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REPORTS

Experimental Studies of Impacts of Harbour Sediments Resuspension to Marine Invertebrates Larvae: Bioavailability of Cd, Cu, Pb and Zn and Toxicity

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The larvae of three marine species (*Artemia salina*, *Crassostrea gigas*, *Paracentrotus lividus*) reared within the suspended particulate phases of contaminated sediments from harbours were used in bioassays firstly to assess their ability to accumulate four metals (Cd, Cu, Pb, Zn) and secondly to show the toxicity of such rearing media by recording delays in growth and possible abnormal larval development. The results show that resuspension processes of dredged harbour sediments may induce both a release of Cd, Cu and Pb which are bioavailable for larvae (levels of bioaccumulation depending on the species) and biological perturbations, i.e. abnormal development in *C. gigas* and *P. lividus* larvae for the more contaminated sediments and growth inhibition in all three larvae for slightly contaminated sediments. The concentrations of Pb reached in the *C. gigas* D-shaped larvae and the *P. lividus* pluteus were unusually high; in contrast, Zn was not accumulated by the three species. The impact of dumping operations thus appears to depend both on the metal considered and on the larvae used in such tests. In this context, Pb seems a more worrying metal than Zn and *C. gigas* and *P. lividus* are better indicator species than *A. salina* larvae. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: heavy metals; bioavailability; toxicity; *Crassostrea gigas*; *Paracentrotus lividus*; larval development.

The marine pollution risk related to dredging activities has received greater attention over the last 20 years, especially in countries bordering the north-eastern Atlantic Ocean (Van Der Burgt, 1994). The bottom sediments involved in these dredging operations are usually considered the final repository of the major part of pollutants discharged into aquatic ecosystems. These toxics are present in the sediment as inorganic (heavy metals) or organic forms (PAHs, pesticides, PCBs) and can be adsorbed onto either mineral or organic particles, especially heavy metals (Robbe, 1984). The physical and chemical features of the sediment, such as the distribution of particle grain size, pH, salinity, organic matter content and oxidization conditions exert considerable influence on the chemical forms of the metal elements (Bourg, 1987; Broman *et al.*, 1994) and thus on their bioavailability. A large number of various sediment bioassays and bioaccumulation studies point out that heavy metals become more bioavailable during oxidization of the sediment as occurs, for instance, during dredging processes (Darby *et al.*, 1986; Förstner, 1989; Salomon and Förstner, 1984; Wollast, 1989).

After the Oslo (1972) and London (1986) agreements which aimed to prevent marine pollution caused by dredging operations, a study group comprising experts from the responsible ministries and national research organisms was created in France to report on the influence of dredging activities upon the marine environment (GEODE Programme). This group proposed reference values for metals and PCB concentrations allowing the classification of harbour sediments according to their levels of contamination

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(Anonymous, 1996). The basis for these guideline values was the concentrations of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) and PCB recorded between 1986 and 1990 within sediments originating from larger French harbours. From these data, a distribution curve (95% extrapolated) was obtained and a median value (Md) was calculated for each toxin. For instance, the median values are 0.6 $\mu g\ g^{-1}$ dry weight of sediment (DW) for Cd, 22.5 $\mu g\ g^{-1}$ DW for Cu, 50 $\mu g\ g^{-1}$ DW for Pb and 138 $\mu g\ g^{-1}$ DW for Zn. According to such a classification, the processes of dumping of dredged sediments were defined: if the concentrations of all these toxics remained under 2Md, the disposal of dredged sediments was allowed into offshore sites, without further study. If the concentrations were higher than 4Md (level 2), the dumping of dredged sediments may be prohibited in an offshore site. For metal or PCB concentrations ranging between 2Md and 4Md (level 1), complementary studies, based on a biological assessment of the toxicity of the dredged sediments (bioassays), has to be carried out before allowing the dumping or not.

In this context, this study focused on the development of sensitive biological tests to identify metal contamination and sediment toxicity. Regularly dredged sediments from harbours of the French Atlantic coast were used in this study. The aim of this work was to observe the behaviour of the larvae of different marine species (*Artemia salina*, *Crassostrea gigas*, *Paracentrotus lividus*) under conditions approximating a dumping process. Two processes were identified. *1*. The respective abilities of the early larval forms to accumulate four metals (Cd, Cu, Pb, Zn) over a short exposure time with the suspended particulate phase of harbour sediments showing different degrees of contamination; in this way, the bioavailability of each metal was assessed for each larva. *2*. The toxicity of such rearing media was assessed by observing delays in growth and possible abnormalities during the development of each larval species.

Materials and Methods

Collection of the sediments and preparation of the suspended particulate phases

Sampled sediments were collected with a 0.1 m^2 grab from the bottom (top 20 cm) of two French harbours: La Pallice, an industrial port and Les Sables d'Olonne, a fishing port. After collection, sediments were stored in clean polyethylene bags and laboratory treatments carried out without delay. Subsamples of sediment were prepared for physical parameters and metal content analyses. Three replicates were prepared for the bioassays: fresh sediments were placed in a clean 5 l glass beaker and mixed with 0.2 μm natural filtered seawater. This dilution (100 $g\ l^{-1}$ wet weight) was close to the dilution which occurs with the hydraulic suction dredging method used locally (Radenac, 1996). The diluted sediments were then stirred strongly for 8 h followed by 2 h of undisturbed settling. The supernatants were then coarsely sieved (25 μm) to obtain the suspended particulate phases of the sampled sediments. Three controls used natural filtered seawater. Lastly, controls and suspended particulate phases were placed in clean 5 l beakers and constituted the larval rearing media. This method was derived from a procedure described by Melzian (1989) ('suspended particulate phase bioassay') and was chosen first to model the local dredging processes but also because it is reported that sediment particles physically interfere with the development of organisms (Davis, 1960; Davis and Hidu, 1969).

Selection of tested sediments

After the preliminary physical analyses, the different sediments were selected according to two criteria: firstly, the homogeneity of their percentage silt/clay and organic matter (which provide the main sources of available metals); and secondly, the presence of a heavy metal concentration gradient (Table 1).

As metal analysis showed, levels of metal contamination of one sediment (according to the GEODE classification) could be different for each metal studied. As a consequence, the global GEODE level of contamination was defined as the higher GEODE level among the four metals: Cd, Cu, Pb, Zn (Table 1).

The coefficient of variation for all the trials was 14.5% for organic matter content and 17.9% for the percentage silt/clay, even if the sediments were characterized by different global levels of metal contamination (Table 1). Despite slight differences between the four metal concentration classes within the same sediment, the 22 tested sediments were characterized by a significant correlation between the four heavy metals analysed (Table 2). The possible effects of synergism or antagonism between metals would therefore be the same for every class of sediments.

Larval rearing and toxicity test

Three marine invertebrates species were selected: *Artemia salina* (Crustacea, Branchiopodia), *Crassostrea gigas* (Mollusca, Bivalvia) and *Paracentrotus lividus* (Echinodermata, Echinoidae). These invertebrate larvae have many advantages: widespread geographical distributions; easily handled and reared under laboratory conditions (need no feeding during the early development period, for example); rapid development; numerous references in the literature to facilitate meaningful comparisons (Chapman and Morgan, 1983; His and Robert, 1985, 1986; Kobayashi, 1971; Okubo and Okubo, 1962; Pagano and Romaña, 1991; Vanahaecke *et al.*, 1980; Woelke, 1967, 1972).

In each experiment, the salinity (34‰), pH (8.1) and temperature (see below) of the rearing media were controlled before the introduction of the larvae. The

TABLE 1

Physical parameters: % H = % water content, % O–M = % organic matter, % S/C = % silt/clay, heavy metal concentrations (μg g^{-1} dry weight) and corresponding GEODE levels (metal specific and global) of the tested sediments. The global GEODE level of contamination was defined as the higher GEODE level among the four metals. *C. gigas*: *Crassostrea gigas*; *P. lividus*: *Paracentrotus lividus*; *A. salina*: *Artemia salina*. Md: median of metal concentrations (μg g^{-1} dry weight), C.V.: coefficient of variation.

	Physical parameters			Heavy metal concentrations				Corresponding GEODE levels				
Bioassay	%H	%O–M	%S/C	Cd	Cu	Pb	Zn	Cd (Md = 0.6)	Cu (Md = 22.5)	Pb (Md = 50)	Zn (Md = 138)	Global
C. gigas	63.5	13.8	75.3	5.77	216	390	1916	> 4Md	> 4Md	> 4Md	> 4Md	> 4Md
	60.6	12.1	89.0	2.44	116	122	1130	> 4Md	> 4Md	2Md < 4Md	> 4Md	> 4Md
	64.6	11.1	86.5	0.81	118	103	1066	Md < 2Md	> 4Md	2Md < 4Md	> 4Md	> 4Md
	67.8	12.9	69.0	0.77	204	57	355	Md < 2Md	> 4Md	Md < 2Md	2Md < 4Md	> 4Md
	64.8	14.2	79.0	5.08	177	178	918	> 4Md	> 4Md	2Md < 4Md	> 4Md	> 4Md
	69.3	11.2	95.0	0.52	34	54	205	< Md	Md < 2Md	Md < 2Md	Md < 2Md	Md < 2Md
	66.5	12.4	98.5	0.23	15	41	147	< Md	< Md	< Md	Md < 2Md	Md < 2Md
	56.4	10.3	50.8	0.36	32	37	137	< Md	Md < 2Md	< Md	< Md	Md < 2Md
	67.4	12.6	81.1	1.16	17	50	168	Md < 2Md	< Md	Md < < 2Md	Md < 2Md	Md < 2Md
	46.3	6.9	82.0	0.17	11	30	107	< Md	< Md	< Md	< Md	< Md
	36.0	9.4	61.4	0.26	15	39	137	< Md	< Md	< Md	< Md	< Md
P. lividus	67.8	12.9	69.0	0.72	162	55	338	Md < 2Md	> 4Md	Md < 2Md	2Md < 4Md	> 4Md
	67.9	13.1	75.4	1.86	117	61	452	2Md < 4Md	> 4Md	Md < 2Md	2Md < 4Md	> 4Md
	64.8	14.2	79.0	4.95	183	178	645	> 4Md	> 4Md	2Md < 4Md	> 4Md	> 4Md
	56.4	10.3	50.8	0.30	32	31	128	< Md	Md < 2Md	< Md	< Md	Md < 2Md
	67.4	12.6	81.1	0.16	17	45	159	< Md	< Md	< Md	Md < 2Md	Md < 2Md
A. salina	69.0	12.6	99.1	3.68	143	125	732	> 4Md	> 4Md	2Md < 4Md	> 4Md	> 4Md
	67.5	11.3	91.8	2.63	126	161	572	> 4Md	> 4Md	2Md < 4Md	> 4Md	> 4Md
	72.0	12.9	92.6	0.57	50	63	242	< Md	2Md < 4Md	Md < 2Md	Md < 2Md	2Md < 4Md
	69.6	11.0	94.9	0.52	37	49	180	< Md	Md < 2Md	< Md	Md < 2Md	Md < 2Md
	70.6	13.8	98.1	0.26	26	42	185	< Md	Md < 2Md	< Md	Md < 2Md	Md < 2Md
	73.2	13.1	97.1	0.26	22	41	170	< Md	Md < 2Md	< Md	Md < 2Md	Md < 2Md
Mean	64.1	12.03	81.7									
C.V.	13.6	14.5	17.9									

rearing volumes were calculated to obtain the minimum of 10 mg DW of larvae required for heavy metal analyses, i.e. 5 l for the oyster and urchin bioassays and 500 ml for the Artemia.

Two criteria were chosen to evaluate the toxicity of the suspended particulate phases: the embryogenesis of the three species and the growth of *Crassostrea gigas* and *Paracentrotus lividus*.

Conditioned adult oysters, *Crassostrea gigas*, were induced to spawn in natural filtered seawater by thermal stimulation. Freshly collected oocytes and sperm were suspended in natural filtered seawater in a sterile glass beaker to achieve fertilization, facilitated by gentle stirring. The number of fertilized eggs was assessed and distributed among all the rearing media at a uniform value of 100 eggs per millilitre at 24 ± 1°C. Breeding was stopped after 24 h. To determine the toxic effects of the suspended particulate phases of each sediment on early larval development, three replicates (20 ml) of each trial were collected into plastic beakers and 100 μl of 4% buffered Formalin was added. One hundred individuals per trial were observed under a microscope (× 100) to distinguish between D-shaped larvae and early stages of development, including eggs, morulae and trochophores. Additionally, the length and width of 10 individuals taken at random per trial were measured (Fig. 1).

Urchins, *Paracentrotus lividus*, were collected from the "Ile de Ré" during the natural spawning period. Spawnings were realized naturally in the laboratory, i.e. without induction. Eggs were suspended in natural filtered seawater (0.2 μm) and a small amount of a diluted sperm solution was added in order to avoid polyspermi. After each sperm addition, the fertilization rate was estimated. Sperm addition was stopped when the fertilization rate was > 90%. Finally, fertilized eggs were distributed among all rearing media at 24 ± 1°C. Breeding was halted after 48 h. After collecting the larvae (using the same method as in the oyster trial), the toxic effects of suspended particulate phases of each sediment were assessed on 100 individuals observed under a microscope (× 40) in order to discriminate the normal pluteus larvae from undeveloped and abnormal ones. In addition, 10 individuals per trial were used at random to measure the length of the normal pluteus larvae (Fig. 1).

TABLE 2

Correlation matrix of Cd, Cu, Pb and Zn concentrations from the sediment (* indicates a probability correlation < 0.05, n = 22).

	Cd	Cu	Pb
Zn	0.793*	0.708*	0.908*
Pb	0.888*	0.716*	
Cu	0.770*		

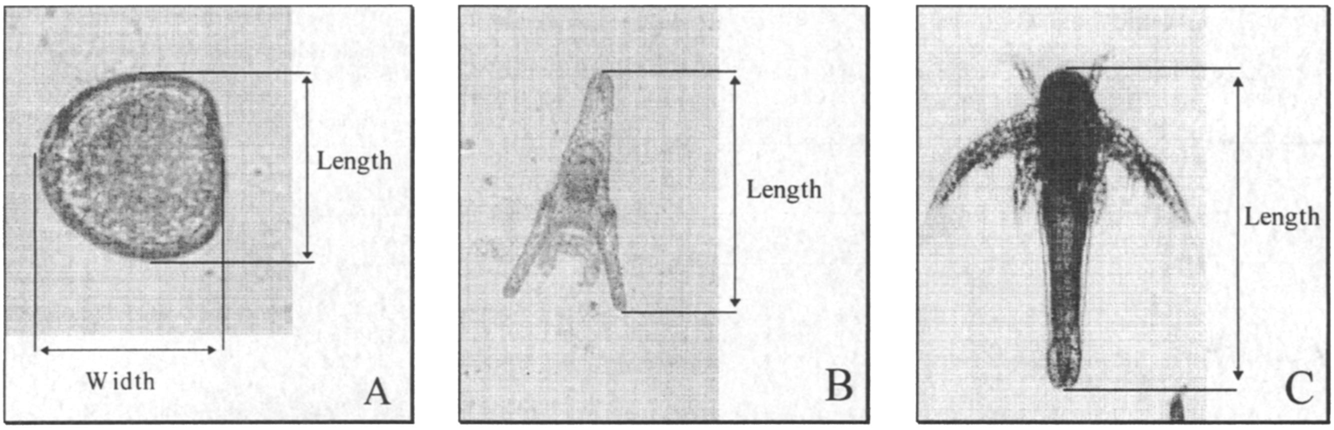

Fig. 1 Biometrical parameters used in the bioassays: (A) *Crassostrea gigas* D-shaped larvae; (B) *Paracentrotus lividus* pluteus larvae; (C) *Artemia salina* nauplius larvae.

For the bioassay on *Artemia salina*, freeze-dried eggs were hydrated for 1 h in freshwater and then immersed in bleach-water (30%) for 7 min to partially digest their envelopes. They were then distributed among all rearing media at 20 ± 1°C. After 48 h of development, three replicates (5 ml) of each trial were collected (using the same method as in the oyster and urchin trials). Additionally, 10 individuals per trial were randomly observed under a microscope ($\times 20$) in order to measure the length of the nauplius (Fig. 1) after the first moult.

Analytical methods

For each sediment, subsamples were prepared for physical parameter and metal content analyses. The percentage organic matter was estimated by the loss of weight on ignition at 450°C for 12 h. The particle grain size distribution was determined by wet sieving using a 56 μm sieve to separate the coarse and fine fractions which were then dried at 80°C and weighed. The loss of weight gave the water content percentage.

For the bioassays, all the larvae were removed from each rearing medium and their Cd, Cu, Pb, Zn concentrations determined. For this determination, larvae were dried at 80°C. Dry samples were then digested with a mixture of nitric/perchloric acids (HNO_3 14 N; $HClO_4$ 17 N) at 80°C for 12 h. After evaporation, the dry residues were dissolved in 0.3 N HNO_3 and stored at 4°C until analysis. Metal concentrations were determined by flame or furnace atomic absorption using a Varian Spectra AA250 atomic absorption spectrophotometer. Deuterium background correction was used. Standard samples of sediment (Quasimeme: QTM007MS) and fish muscle (IAEA: MA-A-2) were used as controls and analysed according to the same protocols. Data obtained from the standards were in agreement with certified values (Table 3).

Analyses of hydrocarbons and PCB (two Arochlor congeners) were performed with a GC/MS method by a certified laboratory (Laboratoire Départemental d'Hygiène, La Rochelle, France).

Data processing and statistical analyses

The heavy metal concentrations within larvae and sediments (grouped according to their global GEODE level) were given as arithmetic mean values ± standard deviations for each GEODE level of the considered metal. A biota-sediment accumulating factor (BSAF) was calculated according to Morisson *et al.* (1996):

BASF = larval concentrations/sediment concentrations.

The results of the toxicity bioassays were expressed as arithmetic mean values ± standard deviations for each global GEODE level of the tested sediment.

The homogeneity of the variances was tested with a Bartlett's test. In the case of heterogeneity, the data were log-transformed (for concentrations and length data) and arcsin-transformed for the data expressed as percentages. One-way ANOVA, with the GEODE levels (metal-specific or global) as fixed factors, was used for testing differences observed in larval concentrations and in the toxicity tests. Differences were accepted as significant at $p < 0.05$ and, in such a case, a comparison between all pairwise values was tested using a Fisher's LSD procedure. In the absence of homogeneity of variance (height of *Crassostrea gigas*

TABLE 3

Concentrations ($\mu g\ g^{-1}$ DW) for Cd, Cu, Pb and Zn in reference materials.

	Sediment QTM007MS		Fish muscle MA-A-2	
	Certified values	Present study	Certified values	Present study
Cd	1.4 ± 0.3	1.6 ± 0.1	0.066 ± 0.004	0.07 ± 0.01
Cu	23.5 ± 2.4	22.0 ± 0.6	4.0 ± 0.7	4.5 ± 0.8
Pb	47.5 ± 7.6	48.1 ± 1.0	0.58 ± 0.07	0.65 ± 0.1
Zn	165 ± 13	158 ± 16	33 ± 1	35 ± 2

larvae), a Kruskal–Wallis non-parametric test was used.

Results

Metal concentrations

The heavy metal concentrations ($\mu g\ g^{-1}$ DW) recorded within larvae of *Artemia salina*, *Crassostrea gigas* and *Paracentrotus lividus* and after either 48 h or 72 h of exposure to the suspended particulate phases of different harbour sediments are given in Fig. 2. The statistical analysis showed that Cd and Cu concentrations were significantly higher in oyster larvae reared in a >4Md medium than those in slightly contaminated media or the controls. Significant differences in Pb concentrations for the oyster larvae were also shown first between the control and the 2Md<<4Md media and second between the 2Md<<4Md and >4Md media. For urchin larvae, a significant difference was only observed for Cu between the >4Md and the other tested media and controls. The metal concentrations remained homogeneous in *Artemia salina* larvae in all suspended particulate phases of the sediments and the controls.

Toxicity tests

The effects induced by the suspended particulate phases of sediments on both parameters used for the toxicity assessment (embryogenesis and larval growth) are given in Figs 3 and 4. Significant differences in the embryogenesis of *Crassostrea gigas* larvae (as evidenced by the proportion of D-shaped larvae) occurred between the control and all the suspended particulate phase media but no significant differences were obtained between each contamination level (Fig. 3). The larval growth parameters (length and width, Fig. 1) were statistically different from the control for the contamination levels > Md value, but significant differences were also observed between the >4Md and <2Md media (Fig. 4).

For the *Paracentrotus lividus* bioassay, there was no significant difference between the percentage of

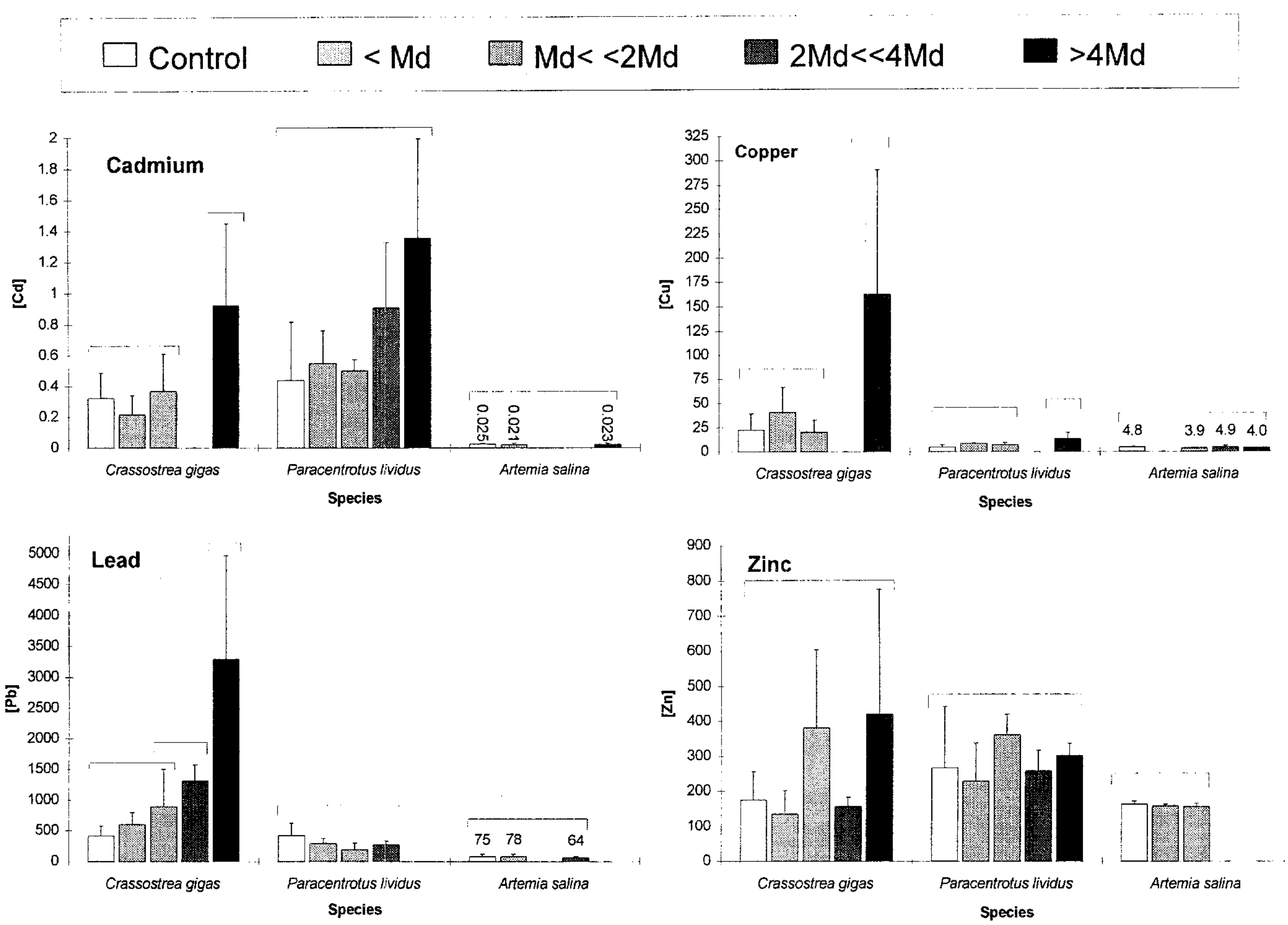

Fig. 2 Cd, Cu, Pb, Zn concentrations ($\mu g\ g^{-1}$ DW) of *Crassostrea gigas*, *Paracentrotus lividus*, and *Artemia salina* larvae in relation to GEODE levels (metal specific) of the suspended particulate phases. Error bars represent ±standard error and, for each species, values (log-transformed) not significantly different from one other (Fisher test, ANOVA, $p<0.05$) are grouped by a common overhead line.

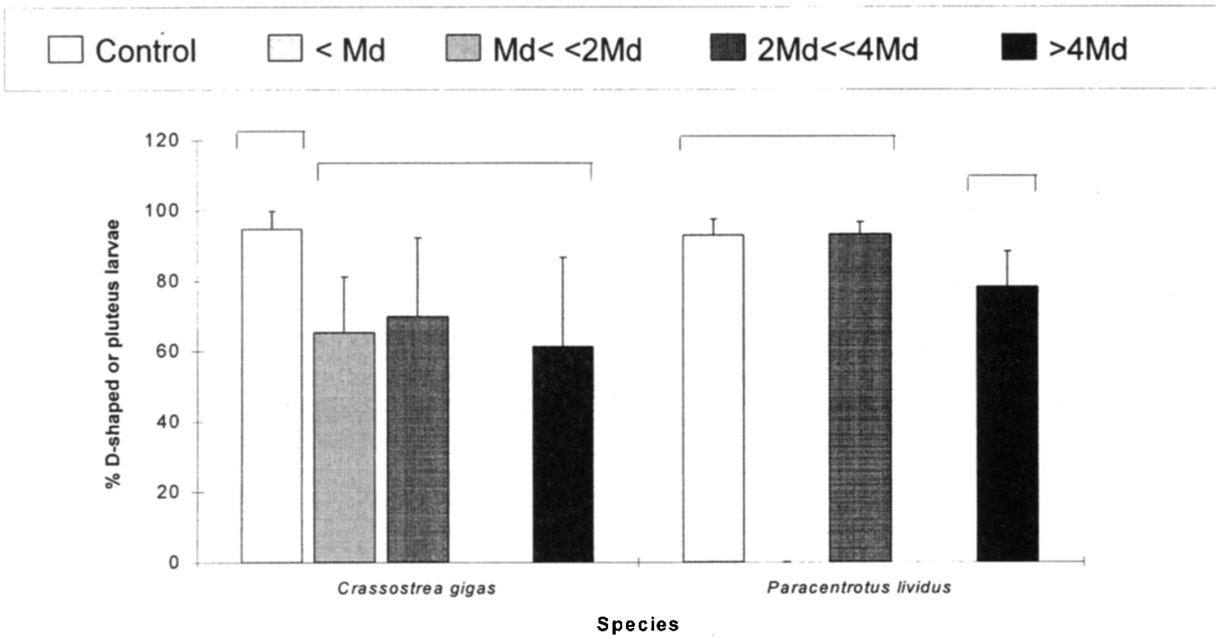

Fig. 3 Average percentage development ($\pm$standard deviation) of normal larvae (D-shaped or pluteus) according to the global GEODE levels of the suspended particulate phases of the tested sediments. The values (arcsin-transformed) which are not significantly different (Fisher test, ANOVA, $p<0.05$) are grouped by a common overhead line.

normal-developed plutei in the control and the <2Md medium, whereas the >4Md medium induced the development of more abnormal larvae (statistically significant, $p<0.05$) (Fig. 3). The biometric study

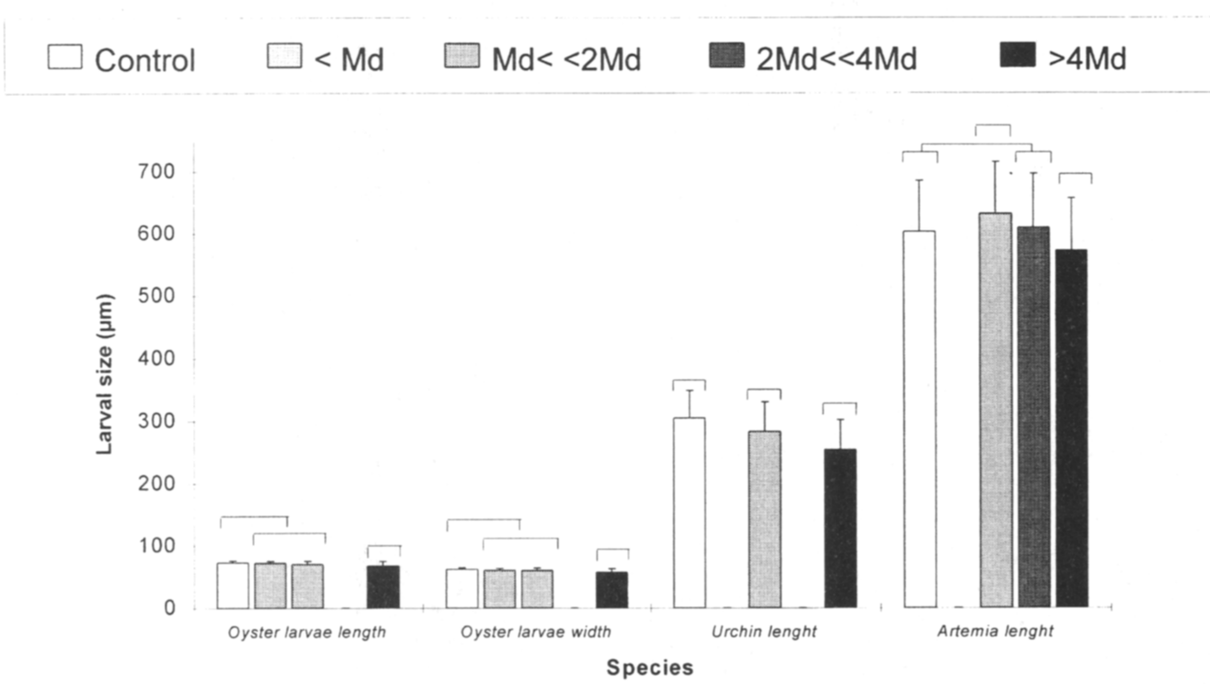

Fig. 4 Average sizes ($\pm$standard deviation) of normal larvae (D-shaped, pluteus or nauplii) according to the global GEODE levels of the suspended particulate phases of the tested sediments. The values (log-transformed) not significantly different (ANOVA with data followed by Fisher test for urchin and Artemia or Kruskal–Wallis test for oyster) from one another are grouped by a common overhead line.

pointed out significant differences in lengths between each rearing medium; the growth performance being inversely proportional to contamination level (Fig. 4).

In the *Artemia salina* bioassay, no obvious developmental abnormality was detected but the length of the larvae (nauplius stage) decreased significantly ($p<0.05$) with an increase in the contamination level of the resuspended sediments (Fig. 4).

Discussion

The levels of heavy metal concentrations at the end of the experiments (Fig. 2) appear very different between metals and between species: in *Artemia salina* nauplii, the four metals were poorly accumulated and the concentrations were within the range of concentrations already recorded for copepods and microzooplankton mainly made up of crustacean species (Table 4).

In *Crassostrea gigas* and *Paracentrotus lividus* larvae, Cd and Cu were more concentrated than in the Artemia nauplii. Moreover, Cd, Cu, and Zn concentrations in oyster and urchin larvae were close to those measured in the gonads of adults used in this experiment for the spawning stimulation and remained within the range of levels recorded for adults collected in the same and in other areas (Table 4). For these metals, the biota-sediment accumulating factor remained as <1.5 for Cd, Cu, and Zn (Fig. 5). These values are lower than those recorded in field studies (Amiard *et al*., 1994; Thomson, 1982) where adults were collected (Table 5). The contact between the larvae and the metals was, however, short in our experiment (48 h).

The concentrations of Pb are noteworthy (Fig. 2). In *Crassostrea gigas* D-shaped larvae, they appear high, ranging from 413 $\mu g\ g^{-1}$ DW for the control to 3290 $\mu g\ g^{-1}$ DW for the $>$4Md medium. Such values are unusual for marine organisms, and far higher than concentrations found in adults and in ♀ gonads of oysters (Table 4). Nevertheless, such values have been recorded previously in other molluscs, such as the gastropod *Policines sordidus* (Ying, 1993). Pb concentrations in the pluteus of *Paracentrotus lividus* were far higher than those measured in the gonads of ♀ individuals used in this experiment and in other adult urchins (Table 4). In the present study, Pb bioaccumulation was not through the food chain, although this is considered the principal input of Pb into *Nephtys* sp. (Polychaeta) and *Echinocardium cordatum* (Echinoidaea) according to Kersten and Kröncke (1991). The biota-sediment accumulating factor of Pb (12.8 ± 4.3 for *Crassostrea gigas*, 4.8 ± 4 for *Paracentrotus lividus* and 1.1 ± 0.8 for *Artemia salina*) were higher than those recorded in field studies focused on adults exposed over a longer period (Table 5).

Heavy metal bioaccumulation in larvae may be discussed in relation to their bioavailability when released during sediment resuspension. Despite the similarities in experimental conditions (i.e. physical characteristics of the sediments (Table 1) and time of exposure), significant differences in metal concentrations were noticed between the controls and larvae exposed to the suspended particulate phases of sediments. Cd, Cu and Pb concentrations in *Crassostrea gigas* D-shaped larvae and Cu concentration in *Paracentrotus lividus* pluteus larvae were higher in the trial using strongly contaminated sediments ($>$4Md). These observations suggest a release of bioavailable chemical forms of these metals from the sediment during the resuspension phase. The bioavailability was, moreover, different according to species and metals.

In the case of Zn, all larval concentrations were homogeneous at every sediment contamination level. This homogeneity does not seem to result from the absence of Zn being released from the sediments as observed in other experiments (Slotton and Reuter, 1995), but could be due to the release of a form of Zn non-bioavailable to the early larval stages.

In our study, Cu was accumulated by *Crassostrea gigas* D-shaped larvae after two days of exposure to the supernatants of natural sediments (Fig. 2). This bioavailability has already been observed in oyster adults exposed for several weeks to artificially contaminated suspended particles (Ettajani *et al*., 1992). Oyster

TABLE 4

Ranges of heavy metal concentrations ($\mu g\ g^{-1}$ DW) in bivalves, echinoids and crustaceans recorded in other studies.

Species (organs)	Cd	Cu	Pb	Zn	Area	References
C. gigas (adults, soft tissues)	3–6.5	150–290	1.5–1.8	2400–2800	Sampling area	RNO, 1993
C. gigas (♀ gonad)	0.38	31	0.25	1152	Marennes Bay, France	Present study
P. lividus	<0.3–2.8	0.8–34.8	<1.0–33.1	<1–710	Mediterranean Sea	Augier *et al*., 1992
E. parma	0.143	6	2.37	20.1	New York Bight	Steimle *et al*., 1994
S. neumayerii (gonads)	0.25–0.80	1.24–3.29	—	18.4–56.1	Antarctic	de Moreno *et al*., 1997
P. lividus (♀ gonad)	0.68	—	2.47	116	Isle of Ré, France	Present study
Copepods	1.6	32.4	2	403	Bay of Cherbourg	Miramand *et al*., 1996
Copepdos	5.5±3.1	47±27	28±18	480±190	Bay of Seine	Miramand *et al*., 1996
Mixed zooplankton	2.4±0.4	16.6±3.1	—	424±122	Mediterranean Sea	Fowler, 1986
Mixed amphipods	0.093	20.9	13.8	43.8	New York Bight	Steimle *et al*., 1994
T. tridentatus (eggs)	0.01	20	ND	32	Japan	Kannan *et al*., 1995

E. parma: *Echinarachinus parma*; *P. lividus*: *Paracentrotus lividus*; *C. gigas*: *Crassostrea gigas*; *S. neumayerii*: *Sterechinus neumayerii*; *T. tridentatus*: *Tachypleus tridentatus*; ND: not detected; —: not measured.

larvae also accumulated Cd in the most polluted medium; such an observation has already been made for other adult bivalves, i.e. *Macoma balthica*, exposed for two days to Cd contaminated water (Bordin *et al.*, 1996). The trend to accumulate Cd in *Paracentrotus lividus* pluteus larvae (though not statistically significant) has already been shown for adults (Warnau *et al.*, 1995). Cd and Cu were not bioavailable to *Artemia salina* nauplii (Fig. 2), contradicting results obtained in field studies on crustaceans (Depledge *et al.*, 1993; Rule and Alden, 1990).

In the case of Pb, Izquierdo *et al.* (1997) showed that 30% of this metal was bound in the sediment to the carbonate fraction in a proportion equivalent to the

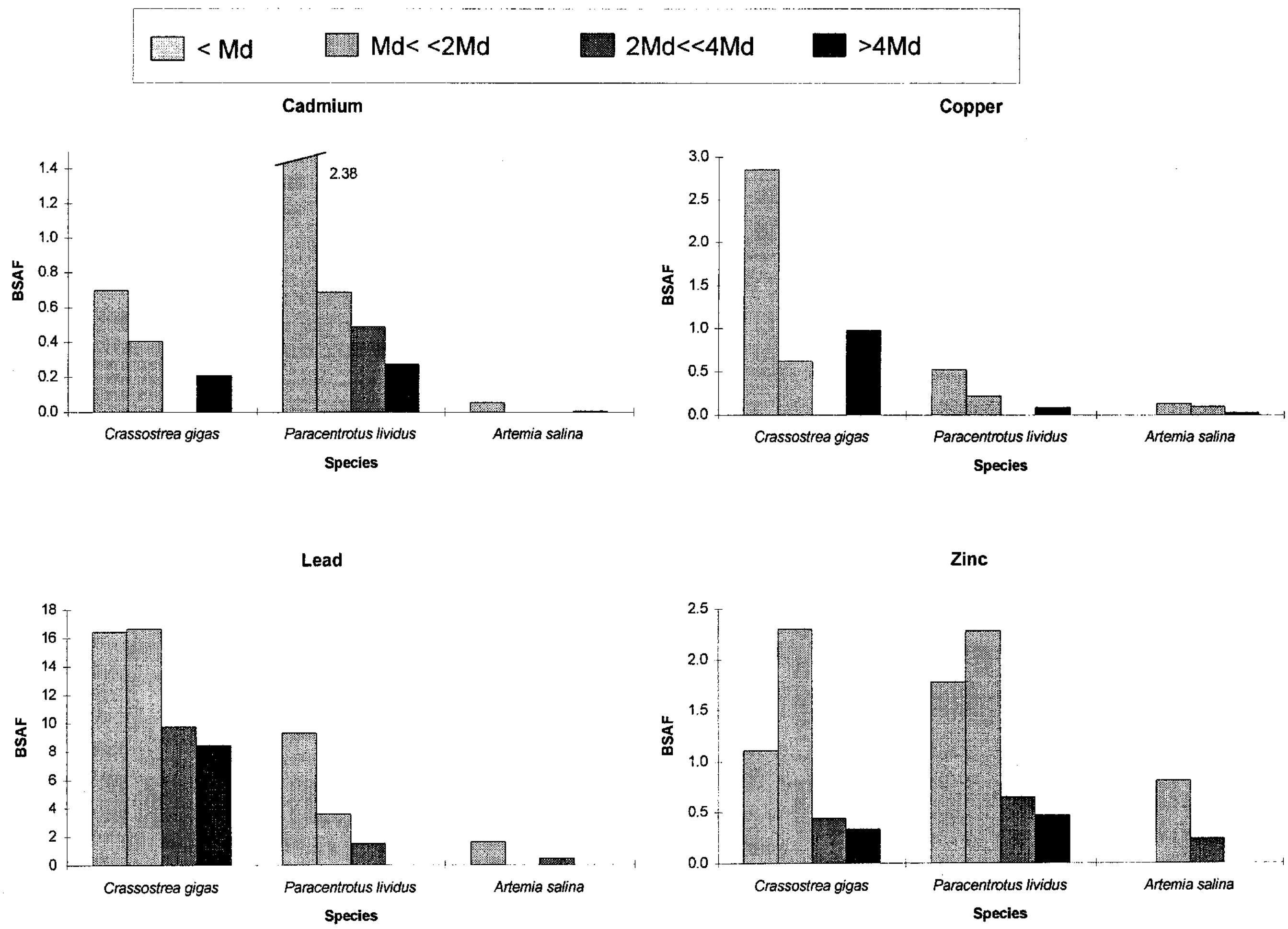

Fig. 5 Cd, Cu, Pb, Zn biota-sediment accumulating factor (BSAF = biota concentration/sediment concentration) of *Crassostrea gigas*, *Paracentrotus lividus*, and *Artemia salina* larvae in relation to GEODE levels (metal specific) of the suspended particulate phases of the sediments.

TABLE 5

Biota-sediment accumulating factor values (BSAF = biota concentration/sediment concentration) recorded in bivalves and crustaceans during field studies.

Species (organs)	Cd	Cu	Pb	Zn	Study	References
M. edulis (adults, soft tissues)	12.1	1	0.1	5	Field study	Thomann *et al.*, 1995
C. virginica (adults, soft tissues)	35.6	19.2	0.04	59.2	Field study	Thomann *et al.*, 1995
M. galloprovincialis (adults, soft tissues)	—	1.08	0.07	2.19	Field study	Puente *et al.*, 1996
S. plana (adults, soft tissues)	0.81	0.67	0.28	2.65	Field study	Saiz-Salinas *et al.*, 1996
M. edulis (adults, soft tissues)	4	0.42	0.2	0.77	Field study	Radenac, 1996
P. pugio (adults, soft tissues)	—	18.9	0.63	1.7	Field study	Rule, 1985

M. galloprovincialis: *Mytilus galloprovincialis*; *S. plana*: *Scrobicularia plana*; *C. gigas*: *Crassostrea gigas*; *M. edulis*: *Mytilus edulis*; *C. virginica*: *Crassostrea virginica*; *P. pugio*: *Palaemonetes pugio*; — : not observed.

organic fraction. The correlation between dissolved Pb in seawater and concentrations in the shells of *Mya arenaria* (Pitts and Wallace, 1994), *Cerastoderma edule* and *Venerupis aurea* (Auernheimer *et al.*, 1996) demonstrated the bioavailability of Pb when this metal is combined with $CaCO_3$. Furthermore, the increase in Pb concentrations was related to a decrease in the size of the planktonic organisms, as noticed in other studies (Michaels and Flegal, 1990). The small body size (<75 μm) of the oyster larvae and the formation of the first shell (prodissoconch I) may explain the high concentrations of Pb within the *Crassostrea gigas* D-shaped larvae. Conversely, the lower concentrations in *Paracentrotus lividus* and *Artemia salina* could be the consequence of a lower calcite metabolism related to the skeleton of the former larvae and of the cuticle in the latter larvae. Consequently, the resuspension process appears to be a source of bioavailable Pb; this bioavailability depending on the calcite metabolism of the organism under consideration. This bioavailability was observed in a previous study where an accumulation of Pb was recorded in the soft tissues of adult *Mytilus edulis* immersed around dredged material disposal sites in our study area (Radenac *et al.*, 1997).

For the four metals, the decrease in the biota-sediment accumulating factor according to metal contamination level of the sediment (Fig. 5) indicated that the amounts of metals accumulated by the larvae were not proportional to their concentrations within the sediment.

For the most contaminated sediments (>4Md), the bioaccumulation of Cd, Cu and Pb has been shown to be concurrent with the toxicity of the corresponding suspended particulate phase (Figs 3 and 4). However, larval growth appears to be a more sensitive quality criteria of the rearing medium than observations of abnormalities in larval development. For less contaminated sediments (<2Md), no statistically significant bioaccumulation of metals was recorded in the larvae (Fig. 2) but growth inhibition may be seen (Fig. 4). This could be due to the presence of other xenobiotics present in our sediments (Table 6), particularly PAH, which may induce toxic effects on invertebrate larvae (Meador *et al.*, 1990).

To conclude, the impact of the dumping operations appears to depend not only on the global GEODE level but also on the metal concentrations which create the sediment classification. For example, for two sediments belonging to the same GEODE level, the sediment whose class was determined by Pb concentrations will have to receive more attention than the sediment whose class is determined by Zn. The assessment of the impact also depends on the species chosen in the bioassay: *Crassostrea gigas* and *Paracentrotus lividus* are better bioindicator species than *Artemia salina*.

In all cases, these results confirm the recommendations of GEODE which suggest taking maximum precautions before authorizing the dumping of highly contaminated sediments (>4Md). They also show that resuspension of dredged material induces biological perturbations (i.e. growth inhibition) even in the case of slightly metal contaminated sediments. In this context, further studies would be useful to assess the impact of dumping of dredged sediments with a level of contamination between 2Md and 4Md.

TABLE 6

Concentrations of total hydrocarbons and two PCB congeners ($\mu g\ g^{-1}$) in three tested sediments with different global GEODE levels.

	<2Md	2Md<4Md	>4Md
Total PAH	196.5	1897.5	2715
Arochlor 1254	12	25	45
Arochlor 1260	14	11.5	50

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