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Comparative radiotracer study of cadmium uptake, storage, detoxification and depuration in the oyster *Crassostrea gigas*: potential adaptive mechanisms

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ABSTRACT: The bioaccumulation of cadmium in the oyster *Crassostrea gigas* originating from a clean (Bourgneuf Bay) and a chronically Cd-contaminated area (Gironde estuary) experimentally exposed to ^{109}Cd-labeled bulk seawater (dissolved and particulate pathways combined) was examined over 21 d. A single-component first-order kinetic model describing the behavior of the bioaccumulation factor (BAF) throughout the experiment showed that the estimated Cd BAF at 21 d was 47% higher for oysters originating from the contaminated estuary than for oysters from the clean area, suggesting an influence of the previous chronic exposure to Cd contamination in the estuarine environment. From the experimental results, the potential adaptive mechanism suggested cannot be attributed to a reduction in Cd permeability but rather to a higher Cd turnover due to the synergy between lysosomes and metallothioneins which, through chelation, are responsible for the reduction in bioavailability and toxicity of cd in oysters. The lower BAF observed for soft parts of oysters previously exposed to chronic Cd contamination corresponded to a faster response to the experimental Cd contamination due to the presence of pre-existing metallothioneins induced by the Cd present in the estuarine environment. Furthermore, based on a 2-component exponential loss kinetic model, Cd complexation to metallothioneins and lysosomes was probably responsible for the slow turnover in the long-term compartment of loss (biological half-life, $T_{b1/2}$ = 495 and 198 d for the Bourgneuf and the Gironde oysters, respectively). Of the total Cd accumulated, 40 to 60% was in the soluble form and 30 to 40% of this fraction had been detoxified by the Gironde oysters through chelation to metallothioneins or to lysosomes, which means that approximately 12 to 24% of the total Cd accumulated was potentially bioavailable to humans through oyster consumption. However, through depuration, it was also more efficiently eliminated from oyster soft parts (the edible portion) previously exposed to Cd than from control oysters. Therefore, in the light of these results, it is suggested that the way in which regulatory thresholds of Cd in oysters are presently calculated should be reconsidered and should take into account the level of Cd already detoxified by the oysters through complexation processes.

KEY WORDS: Cadmium · Radiotracer · Oysters · Uptake · Depuration · Subcellular fractionation · Adaptive mechanisms

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INTRODUCTION

In the marine environment, estuarine zones are subjected to periodic anthropogenic contamination by trace metals. In areas where oyster culture is developed, this situation has a potential socio-economic impact, since oysters are known for their ability to concentrate metals from their environment, in particular the highly toxic contaminant cadmium (Amiard 2002). The Gironde estuary (France) has been devoted to

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oyster culture; however, it is also chronically enriched with Cd (Latouche 1988, 1992). Latouche (1992) demonstrated that the Cd pollution mainly originates from the wastes of a Cd plant in an industrial zone located in a principal drainage basin. Particulate Cd transported by the rivers changes in the Gironde estuary is solubilized as a consequence of the increase in salinity. It is accumulated not only by the oysters on the inner shelf and at the estuary mouth, but also by the sediment, which serves as pollutant reservoir. Geffard et al. (2002) demonstrated that the Cd concentration in oysters from the metal-rich Gironde estuary can be as much as 15 times higher than that in oysters from an area (Bay of Bourgneuf, France) considered to be relatively free of pollution (Amiard-Triquet 1987a).

A key aspect of this study was to define potential adaptive mechanisms developed by oysters subjected to chronic Cd contamination in the environment. A novel experimental approach was used to determine whether the behavior and fate of Cd in the oyster *Crassostrea gigas* are different in organisms originating from a Cd-contaminated area (Gironde estuary) than in those from a relatively clean site (Bay of Bourgneuf) when both are experimentally exposed to an elevated stable Cd level. The bioaccumulation potential of this benthic bivalve was evaluated prior to subcellular analyses. A non-destructive method which allows determination of individual uptake kinetics was used to measure the bioaccumulation potential. In this approach, the radioactive isotope ^{109}Cd was used together with the stable metal to evaluate the bioaccumulation potential of the 2 populations of oysters experimentally stressed by the stable metal. This method is used to identify the relative distribution of the radiotracers at the organism, cellular and subcellular level in order to highlight potential adaptive mechanisms to Cd contamination which could be of ecotoxicological significance. In fact, Cd toxicity (nephrotoxicity, osteotoxicity) has been observed in man in France (itaï itaï disease, in Boisset 1996a), and has led to legal restrictions on oyster production and marketing in regions where Cd concentrations in soft tissues exceed 2 mg kg^{-1} wet wt (Boisset 1996b). As a consequence, oyster farms have been closed in the Gironde estuary. Recently, a European regulation (CE N°466/2001: Communautés Européennes 2001) lowered this threshold to 1 mg kg^{-1}. The results of the present study indicate the need for reconsidering the way in which the regulatory thresholds of Cd in oysters are calculated.

MATERIALS AND METHODS

Uptake of cadmium from food and seawater. Oysters *Crassostrea gigas* of similar size (5 to 6 cm) were harvested in early March at 2 sites along the Atlantic coast of France, the Gironde estuary (GE) near the most highly Cd-contaminated area of the French coast (RNO 1995) and the Bay of Bourgneuf (BB), which is considered to be an uncontaminated area (Amiard-Triquet 1987b). Oysters were transported refrigerated to the laboratory, where they were thoroughly washed to remove encrusting organisms, and then acclimated to laboratory conditions (Mediterranean seawater, 17 ± 1°C, 37‰ psu and 12:12 h light:dark regime) for 2 wk prior to the start of the experiment.

An aquarium filled with 50 l of Mediterranean seawater was spiked with microliter quantities of the radiotracer ^{109}Cd in acidic solution (carrier-free, obtained from AEA Technology, $t_{1/2}$ = 462.3 d) to obtain an activity of 2 kBq l^{-1} in the experimental medium. No change in pH was observed after this addition. The medium, which naturally contained approximately 72.3 pM of stable Cd (Fisher et al. 2000), was also enriched with stable Cd at a concentration of 500 ng Cd l^{-1} (4.45 nM 1^{-1}) to correspond to the environmental level of Cd in the Gironde estuary reported by Latouche (1988). Following radioactive and stable Cd addition, the experimental medium was allowed to equilibrate for 2 h. The initial ^{109}Cd activity in the seawater was checked, and then 60 individuals from each sampling site were placed in the aquarium. The shell of each individual was previously tagged to allow identification. Oysters were fed the prymnesiophyceaen *Isochrysis galbana* at an initial algal cell density of 10^4 ml^{-1} in the experimental medium. This food concentration was chosen to prevent as much as possible the production of fecal pellets (Luoma et al. 1992, Wang et al. 1996) and to avoid recycling of ^{109}Cd. The experimental medium was changed every 2 d to maintain ^{109}Cd activity at a relatively constant level. On each sampling occasion, 10 oysters each from the contaminated and the control site were removed, briefly rinsed in fresh seawater, blotted dry on absorbent paper to eliminate any adhering radioactive medium, and weighed before being gamma-counted. The individual uptake kinetics of ^{109}Cd in each individual oyster were followed over 2 wk.

Cadmium depuration. At the end of the uptake period, the radioactivity of 53 oysters from each group (i.e. sampling cite) was determined; of these 53 individuals, 3 were dissected to evaluate the Cd distribution in their tissues, i.e. shell, remaining soft parts, gills, pallial fluid and the remainder adhering to the dissection tools. Cd distribution was also examined at the subcellular level in 20 individuals. The remaining 30 individuals were placed in fresh flowing seawater and allowed to eliminate the radioisotope. During the depuration phase, the oysters were continuously fed,

and 10 oysters (always the same individuals) from the Gironde estuary and the Bay of Bourgneuf were periodically removed and counted; at each sampling, 3 oysters from those remaining were dissected to determine the tissue distribution of ^{109}Cd.

Subcellular analysis. At the end of the ^{109}Cd exposure period, 20 oysters from both the Gironde estuary and Bourgneuf Bay were dissected and their visceral masses removed for subcellular analysis. Visceral tissues were pooled and homogenized in 0.25 M sucrose, 5 mM 2-mercaptoethanol, 0.1 mM phenylmethyl sulfoxyl fluoride, and 20 mM Tris-HCl at pH 8. Cell fractionation procedures were carried out at 4°C. Subcellular fractions were obtained by differential centrifugation according to the procedure described by Galey et al. (1986). The homogenate was centrifuged successively at 900 × *g* for 10 min to sediment nuclei, cell debris and heavy lysosomes, at 12 000 × *g* for 15 min to sediment lysosomes and the mitochondrial fraction, at 45 000 × *g* for 30 min to sediment the light mitochondrial fraction and cellular membranes, and at 115 000 × *g* for 70 min to obtain the microsomal fraction in the pellet and the cytosolic fraction in the supernatant. Aliquots were taken from each fraction for determination of ^{109}Cd activity and enzymatic activities of a marker for lysosomes (acid phosphatase assay as described by Galey et al. 1986).

The cytosolic fraction obtained in the final centrifugation was fractionated according to the molecular size by gel permeation chromatography on a column of Sephacryl S300 (2.6 × 95 cm) previously equilibrated with 100 mM NaCl, 5 mM 2-mercaptoethanol and 20 mM Tris-HCl at pH 8. The column was eluted at a flow rate of 33 ml h^{-1}. Fractions of 4 ml were collected and analyzed for ^{109}Cd radioactivity and for absorbance at 280 nm (Varian Cary 50 spectrophotometer). Calibration of the column was performed with different molecular weight markers: thyroglobulin 669 kDa, ferritin 440 kDa, catalase 232 kDa, aldolase 158 kDa, bovine serum albumin 67 kDa, chymotrypsinogen A 25 kDa, and ribonuclease 13.7 kDa.

Nuclear detection. The γ emission of ^{109}Cd (88.0 keV) in oysters, seawater and dissected tissues was determined using a well-type NaI detector connected to a multichannel analyzer and a personal computer using Interwinner™ software for spectral analysis. The activity in the tissue fractions was analyzed with an automatic well-type NaI Packard Cobra II counter. The ^{109}Cd radioactivity in samples was determined by comparison with standards of appropriate geometry and was corrected for background, counting efficiency and radioactive decay. Counting times were adjusted to give relative propagated errors <5% at 1 SD level, i.e. 3 min for oysters and seawater, and from 20 min to 1 h for dissected body tissues.

Data analysis. Uptake from water and diet is usually reported as bioaccumulation factors (BAF) which are calculated at each sampling time as: activity in oyster (Bq g^{-1} wet wt)/total ambient activity (Bq ml^{-1}), where total ambient activity corresponds to the bulk seawater representing dissolved and dietary cadmium sources. To reduce the influence of temporal fluctuations of the activity level in the labeled seawater, the radioactivity of the seawater used for each calculation was obtained by computing a running mean of the ^{109}Cd activities in seawater measured on each sampling day and after each new spike.

Cd uptake kinetics were fitted by a simple linear regression model BAF = kt, where k is the regression slope corresponding to the rate of increase in BAF (d^{-1} and t is time in days). Linearity of the uptake kinetics was tested by 1-way ANOVA for regression with replication.

Cadmium loss was determined by dividing the radioactivity in the whole body during the depuration phase (normalized to the wet weight of the organism) by the radioactivity measured at the end of the exposure period (Bq g^{-1} wet wt; time, t = 15 d) to give the percentage of cadmium remaining in the organism at a given time. The % ^{109}Cd retained in the whole body was plotted against time for each individual from each group of oysters. The mean loss kinetics obtained were described by a 2-component exponential model including a short-lived (s) and a long-lived (l) component (Whicker & Schultz 1982):

$$A_t = A_{0s}\, e^{-\lambda s t} + A_{0l}\, e^{-\lambda l t}$$

where A_t and A_0 are the remaining activities (%) in oysters at time t (d) and zero, respectively, and λ is the biological depuration rate constant (% d^{-1}) corresponding to the regression slope. Adjustment of the data distribution to the model was tested by a 1-way ANOVA.

Biological half-lives of Cd were computed for the short-term ($T_{b1/2s}$) and the long-term ($T_{b1/2l}$) components using the corresponding depuration rate constants in the following equation (Whicker & Schultz 1982):

$$T_{b1/2} = \ln 2/\lambda$$

The same procedure was applied for the assessment of Cd retention by the body compartments during the loss period. Difference between the slopes of the regression equations obtained for loss in oysters from the contaminated and from the control area was tested by Student's t-test. The level of significance for statistical analyses was set at $\alpha = 0.05$.

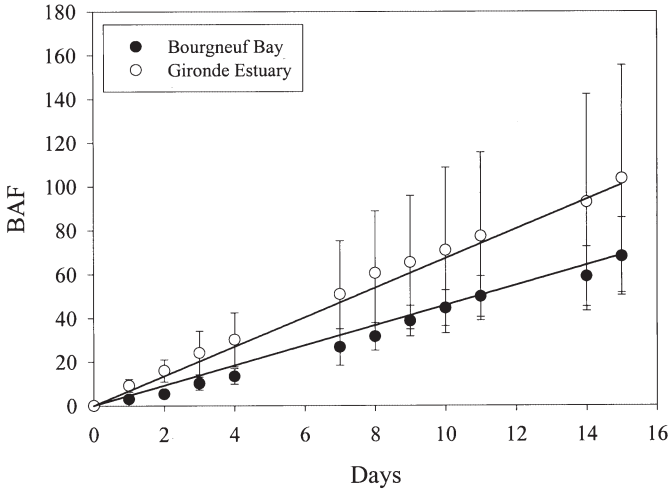

Fig. 1. *Crassostrea gigas*. Uptake of ^{109}Cd in whole oysters originating from a contaminated area (Gironde Estuary) and from clean area (Bourgneuf Bay) following exposure to labeled food and seawater in the presence of 500 ng l^{-1} stable Cd (mean ± 1 SD, n = 10 individuals). BAF: bioaccumulation factor

RESULTS

Cadmium uptake

Stable Cd was added to the experimental medium together with the radiotracer corresponding to dissolved Cd concentrations in the most polluted estuaries, to deliberately stress the organisms in order to demonstrate the presence of any adaptive response in oysters previously subjected to chronic Cd contamination in their natural environment.

The whole-body uptake of radioactive Cd from bulk seawater by both groups of oysters displayed linear kinetics over the 15 d exposure period (Fig. 1). From the fitting of the data, using a linear uptake model (R^2 = 0.988 and 0.975 for the Gironde and the Bourgneuf areas, respectively; $p < 0.0001$), it is estimated that the rate of increase of the bioaccumulation factors in the oysters was k = 6.73 ± 0.23 and k = 4.58 ± 0.23 ($p < 0.0001$) for the contaminated and for the control sites, respectively. The regression slopes were significantly different at the 0.05 level. The ^{109}Cd BAFs obtained at the end of the 2-wk exposure period were 104 ± 52 for the oysters taken from the contaminated zone (Gironde Estuary, GE) and 68 ± 18 for the oysters originating from the clean area (Bourgneuf Bay, BB).

At the end of the exposure period, 3 individuals from each group of oysters were whole-body counted and dissected into shell, soft parts, gills, pallial fluid and remainder. Cd BAFs were then computed for each compartment (Table 1). There was no significant difference between the 2 groups of oysters for the BAFs of shell, gills and pallial fluid, but the soft parts of BB oysters concentrated significantly more Cd (40%) than the soft parts of the GE oysters (t-test, $p < 0.05$).

Table 1. *Crassostrea gigas*. Cd bioaccumulation factors, BAF (mean ± 1 SD; n = 3 ind.) in body compartments of oysters from contaminated Gironde Estuary and from 'clean' Bourgneuf Bay, following 15 d experimental exposure to Cd contamination from both food and dissolved sources

Compartments	Gironde Estuary	Bourgneuf Bay
Shell	29.6 ± 19.2	21.6 ± 3.3
Soft Parts	894.8 ± 131.8	1474.5 ± 404.6
Gills	2771.9 ± 1515.7	1916.5 ± 642.5
Pallial Fluid	7.2 ± 1.9	5.7 ± 0.2

With respect to the distribution of ^{109}Cd (Bq) among body compartments, it appears that the major fraction of radiocadmium is located in the soft parts, followed by gills, shell and pallial fluid, with the remainder containing only a few percent (Fig. 2). The fraction of total Cd in the shell was significantly higher in the GE oysters than in the BB group, whereas ^{109}Cd concentration in the soft parts of BB oysters was higher than that in the from the GE. There were no observed difference for the other compartments in these 2 groups of oysters (t-test, $p < 0.05$).

The subcellular distribution of ^{109}Cd in the digestive gland of oysters at the end of the exposure period was determined by homogenization of the digestive gland, and separation of cellular organelles and membrane fragments from the soluble cytosolic components by differential centrifugation on the basis of their various densities (Table 2). The ^{109}Cd activity was distributed

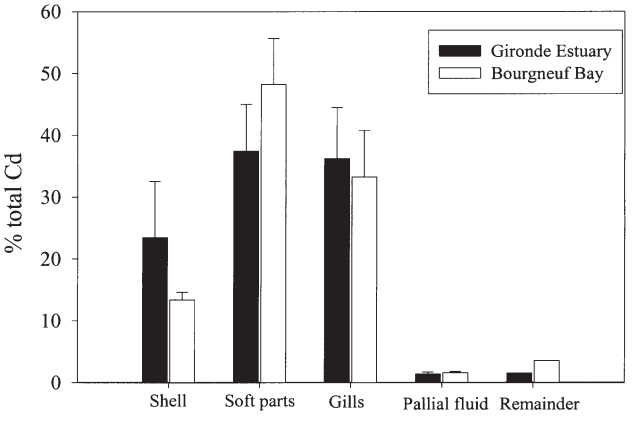

Fig. 2. *Crassostrea gigas*. ^{109}Cd distribution in body compartments of oysters from the contaminated (Gironde) and control (Bourgneuf) areas at end of exposure period. Data are mean + 1 SD (n = 3 ind.)

in the various subcellular compartments with a predominance in the soluble fraction (59.8 and 41.2% of ^{109}Cd for GE and BB oysters, respectively). The remaining ^{109}Cd activity was distributed among the other compartments, principally in the heavy fraction: first pellet (nuclei, heavy lysosomes, cellular debris) = 15.5% GE, 30.2% BB; second pellet (lysosomes, mitochondria) = 15.7% GE, 16.8% BB. The ^{109}Cd activity was approximately 30% higher in the cytosol of GE oysters than in that of BB individuals, with the heavy fraction in BB oysters being more contaminated than in the same fraction of the GE oysters.

The distribution analysis of the enzymatic marker (acid phosphatase) showed that most of the activity was in the first 3 centrifuged pellets, with a maximum in the second pellet for both groups of oysters, indicating the presence of most of the lysosomes in this pellet.

The cytosolic fractions of the digestive gland of oysters which contained 59.8 and 41.2% of ^{109}Cd were fractionated by size-exclusion chromatography on a Sephacryl S300 column to identify the different molecular components responsible for Cd fixation (Fig. 3): 30 to 40% of the cytosolic cadmium was located on a peak with a K_{av} (distribution coefficient) between 0.6 and 0.7 and an apparent molecular weight of 9 kDa, regardless of the origin of the oysters. This fraction cor-

Table 2. *Crassostrea gigas*. Distribution of ^{109}Cd in subcellular fractions of visceral mass as percentage recovered ^{109}Cd radioactivity (% Bq) and enzymatic activities of lysosome markers (acid phosphatase, % AP)

Cell fraction	Gironde Estuary		Bourgneuf Bay	
	% Bq	% AP	% Bq	% AP
Nuclei, cell debris, heavy lysosomes	15.5	16.5	30.2	15.7
Lysosomes, mitochondria	15.7	25.1	16.8	17.5
Light lysosomes, membranes	4.4	14.3	6.4	15.1
Microsomes	4.6	7.7	5.4	9.0
Cytosol	59.8	36.4	41.2	42.7

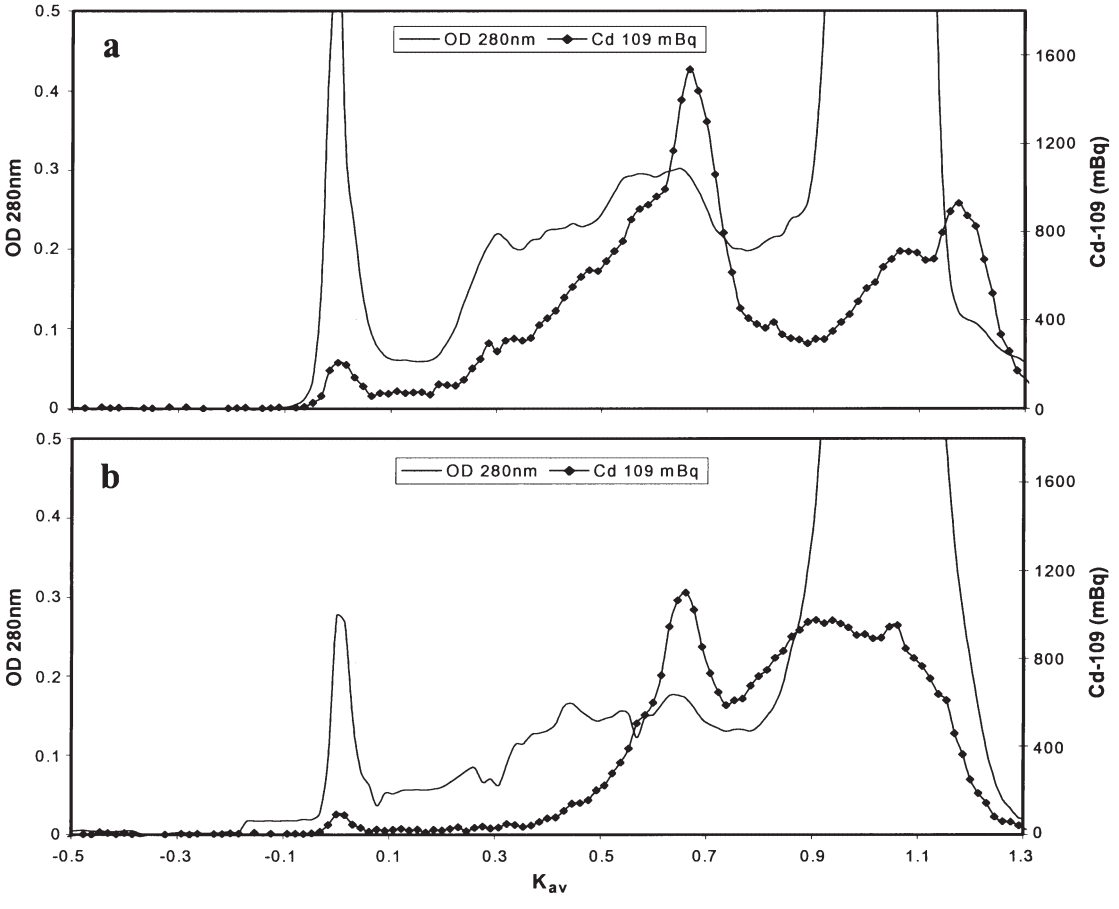

Fig. 3. *Crassostrea gigas*. Fractionation of cytosol from visceral mass of Gironde Estuary (a) and Bourgneuf Bay (b) oysters on a Sephacryl S300 column. Distributions of ^{109}Cd activity (mBq) and protein content measured by absorbance at 280 nm (OD = optical density) are shown; distribution coefficient of the protein is defined by $K_{av} = (V_e - V_o)/(V_t - V_o)$, with V_e = elution volume of the protein, V_o = void volume, V_c = total volume of the column

responds to the elution volume of metallothioneins which play an important role in the metal detoxification process in bivalves and other species.

In the cytosolic fraction of the GE oysters, some proteins of higher molecular weight were associated with the ^{109}Cd activity; whereas in the BB oysters, proteins with molecular weight higher than 9 kDa contained a very low level of ^{109}Cd, which was mainly associated with low molecular weight components.

Cadmium elimination

At the end of the exposure period, the oysters from both groups were transferred to uncontaminated seawater and were maintained for 41 d to follow Cd depuration from each individual oyster. The mean loss kinetics obtained for each group of oysters were best described by a 2-component exponential model (Fig. 4, Table 3).

Depuration of ^{109}Cd appears to be a slow process; the short-lived loss components were characterized by a short biological half-life of 2 and 10 d for oysters from the contaminated and the control areas, respectively. It is estimated that the short-lived loss components (A_{0s}) represent 19.8 ± 2.3 % ($p < 0.0001$) of the total Cd activity of the oysters from the contaminated area and 12.7 ± 9.7 % ($p < 0.0001$) for those from the control estuary. The long-lived loss component was associated with the majority of the Cd accumulated at the end of the exposure period, i.e. 78.3 ± 1.8 % ($p < 0.0001$) and 86.1 ± 10.1 % ($p < 0.0001$) in the GE and the BB oysters, respectively. Depuration from this long-lived loss component, representing the firmly bound Cd, was very slow, resulting in a biological half-life of 198 and 495 d for the contaminated and control groups, respectively.

Comparison of the regression slopes obtained for the short-lived component indicated that the Cd elimination rate was significantly higher for the oysters living in the GE whereas no significant difference between the 2 groups of oysters was noted for the long-lived ^{109}Cd loss components.

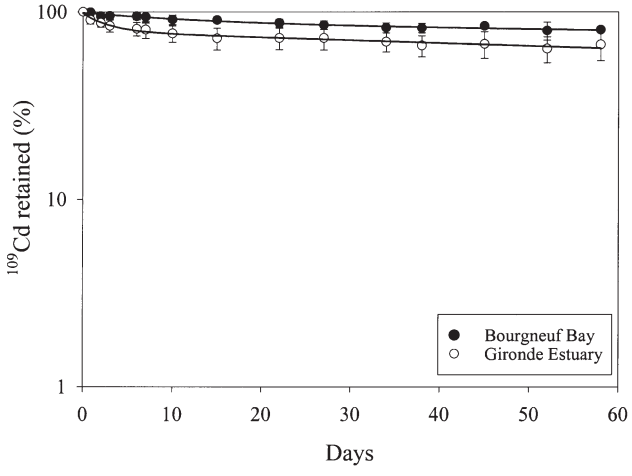

Fig. 4. *Crassostrea gigas*. Loss of ^{109}Cd in whole oysters from contaminated (Gironde Estuary) and clean (Bourgneuf Bay) areas following 15 d exposure to labeled food and seawater (average ± 1 SD individual loss kinetics, n = 10 individuals). Parameters for equation fitting the data are given in Table 3

Cadmium distribution among body compartments during depuration

Comparison of the relative radiotracer content (% Bq) in body tissues of *Crassostrea gigas* over the depuration period (Fig. 5) indicated that the Cd associated with shell, gills and pallial fluid represented a higher fraction of the total Cd accumulated in the GE oysters than in the BB oysters, with the latter containing a higher fraction of Cd in the soft parts. Moreover, the Cd distribution among body compartments did not remain constant throughout the loss experiment. The relative Cd content of the shell and the gills decreased with increasing depuration time, while the relative Cd content of the soft parts increased and that for the pallial fluid remained approximately constant.

DISCUSSION

Analysis of the biokinetics of heavy metals such as Cd using conventional methods in experiments is difficult to undertake at environmental levels of contami-

Table 3. *Crassostrea gigas*. Parameters and statistics of equations describing Cd loss kinetics for oysters originating from Gironde and Bourgneuf Bay areas: 2-component exponential model $A_t = A_{0s}\, e^{-\lambda st} + A_{0l}\, e^{-\lambda lt}$, where A_t = remaining activity (%) in oysters at time t (d), A_{0s} and A_{0l} = remaining activity (%) in oysters at time zero for short-term (*s*) and the long-term (*l*) components, R^2 = determination coefficient, k = rate constant (d^{-1}), $T_{b1/2s}$ and $T_{b1/2l}$ = biological half-life in days, and ASE = asymptotic standard error

Site	A_{0s} (ASE)	k_s (ASE)	$T_{b1/2s}$ (ASE)	A_{0l} (ASE)	k_l	$T_{b1/2l}$	R^2	p
Bourgneuf Bay	12.72 (9.69)	0.0647 (0.0557)	10.7	86.14 (10.06)	0.0014 (0.0019)	495	0.96	<0.001
Gironde Estuary	19.79 (2.28)	0.3410 (0.0941)	2.03	78.26 (1.84)	0.0035 (0.0007)	198	0.97	<0.001

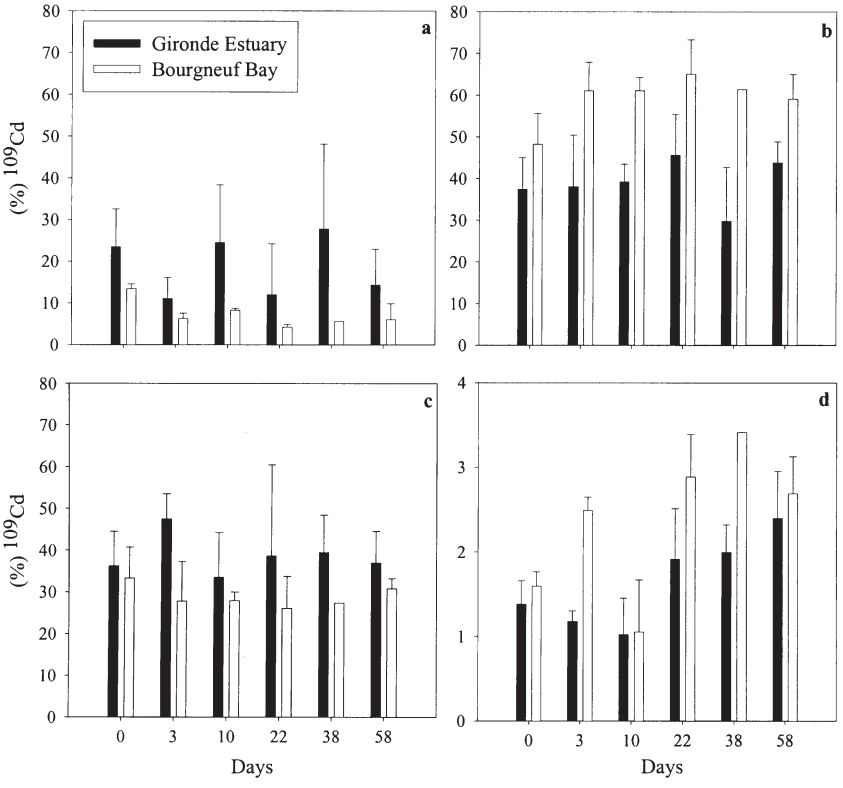

Fig. 5. *Crassostrea gigas*. Percentage of whole-body ^{109}Cd (Bq) retained in shell (a), soft parts (b), gills (c) and pallial fluid (d) over depuration period (mean % activity + 1 SD; n = 3 individuals)

nation because of the relatively low amounts of trace metals present. Moreover, organisms must be sacrificed to determine their Cd content and their Cd uptake kinetics. In contrast, the use of carrier-free radiotracer ^{109}Cd as a non-destructive method allows the uptake and depuration kinetics of a single individual to be followed over time at natural Cd levels, and also a more adequate assessment of the behavior of the Cd in oysters, by reducing the influence of inherent natural variability between different organisms (with dissections being performed as a complementary method for specifically determining intracellular Cd).

The fact that Cd was distributed in both the gills and soft parts indicates that both the dissolved and the food pathways contributed to the transfer of Cd to the oysters. The BAF had not approached a steady state by the end of the 15 d exposure period. The single-component first-order kinetic model describing the behavior of the BAF throughout the experiment showed that the estimated Cd BAF after 15 d exposure was 47% higher for oysters previously exposed to elevated levels of Cd in their natural environment than for oysters originating from an uncontaminated area. This suggests that the former had not developed a resistance to Cd contamination through a reduction in Cd permeability. Part of this additional Cd was associated with the shell which represented a significantly higher percentage of the total body burden than was the case for oysters from the control area. This was probably connected with the rough shell surface of the Gironde oysters, which offered a greater surface area for adsorption than the relatively smooth shell of oysters from the Bay of Bourgneuf. The other fraction of additional Cd was located in the gills: the previously contaminated oysters bio-concentrated 30% more Cd than the gills of oysters from the control area (although this difference was not significant due to a high SD). A similar linear Cd uptake pattern was reported by Lu et al. (1998) for the oyster *Crassostrea rivularis* experimentally contaminated by dissolved Cd. Furthermore, it has been demonstrated in some molluscs that Cd accumulation rates and body burdens are greater in organisms that produce metallothioneins than in those in which induction is weak (Langston & Spence 1995). Thus, our results in general suggest that oysters chronically exposed to environmental Cd contamination develope an adaptive mechanism through an enhanced ability to detoxify the metal internally.

In order to test this hypothesis of adaptive response, we chose the visceral masses of the oysters to examine the subcellular fractionation, since a predominant proportion of Cd was present in their soft parts. The highest percentage of Cd was found in the cytosolic fraction of oysters from both sites. The other fraction with a notable amount of Cd was the 'heavy' fraction, which contained the nuclei, heavy lysosomes and cellular debris. This latter fraction is comparable to the 'insoluble' fraction obtained by other authors using different methods (Mouneyrac et al. 1998, Geffard et al. 2001). In the BB oysters, the heaviest fractions—nuclei, heavy lysosomes and cellular debris or lysosomes and mitochondria—contained up to 47% of the total Cd.

In the cytosol of oysters from both areas, chromatographic analysis revealed an elevated fraction of Cd in the elution range of metallothioneins. Metalloprotein, ubiquitous in various organisms, is a cystein-rich protein which plays an important role in metal detoxification (Kägi & Vallee 1960, Binz & Kägi 1999), and has been well documented in oysters (Butler & Roesijadi 2001, Tanguy et al. 2001). The distribution of Cd between the metallothioneins and the lysosomal vacuolar

system has been described by other authors (George 1990, Viarengo & Nott 1993).

Chromatography of the cytosol of GE oysters indicated a low amount of Cd associated with the high molecular weight proteins. The presence of high molecular weight Cd-binding proteins in cytosols of contaminated marine animals was discussed by George (1982), who suggested that 'spill over' of Cd occurred when the binding capacity of metallothioneins was exceeded. Nevertheless, there is still some doubt about the origin of these high molecular weight proteins, because some may comprise an aggregation of metal-binding proteins or metallothionein dimers (Engel 1999).

Some Cd appeared to be associated with low molecular weight molecules in the cytosolic fraction of the BB oysters. This observation has been reported frequently for oysters (Coombs 1974, Frazier & George 1983, Fayi & George 1985, Isani et al. 2000). Fayi & George (1985) showed that these metal-binding components, with molecular weights lower than <2 kDa may be peptides such as Gly-Gly and Cys-Gly and some complexes of glutathion (GSH: γglutamyl-cysteinyl-glycine). According to Mason & Jenkins (1995), this component may be important during the first phase of contamination when the concentration of induced metallothioneins is still too low to have a protective effect.

In our study, we observed different proportions of Cd radiotracer between the 2 compartments (i.e. lysosomes and cytosolic metallothioneins) involved in Cd accumulation, depending on the origin of the oysters (contaminated or clean site). The abundance of this metal in the cytosol of GE oysters at the end of the exposure period could be attributable to the presence of pre-existing metallothioneins induced by chronic Cd contamination of the estuarine environment. For the BB oysters, constitutive forms of Zn metallothioneins (Engel 1999) could be present. These metallothioneins are necessary for the regulation of the high levels of Zn naturally present in feral oysters (Roesijadi 1996). Since Cd shows a greater affinity for metallothionein SH-groups than zinc (Geret & Cosson 2000), it is possible that Cd replaces Zn in the metallothioneins during contamination (Viarengo et al. 1985, Viarengo & Nott 1993). This would explain the relatively high content of ^{109}Cd in the cytosol of the BB oysters after experimental contamination. In the lysosomes of the BB oysters, the higher percentage of Cd could result from a more efficient lysosomal system compared to the GE oysters. The presence of the contaminating metal in the environment may have modified the latter's lysosomal physiology, since heavy metals are known to destabilize the lysosomal membranes and disrupt the hydrolytic activity of lysosomal enzymes (Regoli 1992, Viarengo & Nott 1993).

When contaminated individuals were transferred to clean seawater, the 2 groups of oysters displayed 2-compartment, exponential depuration kinetics. In the short-lived compartment, which contained only 13 to 20% accumulated Cd, the Cd was rapidly eliminated in a few days and corresponded to the fraction adsorbed to shell, gills or digestive epithelium after exposure to contaminated water. The significantly higher depuration rate in the short-lived loss component, observed for previously contaminated oysters, could be due to Cd desorption from the rugose shell, which represented a significantly higher percentage of the Cd body burden than in oysters from the uncontaminated area.

Based on the results of the subcellular fractionation, the component representing the major portion of accumulated Cd (78 and 86% with a biological half-life of 6 mo and 1.5 wk for oysters originating from the contaminated and clean areas, respectively) corresponded to the Cd bound to induced ligands (e.g. metallothioneins) or to lysosomes. Since Cd in the soluble fraction of oysters is more easily eliminated than the insoluble form (Roesijadi & Klerk 1989, Geffard et al. 2002), the higher Cd turnover observed for the GE oysters is probably a consequence of the higher percentage of cytosolic Cd, a finding which indicates the presence of an adaptive response in oysters to chronic Cd contamination of marine waters.

The distribution of Cd between the lysosomes and the cytosolic metallothioneins could also affect the Cd elimination rates observed for the long-term compartment of loss. Such a mechanism has been hypothesized by Engel (1999), but the elimination process differs according to the metal concerned. During copper contamination, some experiments have shown a transfer of copper from metallothioneins to lysosomes whereby the metal was trapped by the lipofuschins and subjected to exocytosis (George 1990). On the other hand, the transfer of Cd from metallothioneins to lysosomes does not induce an exocytosis mechanism. The Cd metallothioneins (which are unstable in the acidic intralysosomal environment) are destroyed, and the metal returns to the cytosol, where it is again trapped by the newly synthesized metallothioneins. In this manner, the toxicity of Cd is neutralized although the metal remains inside the cell (Viarengo & Nott 1993).

The presence of rapid and slow Cd elimination processes is comparable to the results obtained by Geffard et al. (2002), who studied Cd elimination in the same species of oysters translocated from the metal-enriched GE to a clean site in the BB. The authors, who examined depuration from soft parts of oysters, observed only a single long-term compartment of Cd elimination with an estimated half-life of 137 d, a value similar to 198 d recorded in our study. Never-

theless, in our study the use of a radiotracer demonstrated the presence of a short term compartment of Cd elimination corresponding to the fraction adsorbed to oyster shell. In another depuration study carried out in the field, Van Dolah et al. (1987) recorded a biological half-life of 150 d for Cd in the soft tissues of *Crassostrea virginica*, a value which is also in agreement with our data.

Exposure to chronic Cd contamination in the marine environment appears to induce a degree of resistance to Cd in the oyster *Crassostrea gigas*. From the results obtained at the organism and tissue level, the potential resistance mechanism involved cannot be attributable to a reduction in Cd permeability but rather to a higher Cd turnover. Furthermore, the results obtained at the subcellular level revealed a significant amount of Cd in the cytosolic fraction, in particular associated with metallothioneins, indicating that Cd is complexed. However, a significant amount of Cd was also found in the insoluble fraction (termed herein the 'heavy fraction'), mainly associated with the lysosomes. Therefore, through chelation, lysosomes, in parallel with metallothioneins, may be responsible for the reduction in bioavailability and toxicity of Cd in oysters. Among the differences observed between the 2 populations of oysters is a more rapid response to Cd contamination in oysters previously exposed to chronic contamination in the marine environment; this is due to the presence of pre-existing metallothioneins induced by the elevated Cd in the contaminated estuarine environment, whereby no essential modification of the metabolic Cd pathway occurs. In addition, the fact that 30 to 40% of the soluble Cd (which represents 40 to 60% of the total Cd) was detoxified by the Gironde oysters through chelation to metallothioneins or to lysosomes. This would result in approximately 12 to 24% of the total Cd accumulated being potentially bioavailable to humans consuming oysters. However, through depuration processes, Cd is also more efficiently eliminated from the soft parts (edible parts) of oysters previously exposed to Cd than from control oysters. Therefore, the manner in which regulatory thresholds of Cd in oysters are presently calculated should be reconsidered, by taking into account the amount of Cd already detoxified by the oysters through complexation process.

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