critical body burden of PAHs (Fluo, Pyr, BaA, Triph, Chrys, BbF, BkF, BjK, BeP, BaP, Per, IP, BPer and the DahA) in the larvae was 0.3 $\mu g\,g^{-1}$, above which abnormalities were observed. This value corresponds to concentrations observed for most vertebrate and invertebrate species. The bioavailability of PAHs is determined by their solubility; only the soluble fraction of PAHs is accumulated by the embryos. The bioavailability of metals for the larvae is substantiated by MT induction, correlated with cytosolic metal concentrations. MT induction provided a better early-warning response than the embryotoxicity test currently used for evaluating environmental contamination by metals. This study recommends choosing oyster embryos as a particularly sensitive tool for evaluating sediment quality.

Keywords: Sediments; Oyster larvae; Embryo toxicity; Contaminant body burdens; Biomarkers; PAHs; Heavy metals

1. Introduction

Sediments represent a vast sink for contaminants in aquatic systems and may pose a threat to pelagic and benthic organisms. Various bioassays have been proposed to assess the biological effects of contaminated sediments, using amphipods, polychaetes, oligochaetes or bivalves (Carr et al., 1989; Chapman, 1990; ASTM, 1992; Eertman et al., 1993; Burgess and Morrison, 1994; Matthiessen et al., 1998). The embryotoxicity test with the oyster *Crassostrea gigas* is recognized as one of the most sensitive of all classically-used bioassays (Stebbing et al., 1980; His et al., 1999). First promoted by Woelke (1972), to evaluate industrial effluents, it was later adapted for studying the biological effects of contaminated marine sediments (Cardwell et al., 1976; Chapman and Morgan, 1983; Long et al., 1990; Miller et al., 2000; Geffard et al., 2001, 2002a). Three different sediment treatments, whole sediment, pore water and elutriate exposures, are proposed. Although bioassays allow the toxicity of sediment-associated contaminants to be evaluated, they do not identify the compounds inducing the biological effects observed.

The bioavailability of contaminants depends on several factors: physical (grain size of the sediment and suspended particulate materials), chemical (solubility, reactivity of compounds, complexing agents), and

* Corresponding author. Tel.: +33-5-56-84-33-84; fax: +33-5-57-96-22-67.
E-mail address: ogeffard@lptc.u-bordeaux.fr (O. Geffard).

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biological (benthic or pelagic organisms, mode of exposure) (Borgmann, 2000). One of the best methods for assessing the bioavailability of sediment-associated contaminants is to observe their accumulation in organisms. This method takes into account all factors controlling the bioavailability of the contaminants (Connell et al., 1999; Borgmann, 2000).

In marine ecotoxicology, several biomarkers are applied. Different categories of these are identified, e.g. biomarkers of exposure and response, or general and specific biomarkers. Specific biomarkers may also be used, in conjunction with internal concentration determinations of contaminants, to assess the sublethal effects of metals (Geffard et al., 2002b), particularly metallothioneins (MT). MT protein had previously been detected in *Mytilus galloprovincialis* (Pavicic et al., 1985) and *C. virginica* larvae (Roesijadi et al., 1996, 1997), but using unrealistically high experimental concentrations. The induction of MT in *M. galloprovincialis* larvae exposed to naturally contaminated sediments, has also been observed recently (Geffard et al., 2002b).

The aim was to study the bioavailability and toxicity of sediment-associated PAHs and heavy metals, using embryos and larvae of the oyster *C. gigas* exposed to whole sediment and its elutriates. Two sediment sites, one contaminated by metals, the other by PAHs, were studied. The final aim was to study the biological accumulation of heavy metals and PAHs of sedimentary origin, and the possible induction of MT in *C. gigas* larvae, in the case of the Bidassoa heavy metal contaminated sediment.

2. Material and methods

2.1. Sediment

2.1.1. Sampling and conservation

In previous studies (Geffard et al., 2002a), 10 sediment samples, taken from contaminated sites in the Spring of 1998, were used in *C. gigas* embryotoxicity experiments, after physical and chemical characterisation. Two sediments, chosen for their type of contamination, were used in the present study. The sediments were collected at the Bidassoa Estuary (French-Spanish border) in April 2000, and at Arès (Arcachon Bay, France) in July 2000. Superficial sediments (oxic fraction, 2 cm) were scraped, using a plastic blade, and placed immediately in a plastic box that was filled to the brim to eliminate air bubbles. Samples were then transported to the laboratories in an ice-pack refrigerated isothermal container. They were subsequently wet sieved at 2 mm, to eliminate debris. They were then homogenised and placed into glass bottles at 4 °C and kept in the dark for less than a week prior to being used in the bioassays.

2.2. Sediment treatments

Whole sediments: Sediment suspensions of 0 (control), 0.6, 1.25, 2.5 and 5 $g\,l^{-1}$ (wet settled sediment), in filtered (0.2 μm) seawater (FSW) of 33 psu salinity, were prepared in 2-l glass beakers. To avoid any mechanical effects, the sediments were allowed to settle for 2 h before adding the biological material (Geffard et al., 2002a). The seawater used in the present study was obtained from the Bay of Arcachon, which is devoted to substantial oyster farming (*C. gigas*) and was thus assumed to have good biological quality (His et al., 1999).

Elutriates were prepared using a modified Melzian method (1990). The sediments were shaken mechanically (multi-wrist shaker, 500 rpm) in glass bottles, in FSW at a ratio of 1:4 (volume sediment/water) for 8 h and allowed to settle for a further 8 h before removal of the supernatant (elutriate). The elutriated concentrations of the Arès and Bidassoa sediments corresponded to 326 and 340 $g\,l^{-1}$, respectively. The two elutriates were diluted with FSW to the following concentrations: 0% (control), 6.25%, 12.5%, 25%, 50% and 100%.

For analytical characterisation, one part of the Arès elutriate was filtered at 0.7 μm (GF/F Whatman), to evaluate PAH concentration in the soluble fraction of its elutriate.

2.3. Larval rearing

Mature oysters (*C. gigas*) were conditioned in a hatchery, and then induced to spawn by thermal stimulation (His et al., 1997, 1999). Females in the laying process were isolated in 1 l of FSW, while spawning males were placed in a small amount (≈100 ml) of FSW, to obtain a sperm-dense solution. The oocytes and sperm of different oysters were observed under an inverted microscope, and the best reproductive pair (regular oocytes and very mobile spermatozoa) was selected for the experiment. The oocytes were fertilized using 5 ml of the sperm-dense solution. Fifteen minutes after fertilization, the embryos were counted and placed in 2-l beakers (60,000 fertilized eggs l^{-1}) filled with the different media to be tested (three replicates per treatment).

The embryos were incubated at 24 ± 1 °C for 24 h until D-larvae stages were obtained (His et al., 1997). After incubation, the larvae were recovered through a sieve (32 μm), and counted. They were then washed with 0.9% aqueous ammonium formiate (Holland and Hannant, 1973, to eliminate NaCl), freeze dried, and stored for analysis.

Only larvae from treatments below the abnormality threshold of 20% were analysed. EC_{20} was chosen because studies have shown that 20% of abnormalities are commonly observed at NOEC (Crane and Newman, 2000).

2.4. Embryo toxicity evaluation

After each incubation period, approximately 100 larvae per treatment were subsampled and killed with 100 μl of 40% buffered formalin, to determine the percentages of abnormal larvae, based on His et al. (1997).

2.5. Sample preparation for chemical analyses

The analyses were performed on three sample types: whole sediment, elutriates, and larvae. The method for extracting sediment PAHs is described in detail by Budzinski et al. (1995). The content of PAHs in elutriates was determined in unfiltered and 0.7 μm filtered elutriates, based on Geffard et al. (2002a). For larvae PAH contents, freeze-dried larvae were homogenised, using a hand-held glass grinder, in dichloromethane. Prior to the microwave-assisted extraction (Budzinski et al., 1995), internal standards (Pyrene, Benzo(e)pyrene, Benzo(a)pyrene, Benzo(g,h,i)perylene perdeuterated) were added to each sample; extraction conditions were 10 min at 30 W. The organic extract was then filtered, purified on a micro-column containing alumina and then fractionated on a micro-column containing silica, to collect only the aromatic compounds.

For the PAH extraction, the quality assurance control was performed using blanks. The quantity of each PAH in the blank was subtracted from the quantity in each sample. The quantification of each PAH was considered possible only when its blank amount was lower than 10% of its value in the sample; in other cases, the PAH value was not used.

For metals, aliquots of freeze-dried sediment (0.5 g) were taken from the well-homogenised total sample and placed into acid-washed glass tubes. Hot mineralisation (95 °C) was performed by adding 5 ml of 3 N HNO3 and 3 ml of 12 N HCl. This process was conducted until dryness was achieved; the residues were then re-suspended in 10 ml of 1 N HCl for metal analysis (Aminot and Chaussepied, 1983). The assays were validated using certified sediment (SD-M-2/TM IAEA Monaco). The method used for the elutriates is described in Geffard et al. (2002b). Analyses of Cd, Cu and Zn were performed after preconcentration by liquid–liquid extraction, using methods described by Danielsson et al. (1982).

Metal and MT extractions in larvae were performed simultaneously. The three replicates of lyophilised larvae were homogenised, using a hand-held glass grinder, in 0.02 M Tris-NaCl buffer, pH 8.6, at a ratio of 10 ml of buffer/gram of larvae (wet weight). Proteolytic reactions and the oxidation of MT molecules were avoided by working at 4 °C in the presence of β-mercaptoethanol (10 mmol l^{-1}) added to the Tris buffer. Cytolsolic (S1) and insoluble (P1) fractions were previously separated by centrifugation (25,000 g, 55 min at 4 °C) after heating at 75 °C for 15 min. A second fraction (S2) containing the MT was thus obtained, as well as a second insoluble fraction (P2) containing heat-sensitive compounds. S2 supernatants were frozen at −80 °C for MT analysis.

Before metal analysis, an acid digestion step at 60 °C was required for the soluble (S1) and insoluble (P1) fractions. This step lasted for 12 h and involved the addition of Suprapur nitric acid (Carlo Erba) at a ratio of 1 ml/ml of S1 supernatant and 1 ml/0.5 g of larvae (P1). The solutions obtained were supplemented with deionised water to a pre-determined volume.

2.6. Chemical analyses

The PAHs were analysed using gas chromatography combined with mass spectrometry, based on a protocol described by Baumard and Budzinski (1997). The Tri-aromatic compounds (Phe and An) were not used in this study because their blank quantities were too high (between 1 and 3 ng for Phe; 0.1 and 0.2 ng for the An) as compared to those in samples of larvae. Consequently, only the following PAHs were analysed: Fluoranthene (Fluo), Pyrene (Pyr), Benzo(a)Anthracene (BaA), Triphenylene + Chrysene (Triph + Chrys), Benzo(b)Fluoranthene + Benzo(k)Fluoranthene + Benzo(j)Fluoranthene (BbF + BkF + BjK), Benzo(e)pyrene (BeP), Benzo(a)pyrene (BaP), Perylene (Per), Indenol(1,2,3-cd)pyrene (IP), Benzo(ghi)perylene (BPer) and Dibenzo(a,h)anthracene (DahA),

After the acid digestion phase, metals were analysed using flame atomic absorption spectrophotometry (AAS) for Cu and Zn, or electrothermal AAS with Zeeman (Hitachi Z8200) for Cd (Amiard et al., 1987). The total bioaccumulation of metals (QCd, QCu and QZn for Cd, Cu and Zn) in larvae was calculated by adding the amounts measured in the soluble (QS1) and insoluble (QP1) fractions. The MT larval contents were measured in the S2 fraction using differential pulse polarography (Geffard et al., 2002b).

2.7. Statistical analysis

For each series of results, means were compared using *t*-test or a one-way ANOVA. Significant differences (at the 95% level) were then determined using the Tukey test. Linear regressions between metal and MT contents in the larvae were performed, and correlation coefficients (r) were determined using Excel software.

3. Results

3.1. Characterisation of the sediments and elutriates

The total PAH concentrations varied by two orders of magnitude between the Arès (31,665 ng g^{-1} dry weight) and the Bidassoa sediment (813 ng g^{-1} dry weight)

Table 1
Concentrations of PAHs (in ascending order of molecular weight) in the sediments and elutriates obtained from Arès; sediment concentrations of heavy metals in the tested sediments

Sample	Fluo	Pyr	BaA	Triph + Chrys	BbF + BkF + BjF	BeP	BaP	Per	IP	BP	DA	ΣPAH	Cd	Cu	Zn
Sediment	*Polycyclic aromatic hydrocarbons (ng g^{-1} dry weight)*												*Heavy metals (ng g^{-1} dry weight)*		
Arès	5285[a]	3951[b]	3536[a]	3322[a]	5331	1757	2585[a]	710	2299	2170	720[a]	**31,665**	0.08	9	80
Bidassoa	112	89	56	75	154	66	72	43	68	13	65	**813**	0.84	83[b]	328[b]
Elutriate	*Polycyclic aromatic hydrocarbons (μg l^{-1})*												*Heavy metals (μg l^{-1})*		
Arès															
Unfiltered	0.152	0.149	0.059	0.082	0.164	0.065	0.084	0.025	0.073	0.075	0.014	**0.941**			
Filtered	0.035	0.036	0.015	0.022	0.020	0.009	0.010	0.003	0.005	0.007	0.001	**0.164**			
Bidassoa															
Unfiltered													0.1	14	26

Compounds for which Long et al. (1995) provide effect ranges are underlined.
[a] Value exceeds effect range-medium (ER-M).
[b] Value exceeds effect range-low (ER-L).

(Table 1). In the unfiltered Arès elutriate, the total PAH concentration was six times higher (0.94 ng ml^{-1}) than in the filtered one (0.16 ng ml^{-1}) (Table 1). In the unfiltered Bidassoa elutriate, the concentrations of Cd, Cu and Zn were 0.1, 14 and 26 μg l^{-1} respectively.

3.2. Embryotoxicity of sediment treatments

The percentages of abnormal larvae in controls ranged from 5% to 9.3%. The Arès and Bidassoa whole sediments lead to similar effects (27% and 32% abnormalities respectively at 5 g l^{-1}, the highest concentration tested) (Fig. 1). At 100%, the Bidassoa elutriate was more toxic than the Arès elutriate with 43% and 27% of abnormal larvae, respectively. At 50% of elutriate, the Arès and Bidassoa elutriates showed significant biological effects (17% and 14.6% abnormalities), but these values were lower than the threshold of 20%, beyond which the tested treatment is considered as toxic (Fig. 1).

3.3. PAH bioaccumulation and distribution in larvae with the Arès sediment

PAHs in larvae increased with increasing concentrations of both treatments (whole sediment and elutriate, Fig. 2). In controls, the PAH concentrations in larvae ranged from 0.04 to 0.06 μg g^{-1}. At the highest tested concentrations, PAH contents in the larvae were 0.31 μg g^{-1} dry weight (whole sediment) and 0.37 μg g^{-1} dry weight (elutriate). In both cases, the PAH bioaccumulation factors (the ratio between the PAH concentration in larvae and the PAH concentration in sediment treatments), were negatively correlated with the log K_{ow}. The correlation coefficients (r^2) with the whole sediment, unfiltered and filtered elutriate, were 0.73, 066 and 0.51 respectively.

The relative concentrations (%) of the tetra- (Fluo + Pyr + BaA + Triph + Chrys; m/z of 202 and 228), penta- (BbF + BkF + BjF + BeP + BaP + Per; m/z of 252) and hexa-aromatic (IP + Bper + DahA; m/z of 276 and 278) PAHs in the Arès sediment, unfiltered and filtered elutriate, are indicated in Fig. 3A. In all cases, the tetra-aromatic PAHs were more abundant (50%) than the penta-aromatic PAHs (35%) which were, in turn, more abundant than the hexa-aromatic PAHs (15%). Although the sediment and unfiltered elutriate presented similar PAH profiles, the lowest molecular weight PAHs were more abundant in the filtered elutriate.

The average tetra-, penta- and hexa-aromatic PAH percentages (S.D.) in larvae exposed to the different whole sediment concentrations are shown in Fig. 3B. The lowest molecular weight PAHs were more abundant in the larvae (80% of total PAHs) than in the sediment itself (45% of total PAHs). The PAH distribution in the larvae and both elutriates was similar (Fig. 3C).

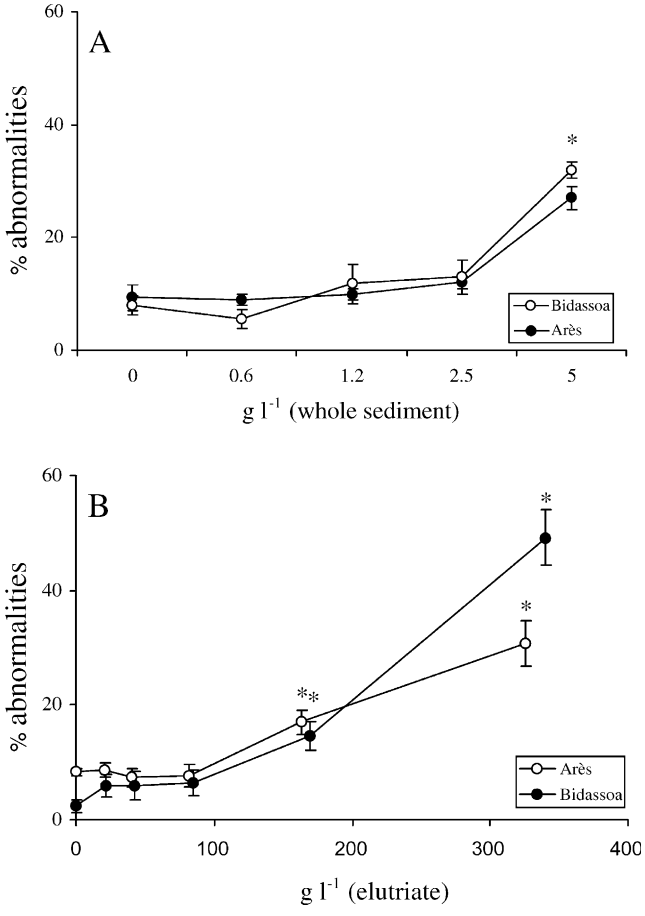

Fig. 1. Whole sediment (A) and elutriate sediment (B) *C. gigas* embryotoxicty. Percentages of abnormal larvae (±SD). (●) Arès sediment; (○) Bidassoa sediment. (∗) abnormal larvae (%) significantly higher than in controls.

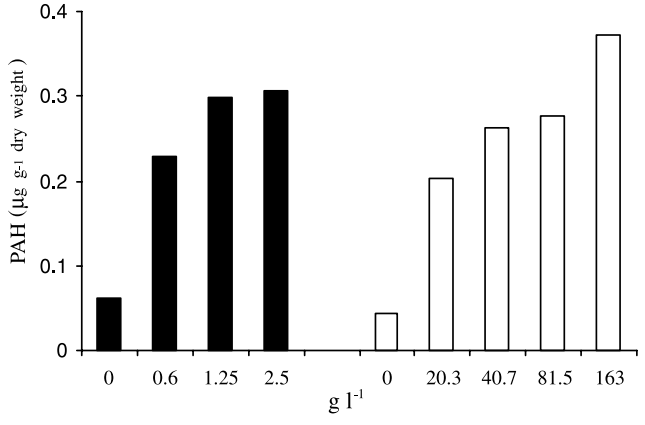

Fig. 2. Concentrations of total PAHs in the *C. gigas* larvae (ng g^{-1} dry weight) after exposure to a range of Arès whole sediment (black bars) and elutriate (white bars) concentrations.

The ratio between BeP and BaP (Bep/BaP), an index of possible PAH metabolisation by larvae, was <1 in the different sediment treatments (0.66, 0.76 and 0.95 in the Arès sediment, unfiltered and filtered elutriate respectively). The same ratio, however, was >1 in contaminated larvae (2.3 ± 0.3 and 2.7 ± 0.3 in larvae exposed to whole sediment and elutriate respectively).

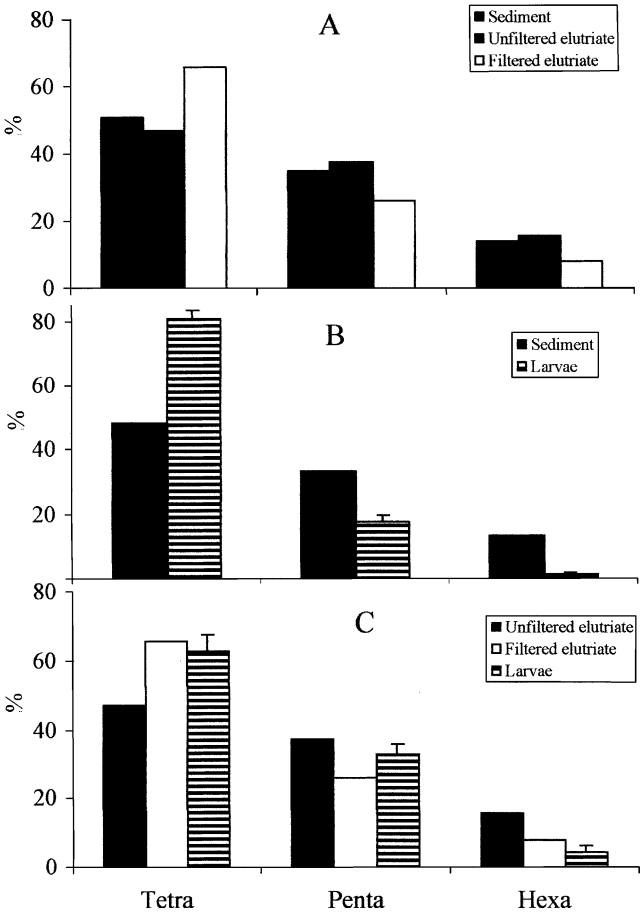

Fig. 3. Relative contents (%) of tetra-, penta- and hexa-aromatic compounds in the different Arès sediment treatments and larvae. (A) In Arès sediment and its unfiltered and filtered elutriates, (B) in Arès sediment and larvae exposed to it and (C) in Arès unfiltered and filtered elutriates and larvae exposed to unfiltered elutriate.

3.4. Metal bioaccumulation and MT induction in larvae with the Bidassoa sediment

The concentration of total Cd (QCd; Fig. 4A) in larval tissue increased as a function of the whole sediment and elutriate concentrations, becoming significant for concentrations above 2.5 $g l^{-1}$ ($p = 0.0016$) and 85 $g l^{-1}$ ($p = 0.0042$), respectively. The concentration of total Cu (QCu; Fig. 4B) in larvae increased, as previously, with increasing concentrations of the two sediment treatments. The QCu was significantly higher than in the control experiment, after exposure to a concentration of 2.5 $g l^{-1}$ ($p = 0.0073$) with the whole sediment, and of 85 $g l^{-1}$ ($p = 0.0012$) with elutriate. For Zn (QZn; Fig. 4C), a significant increase was observed only in the case of exposure to the whole sediment ($p = 0.0008$) above 0.6 $g l^{-1}$. In every case, total concentration in the ambient environment of each metal was significantly

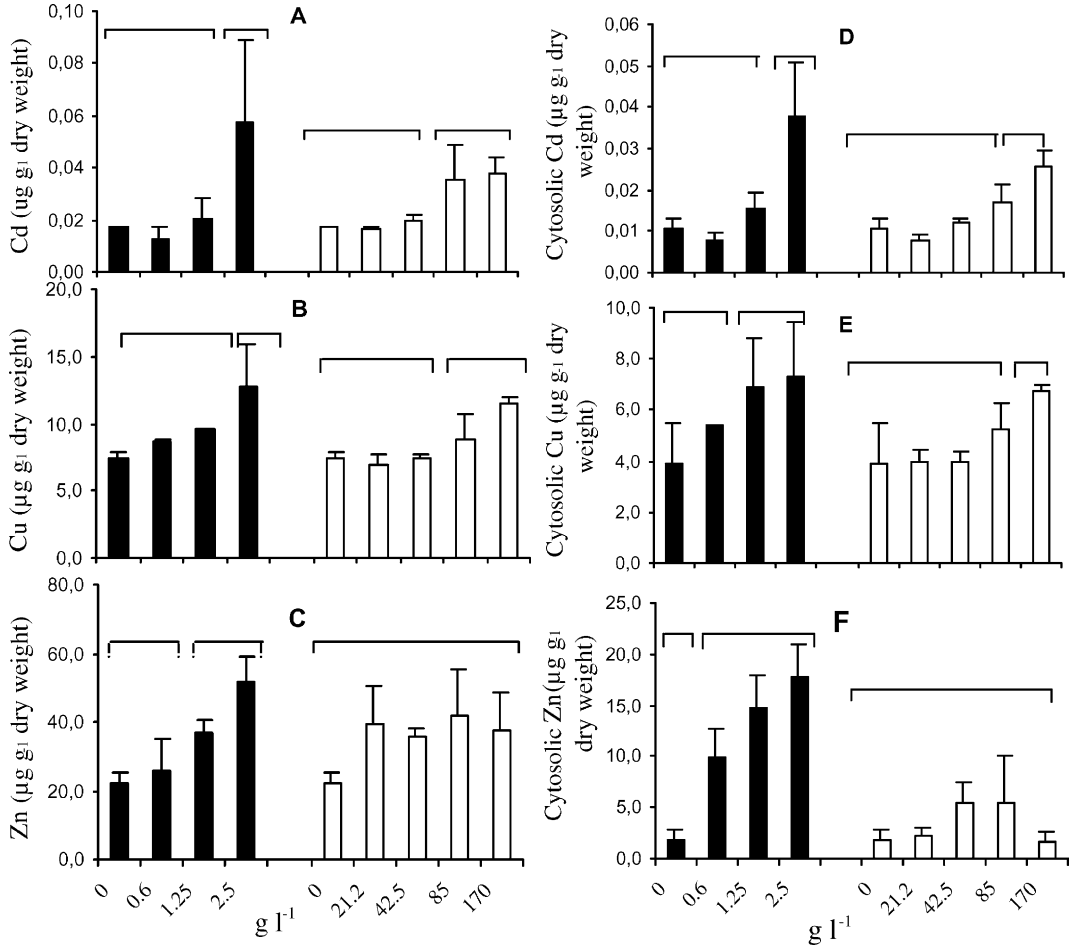

Fig. 4. Total Cd (A), Cu (B), Zn (C) concentrations ($\mu g\,g^{-1}$ dry weight ± SD) and cytosolic Cd D), Cu (E) and Zn (F) concentrations ($\mu g\,g^{-1}$ dry weight ± SD) in oyster larvae after exposure to a range of Bidassoa whole sediment (black bars) and elutriate concentrations (white bars). Values not significantly different from one other are grouped under a common overhead line (ANOVA, $p < 0.05$; Tukey test).

Table 2
Equation of the relation between the concentration of cytosolic Cd, Cu, Zn or Cd + Cu + Zn and MT in larvae after exposure to a range of whole sediment or elutriate concentrations of Bidassoa sediment

		Equation	n	r^2
Cd	Whole sediment	$Y = 13693x + 730.5$	12	0.304*
Cu	Whole sediment	$Y = 117.2x + 279.8$	12	0.733**
Zn	Whole sediment	$Y = 30.52x + 603.9$	12	0.438**
Cd	Elutriate	$Y = 8532.2x + 672.8$	15	0.262*
Cu	Elutriate	$Y = 100.1x + 330.9$	15	0.748**
Zn	Elutriate	$Y = -21.81x + 825.2$	15	0.106

n: number of samples. r^2: correlation coefficient. * and ** show that the relation is significantly correlated at 95% and 99% respectively.

($r > 0.75$) correlated with concentration in the cytosolic fraction, especially for Cd and Cu (Fig. 4D, E, F).

MT increased significantly ($p < 0.001$) in larvae exposed to 1.25 $g\,l^{-1}$ or more of the Bidassoa whole sediment whereas, in the presence of elutriate, the observed MT induction was not significant ($p = 0.0832$) Fig. 5.

The correlation between MT and metal concentrations in larvae, studied for each different metal in the cytosolic fraction (Table 2), was significant for Cd and Cu for both sediment treatments and, in the case of Zn, for the whole sediment only.

4. Discussion

As previously mentioned (Geffard et al., 2001), the Arès sediment is characterised by high PAH contamination, the Bidassoa sediment by heavy metal contami-

nation. In the Arès sediment, the different PAH level was higher than the effect range-low (ER-L) values of Long et al. (1995), above which toxicity can be occasionally observed. In the same way, in the Bidassoa sediment, the Cu and Zn concentrations were over the ER-L levels.

If we compare the contamination of the Arès and Bidassoa elutriates with the data from Law et al. (1997) and Fernandes et al. (1997) for natural brackish water, and with data from Geffard et al. (2002b) for elutriates of contaminated sediments, the Arès and Bidassoa elutriates were characteristic of PAH and metal contamination respectively, and revealed contamination levels similar to those observed in seawater from contaminated coastal areas.

4.1. Embryotoxicity of the sediments

In previous data (Chapman and Fink, 1984; Geffard et al., 2002a), it was shown that whole sediment treatments had biological effects at lower concentrations than was the case for elutriates. Because the biological effects for the highest concentrations tested in the Arès and Bidassoa whole sediment and elutriates exceeded the 20% abnormality threshold, larval rearing could not be used for chemical and biochemical analyses in the present study.

4.2. PAH bioavailability and toxicity (Arès sediment)

PAH levels in the larvae increased with the whole sediment and elutriate concentrations tested, indicating that these contaminants were potentially available during *C. gigas* embryonic development (Fig. 2). Connell et al. (1999) for PAHs, and Borgmann (2000) for heavy metals, have shown that the body concentration measurements of contaminants provide an effective tool for predicting biological effects. In the present study, using the two sediment treatments, PAH internal concentrations of 0.3 $\mu g\,g^{-1}$ (dry weight) in larvae represented the maximum level above which biological effects (abnormalities) could be observed, this value being the critical body PAH concentration for *C. gigas* larvae. Estimated in μmol/g wet weight of larvae, this concentration (0.016) corresponds to the critical body burden for non-ionic organic compound concentrations (0.01–6 $\mu mol\,g^{-1}$ wet weight), above which sublethal effects are observed for most vertebrate and invertebrate species (Connell et al., 1999). This value also corresponds to the threshold level for adult mussels, above which they cannot transform PAHs but merely accumulate them (Baumard et al., 1998).

The proportion of the tetra-, penta- and hexa-aromatic PAHs in the different sediment treatments and larvae was compared, to study their different bioavailability and metabolisation by *C. gigas* larvae. Previous

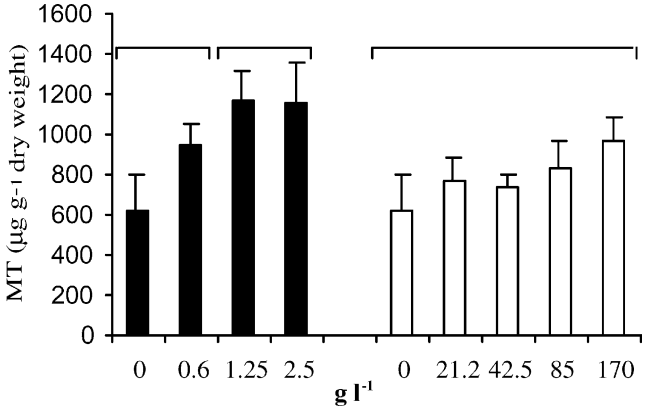

Fig. 5. Concentrations of MT ($\mu g\,g^{-1}$ dry weight ± SD) in the oyster larvae reared in the presence of a range of Bidassoa whole sediment (black bars) and elutriate (white bars) concentrations. Values not significantly different from one other are under a common overhead line (ANOVA, $p < 0.05$; Tukey test).

data from *C. gigas* embryotoxicity experiments using the Arès sediment (Geffard et al., 2001) showed that unfiltered and filtered elutriates induced the same biological effects, suggesting that the toxicity was mostly due to soluble PAHs. This explains the observations on the chemical differences between unfiltered and filtered elutriates, with regard to the proportions of tetra-, penta- and hexa-aromatic PAHs in both elutriates, as compared to whole sediment concentrations. In the unfiltered elutriate, 84% of the total PAHs were adsorbed to suspended particulate matter, demonstrating that sediment and unfiltered elutriate had similar PAH distribution. The filtered elutriate, unlike the whole sediment and unfiltered elutriate, was enriched with the lowest molecular weight PAHs. During the elutriation procedure, the release of dissolved PAHs seemed to be determined by the water solubility of each compound, represented by the octanol–water coefficient (K_{ow}, Karickhoff et al., 1979). The lowest molecular weight PAHs were more soluble than the heaviest ones, and were probably more intensively released.

Despite direct contact between embryos and the sediment, the PAH profiles of whole sediment and larvae were very different. The larvae were particularly enriched in the lowest molecular weight PAHs (80% of total PAHs), indicating that PAH solubility (K_{ow}) governed PAH bio-availability. These results correspond to those of Porte and Albaigès (1993) and of Baumard et al. (1999), who found that the concentrations of the lowest molecular weight PAHs in bivalves and fish were higher than those of the heaviest molecular weight compounds. The bioavailability and toxicity of hydrophobic organic contaminants of sedimentary origin depends on the quantity and quality of organic matter in the sediment (Di Toro et al., 1991; Baumard et al., 1998; Fleming et al., 1998; Ferguson and Chandler, 1998). The Arès sediment is characterised by high organic matter

concentration (9.7%, Geffard et al., 2001, which may explain the weak bioavailability of heavier molecular weight PAHs.

PAH distribution in the larvae exposed to the unfiltered elutriates differed slightly from the distribution in the elutriate itself but was similar to that of the filtered elutriate, indicating that only the soluble fraction of PAHs is available to the embryos. This confirms our previous data (Geffard et al., 2001) concerning the same effects observed on *C. gigas* larvae in the presence of both filtered and unfiltered elutriates, indicating that the toxic effects are exclusively induced by the soluble fraction of this type of contaminant.

Whereas, in the larvae, BeP concentrations exceed BaP concentrations, in whole sediment, unfiltered and filtered elutriates, BaP concentrations exceed those of BeP. Similar observations have been made for adult mussels (Baumard et al., 1998). The relative depletion of BaP in the larvae may well be due to the preferential metabolisation of BaP, corroborating previous findings by Michel et al. (1995) for *M. galloprovincialis*, and Borchert et al. (1997) for *Sphaerium corneum*. Even though the metabolisation of organic compounds is lower for bivalves than for other taxa, oyster larvae can, nonetheless, metabolise organic compounds.

4.3. Heavy metal bioavailability and toxicity (Bidassoa sediment)

The bioavailability of metals (Cd, Cu and Zn) from the two sediment treatments was studied by determining the total metal larval contamination, and by measuring the MT induction in larvae. After 24 h exposure, metal concentrations, both in tissues and in the cytosol (excluding Zn), increased as a function of the metal contamination level in the experimental medium in which larvae were reared. As for PAH contamination, the critical body concentration for each metal was calculated and was respectively 0.6, 13 and 50 $\mu g\, g^{-1}$ for Cd, Cu and Zn. These values for *C. gigas* larvae are lower than those found by Geffard et al. (2002b) for Cd and Zn in mussel *M. galloprovincialis* larvae, and than those found by Radenac et al. (2001) for *Paracentrotus lividus* larvae. Different exposure times (24 h to obtain oyster larvae, and 48 h in the case of mussel and sea urchin larvae) may explain these observations. Oyster embryos which develop faster than those of the other two species, may also show greater, more sensitive metabolism. The apparent discrepancy between our study and that of Radenac et al. (2001) could be explained by the fact that Radenac et al. used larvae and embryos with significant abnormalities.

The bioavailability of sediment-associated metals was also demonstrated by MT induction in oyster larvae, which clearly correlated to metal contamination levels (Table 2). MT levels in the larvae exposed to the Bidassoa whole sediment and unfiltered elutriate were significant only in the presence of whole sediment. This difference may be explained by cytosolic metal concentration being lower in larvae exposed to the elutriate, particularly for Zn, than for larvae exposed to whole sediment (Fig. 4).

According to Pavicic et al. (1994) and Geffard et al. (2002b), results obtained with whole sediment confirms than MTs constitute a distinctly enhanced early-warning response compared with currently-used embryotoxicity tests. The highest MT concentrations observed for whole sediment concentrations of 1.25 and 2.5 $g\, l^{-1}$ (Fig. 5) indicated that the maximal detoxification processes probably involved MT induction.

In the presence of the Bidassoa unfiltered elutriate, despite the good correlation between metal concentrations in the cytosolic fraction (excluding Zn) and the MT levels of the larvae (Table 2), significant biological effects (% abnormal larvae) appeared before significant MT induction. This may indicate that metal contamination does not totally explain the toxicity observed, as other toxic substances probably disturb the embryonic development. For instance, the principal bias observed in bioassays with elutriates is due to ammonia (Ankley et al., 1990; Huber et al., 1997; Van Sprang and Janssen, 1997). This compound is highly toxic to marine organisms (Kohn et al., 1994), particularly to the embryos and larvae of *C. gigas* (Geffard et al., 2001).

5. Conclusion

Sediment-associated PAHs and heavy metals were readily available, as indicated by their accumulation in *C. gigas* larvae, and by the abnormalities induced during larval development, thereby confirming the choice of this biological model as a sensitive tool for evaluating the biological quality of contaminated sediments (Geffard et al., 2001, 2002a).

Both the toxicity assessment of sediment and the bioavailability of contaminants were more easily determined using the whole sediment treatment.

For the first time, it proved possible to determine PAH and metal critical body concentrations for *C. gigas* embryos, one of the most sensitive organisms used in marine ecotoxicology.

MT induction in oyster larvae provided an enhanced early-warning tool for evaluating environmental contamination by metal of sedimentary origin. Further investigations are needed to develop an equally effective PAH contamination biomarker using *C. gigas* embryos.

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