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Bioavailability and toxicity of sediment-bound lead to a filter-feeder bivalve *Crassostrea gigas* (Thunberg)

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Two different approaches were used to study the bioavailability of sediment-bound lead. *In vitro* techniques simulating the potential metal desorption under conditions prevailing in the digestive tract were assayed on a contaminated sediment. An experimental model of a food chain was designed to determine the retention of lead in the soft tissues of oysters according to the environmental source of the metal (water or sediment). Neither enzymatic action nor leaching at low pH (both aspects of digestion) induce the release of important lead amounts from particles. Therefore, after 3 weeks of exposure, the retention of lead from the trophic source is lower (1%) compared with direct contamination (5%). Lysosomes are the major intracellular structures responsible for lead storage in the gills, digestive tract and digestive gland. The abundance of lysosomes and their lead amount vary according to the gross concentrations in the soft tissues. The cytopathological data are in agreement with the results about lead accumulation: in oysters exposed to sediment-bound lead, impairments are not so marked as in individuals contaminated directly from water but the same organelles are concerned. Mitochondrial impairments may be related to the effect of lead on cellular respiration processes and changes involving the granular endoplasmic reticulum may have an effect on the level of protein synthesis. Cellular extrusions carrying away numerous lysosomes loaded with lead could account for the balancing of lead incorporation between 2 and 3 weeks of exposure.

Keywords: lead, suspended sediment, oyster, bioavailability, cytopathology, electron probe microanalysis

Introduction

In the aquatic environment, a great number of pollutants are bound to sedimentary particles. Among metals, lead exhibits a high affinity towards clay minerals and organic substances (Förstner 1979).

The contamination of sediments represents a possible source of stress to the benthic biota. Indirect evidence of pollutant exchanges between sediments and organisms has been provided by several studies in which aquatic organisms were exposed to sediments contaminated in environmental situations (Pavillon 1990, Krantzberg & Boyd 1992, Tay *et al.* 1992, Burgess *et al.* 1993). To characterize sources of toxicants, sediments have been chemically manipulated. The results evidenced the contribution of trace metals to sediment toxicity (Krantzberg & Boyd 1992, Burgess *et al.* 1993).

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The toxicity associated with contaminated sediment may be introduced into the water column or pore water (Giesy *et al.* 1990, Burgess *et al.* 1993). It is known that chemical substances have a biological activity only when soluble (Jouany 1991). However, a sedimentary particle ingested by a benthic organism is submitted to digestion processes, including pH changes and the action of a great variety of digestive enzymes (Lebesnerais 1985). Benthic organisms (molluscs, crustaceans, echinoderms, fishes) are capable of transforming the crystal structure of various sediment components (Anderson *et al.* 1958, Pryor 1975, Syvitski & Lewis 1980). In addition, a portion of the particle-bound organic matter is digested and any associated pollutant may be released in the gut. Thereafter metals, initially bound to sediment, may give rise to the various uptake mechanisms through membranes.

The toxicity that contaminated sediment may introduce through metal transfer in the food webs including filter-feeders has not been examined extensively. Furthermore, the benthic species affected (oysters, mussels) in areas such

as estuaries and coastal zones which are particularly at risk for metal contamination (Bryan 1984) are often interesting from the economic point of view.

Pollutant transfer through the particulate phase has given rise to several studies (Schultz-Baldes 1974, Lee *et al.* 1975, Kirchmann *et al.* 1977, Terhaar *et al.* 1977, Nakahara & Cross 1978, Amiard 1979, Ballester & Castellvi 1979, Hamdy & Prabhu 1979, Mann *et al.* 1979, Dahlgaard 1981, Borchardt 1983, Flatau & Gauthier 1983, Harvey & Luoma 1985, Abbe & Sanders 1988, Martoja *et al.* 1988, Amiard *et al.* 1989), but in most cases the particulate phase consisted of phytoplankton or bacteria, excluding sedimentary particles. In filter-feeders, the importance of metal accumulation through direct uptake is due to the fact that metal quantities available from water are considerably higher than those associated with particles (an oyster filters several liters of seawater per hour whereas particle concentrations higher than 3–4 $mg\,l^{-1}$ are sufficient to induce the formation of pseudo-feces). In the short term, this difference at the level of the source induces a higher incorporation of metals from the dissolved phase than from the particulate phase. The data about copper accumulation in oysters (*Crassostrea gigas*) through direct uptake (seawater) or via sedimentary particles are in agreement with these general findings (Ettajani *et al.* 1992). However, the studies revealed that the percentage of copper retained from suspended particles fed to oysters (70%) was always higher than the fraction incorporated from the dissolved phase (39%). Thus the food pathway, and more precisely the sedimentary phase, could play a significant role in the accumulation of metals in oysters, but only as a consequence of chronic exposure (Amiard-Triquet *et al.* 1988, Ettajani *et al.* 1992). This seems to be in agreement with a field study by Kamimura (1980). The results of these investigations suggest that copper and zinc accumulation in food substances is one of the major factors influencing trace element levels in oysters.

The purpose of this study is (1) to predict the bioavailability of sediment-bound lead by using *in vitro* techniques to simulate the potential metal desorption under conditions prevailing in the digestive tract as recommended by Amiard (1992), (2) to validate these assumptions by determining lead transfer in an experimental model of a food chain as proposed by Amiard-Triquet *et al.* (1993) and (3) to examine the potential cytopathological effects associated with additional bioaccumulation.

Materials and methods

Pre-treatment of the sediment

Mud has been sampled in a coastal area devoted to oyster culture (Bay of Bourgneuf, France) and the finest particles were selected by using elutriation in order to fulfil the feeding requirements of oysters (Razet *et al.* 1990, Ettajani & Pirastru 1992). This sediment was then submitted to overloads of lead introduced in seawater (70 $\mu g\,l^{-1}$ as $Pb(NO_3)_2$ in nitric solution 0.5 $mol\,l^{-1}$). The ratio between the particulate and the dissolved phases was 1 $g\,l^{-1}$, a realistic value compared with the average turbidity determined in the Bay of Bourgneuf (0.224 $g\,l^{-1}$). Preliminary experiments have shown that no additional accumulation occurred between 24 and 48 h of contact. Thus the definite experiment was limited to 24 h. In order to avoid particle deposition able to decrease the contact with lead, experimental units were shaken permanently. Then seawater and residual lead were eliminated after centrifugation and the pellets were rinsed for 1 h. After a second centrifugation, the pellets were distributed into three groups devoted (1) to lead determination by atomic absorption spectrophotometry (AAS), (2) to desorption tests and (3) to be used as a vector of transfer in an experimental model of a food chain.

Desorption tests

The major enzymes involved in the digestion processes in molluscs are α-amylase, pepsin, lysozyme, peptidase, and acid and alkaline phosphatase (Boucaud-Camou *et al.* 1985, Lebesnerais 1985, Deslous-Paoli 1987). The sediment contaminated with lead was put into contact with enzymes at pHs identical to those at which they intervene *in vivo* (pepsin, pH 4; lysozyme, peptidase and acid phosphatase, pH 5.5; alkaline phosphatase and α-amylase, pH 6.8). Mammal enzymes (Merck-Clévenot, Sigma) were used to mimic the digestive activity. The different enzymes which have been shown to be active at the same pH were tested concomitantly according to two procedures: independent tests in which different samples of sediment were submitted to each pH and corresponding enzyme mixture, and sequential tests in which the same sample of sediment was submitted successively to the different enzymes according to increasing pHs.

In each experimental unit, 1 mg of each enzyme was added to 50 mg of sediment dispersed into 2 ml of buffer (acetic acid 1%). The required pH was then adjusted by adding 5 N NaOH. The digestion time was 2 h. Since oysters are poikilothermic organisms, enzymatic attacks have been carried out at ambient temperature. At the end of each independent test or each step of sequential tests, sediments were centrifuged, rinsed with the buffer at convenient pHs and centrifuged again. In order to distinguish the relative importance of physiological pH influence and enzymatic processes, desorption tests were carried out concurrently in the absence of enzymes.

Moreover desorption tests have been carried out with contaminated sediment dispersed in weakly-concentrated acid (50 mg in 2 ml 0.1 N HCl).

Under each experimental condition, the tests were carried out in duplicate. At the end of the experiments, the pellets were prepared for lead analysis by AAS.

Experimental model of a food chain

The experimental model was used previously to study the bioaccumulation of silver, copper and lead in filter-feeding

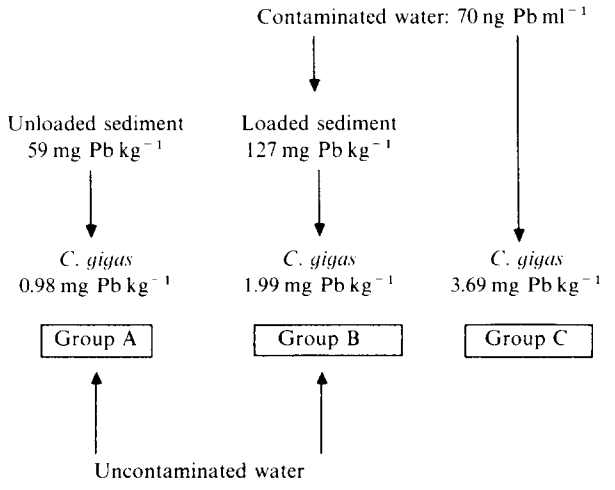
molluscs through the particulate phase: phytoplankton (Martoja *et al.* 1988, Amiard *et al.* 1989) and suspended sediment (Ettajani *et al.* 1992).

In the present study, young oysters (5 months, average dry weight of soft tissues <200 mg) were exposed to lead dissolved in natural seawater or loaded to particles as described above. Controls (Group A, Table 1) were reared in uncontaminated seawater and each individual received about 3 mg of uncontaminated sediment five times a day. Oysters of Group B were provided with identical quantities of contaminated sediment. In the case of direct contamination the overloads of lead introduced into the rearing medium of oysters were similar to those used to contaminate sediment ($70\,\mu g\,l^{-1}$). Each group was distributed into several experimental units with 10 specimens in 10 l of seawater. Seawater and contaminant were renewed daily. Under these conditions, the measured lead concentration was at least 10% lower than the nominal concentration (Amiard-Triquet *et al.* 1981). One hour before the renewal mentioned above, all the oysters were provided with freeze-dried *Spirulina*.

Thirty oysters were sampled from each experimental group after 2 and 3 weeks. Their soft tissues were prepared for lead analysis by AAS. At the end of the experiment, several factors were determined for each specimen in order to calculate the condition index: dry weight of soft tissues/total weight minus shell weight (Lawrence & Scott 1982). Additional oysters were sampled for cytopathological and microanalytical studies.

The transfer parameters of the experimental model have been calculated as described earlier (Martoja *et al.* 1988). In the case of direct uptake, the overload of lead, introduced and available daily to each oyster, is $70\,\mu g$ since the experimental density was one oyster per liter. The additional quantity of lead ingested with contaminated suspended particles was determined over a finite period (2 or 3 weeks) after abatement of lead quantities present in uncontaminated sediment. Since the contaminated sediment had been rinsed for 1 h, the release of lead in the rearing medium of oysters during the short stay of particles before ingestion is negligible. Using the quantities of lead accumulated by oysters for each exposure period (minus environmental quantities measured in controls), it was possible to calculate the percentage of the metal retained and thus to evaluate the transfer to oysters (see below, Table 5).

Table 1. Influence of the way of contamination on the lead concentrations in oysters *Crassostrea gigas* at the end of the experiment (21 days) - data based upon dry weight



Trace element analysis at the global level (AAS)

Sedimentary particles of known dry weight were heated (95°C) with HNO_3. The non-digested residue was eliminated and the total volume was adjusted to 4 ml with deionized water. Soft tissues of individual oysters were dried (85 C) and then digested with acid as described for the sediment.

Lead was analyzed in these solutions by AAS according to the method described by Amiard *et al.* (1987) and validated by international intercalibration exercises (IAEA, 1987, 1988).

Microscopy and microanalysis

Only the oyster target organs for metal sequestration and detoxification were chosen for examination. The gills, digestive gland and digestive tract were fixed in 3% glutaraldehyde in a 0.2 M sodium cacodylate buffer (pH 7.4), with 0.35 M sucrose added for 1.5 h at 4°C. For ultrastructural studies, the samples were post-osmicated in 2% osmium tetroxide in H_2O for 1 h, while for the electron probe microanalysis (EPMA) studies, post-osmication was omitted. The samples were dehydrated in ethanol and embedded in Epon-Araldite.

Ultrathin sections stained with uranyl acetate and lead citrate were examined in a Phillips 201 at 80 kV.

EPMA was carried out with a CAMECA MBX (Camebax) equipped with a TEM and wavelength dispersive spectrometers (WDS). The probe was about 500 nm diameter for a beam intensity of 150 nA at 45 kV. The following monochromator crystals were used: TAP (thallium acid phthalate) for phosphorous, aluminium and silicon; PET (penthaerythritol) for sulphur; and lithium fluoride for lead, zinc, copper and iron.

For each beam area and for each element, intensities were measured at the peak and at the background for 100 s counting periods. The intensity of each element was calculated as $N_c = \overline{N_p} - \overline{N_b}$ where $\overline{N_p}$ is the mean of two values, expressed as the number of impulsions obtained for the peak line, and $\overline{N_b}$ the mean value between the left and the right background. The intensities were retained only in the case of significant values (confidence interval $N_c \pm 2\sigma$, with a standard deviation $\sigma = \sqrt{\overline{N_p} + \overline{N_b}}$). In this case, we consider that intensity variations mean concentration differences. The EPMA was carried out on 20 points for each organ and each experimental condition.

Results

In vitro *study of lead desorption*

At the pH conditions prevailing in the gut, some desorption of lead loaded to sediment was observed. The desorption of lead was not evidenced at the highest pH assayed (6.8) in independent tests (Table 2). In these tests, no significant desorption occurred in the sediment samples submitted to enzyme mixtures at pH 5.5 and 6.8. In the case of sequential tests, the desorption due to the pepsin activity at pH 4 did not increase during the next steps of the experiment (Table 2). It is somewhat surprising that in several cases the highest desorption was observed in samples submitted to the pH effect alone rather than to the combined effect of pH and enzymes.

In sediment samples submitted to 0.1 N HCl, a fraction as high as 87% of lead initially loaded to sediment was released (Table 3).

Lead transfer in the experimental model of a food chain

At the end of the experiment (3 weeks) the concentrations reached in oysters exposed through water were considerably higher than those determined in specimens fed with sedimentary particles (Table 1). In the first case, the concentration factor (concentration in oysters, mg Pb kg^{-1}/concentration in water, mg Pb l^{-1}) was 5271, whereas the contamination through the particulate phase led to a concentration factor (concentration in oysters, mg Pb kg^{-1}/concentration in the contaminant medium of particles, mg Pb l^{-1}) as low as 28.

The lead body burdens at the different steps of the experiment are shown in Table 4. They increased in oysters exposed to sediment-bound lead and after 2 weeks they had doubled compared with the controls. They did not increase between the second and the third week of exposure. In the case of a direct exposure, a slowing down of uptake was also observed between 2 and 3 weeks.

The transfer parameters are shown in Table 5. Whatever the way of transfer may be, the oysters retained only a small fraction of lead: 1–5% over the first 2 weeks of exposure. These percentages decrease later on since the additional bioaccumulation between 2 and 3 weeks was practically negligible.

The intracellular localization of lead was examined at the end of the experiment (Figure 1). In controls, lead was sometimes detected by EPMA in the studied organs at low concentrations near the input signal threshold. The intracellular structures involved in lead storage were lysosomes. In oysters exposed to soluble lead in water, the

Table 2. Residual lead concentrations ($\mu g\ g^{-1}$) in sediments submitted to desorption tests [initial concentration in untreated sediment: 127 (8) $\mu g\ g^{-1}$]

Experimental conditions	Independent tests	Sequential tests
pH 4	103 (2)	103 (2)
pH 4 + pepsin	107 (1)	107 (1)
pH 5.5	108 (2)	88 (8)
pH 5.5 + acid phosphatate, lysozyme and peptidase	130 (0)	106 (7)
pH 6.8	131 (6)	95 (11)
pH 6.8 + α-amylase and alkaline phosphatase	126 (6)	108 (2)

Mean and SD.

Table 4. Lead accumulation in oysters exposed through water and sedimentary particles and associated condition index

Length of exposure (weeks)	Control	Experimental conditions Uptake from sediment	Direct uptake
	Average lead body burden (ng)		
0	105 (11)	–	–
2	123 (13)	244 (21)	54 906 (5735)
3	125 (14)	231 (18)	55 512 (4897)
	Condition index		
3	58 (2.6)	60 (2.3)	57 (2.5)

Mean and SD.

Table 3. Comparative desorption of sediment-bound lead according to the conditions of contamination and the initial lead concentrations in the suspended sediments

Conditions of contamination	Initial lead concentration ($\mu g\ g^{-1}$)	Desorption (%) due to 0.1 N HCl	Source
Elutriated sediment + artificial overload			
2.5 $\mu g\ l^{-1}$	48	0	Ettajani, unpublished
20 $\mu g\ l^{-1}$	60	0	Ettajani, unpublished
70 $\mu g\ l^{-1}$	127	87	present study
Natural conditions			
Loire estuary, France			
Spring	120	60	Ettajani, unpublished
Summer	96	68	Ettajani, unpublished
Sepetiba Bay, Brazil	139	67	De Lacerda *et al.* 1987

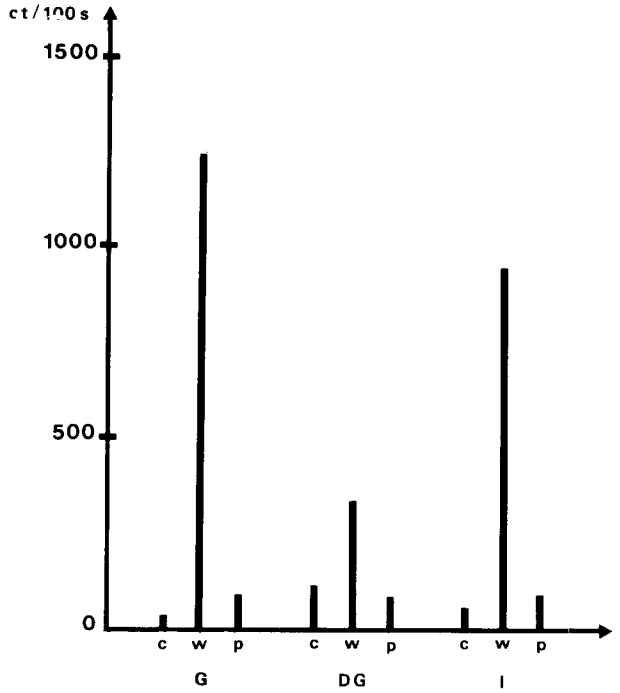


Figure 1. Intensity scales obtained for lead on 20 points of analysis for gills (G), digestive gland (DG) and intestine (I) from the control (c), the water experimental group (w) and the particulate experimental group (p) during 100 s of counting.

Table 5. Parameters of the experimental model of a food chain used in the study of lead transfer

Way of exposure	Length of exposure	
	2 weeks	3 weeks
Lead burden (μg) in soft tissues of a standard oyster		
1. Particulate phase	0.116	0.110
2. Direct uptake	53.335	54.871
Lead overload (μg) available to a standard oyster from seawater		
3. Direct uptake	980	1470
Lead overload (μg) ingested with contaminated sediment		
4. Particulate phase	11.3	17.0
Percentage of lead at disposal accumulated in soft tissues		
5. Particulate phase	1.03	0.65
6. Direct uptake	5.44	3.73

amounts of this metal increased considerably in lysosomes of the three analyzed organs, especially in the gills and the digestive tract. After exposure to sediment-bound lead, the metal amounts in individual lysosomes were not higher than in controls but lead was detectable in more numerous lysosomes. In both controls and contaminated oysters, other elements such as iron, zinc, copper, nickel, aluminum, silicon, phosphorous and sulphur were stored concomitantly with lead in the lysosomes. The presence of these elements was not observed in any other cellular structure.

Biological effects of lead

No mortality occurred during the experiment. The condition index (Lawrence & Scott 1982) was not modified significantly (F-test of Snedecor) in contaminated individuals (Table 4).

Cytopathological features were observed in different organs.

Gills. In comparison with controls (Figure 2), the various cellular types of the gill epithelium showed ultrastructural damage in oysters exposed to lead through water, especially in the latero-frontal cells. In the apical area, the plasma membrane was occasionally disrupted in some cells, whereas microvilli disappeared in some cases and apocrine extrusions were observed (Figure 3). Sometimes cytoplasmic regions appeared completely devoid of organelles. Other cells

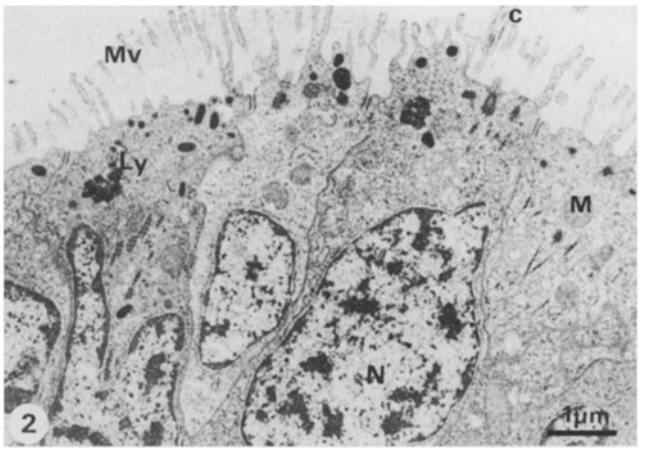


Figure 2. Gill epithelium of control oyster. The lysosomes (Ly) are loaded with various elements (Ca, Zn, Cu, Fe, P and S). ×9240. C = cilia, Ly = lysosomes, M = mitochondria, Mv = microvilli, N = nucleus.

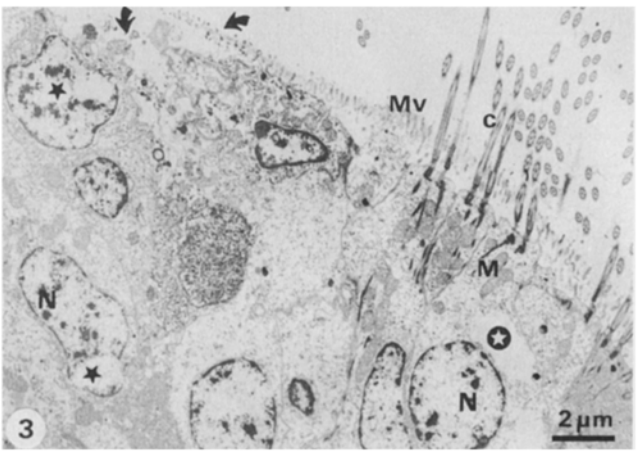


Figure 3. Gill epithelium of oyster exposed to lead through water. Ultrastructural damages occur at the apical area (arrows) and in the cytoplasm or nucleus which are vacuolized (stars). ×3960. C = cilia, Ly = lysosomes, M = mitochondria, Mv = microvilli, N = nucleus.

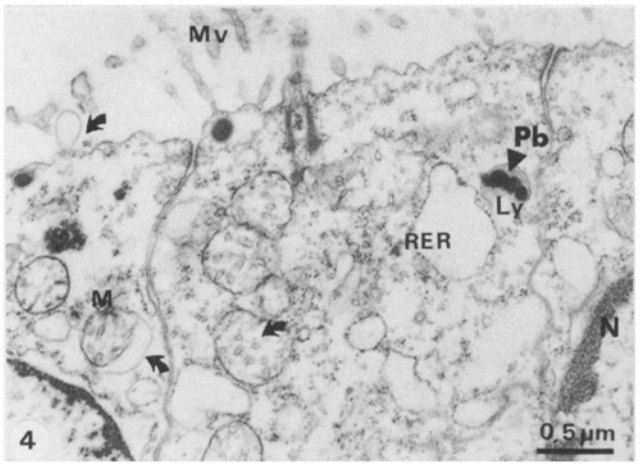


Figure 4. Apical area of an epithelial gill cell. Oyster exposed to lead through water. The mitochondria are deprived of cristae and microvilli disappear in some cells (arrows). In the lysosomes, lead is significantly detected. × 19 800. Ly = lysosomes, RER = vacuoles of the rough endoplasmic reticulum, N = nucleus, M = mitochondria, Mv = microvilli.

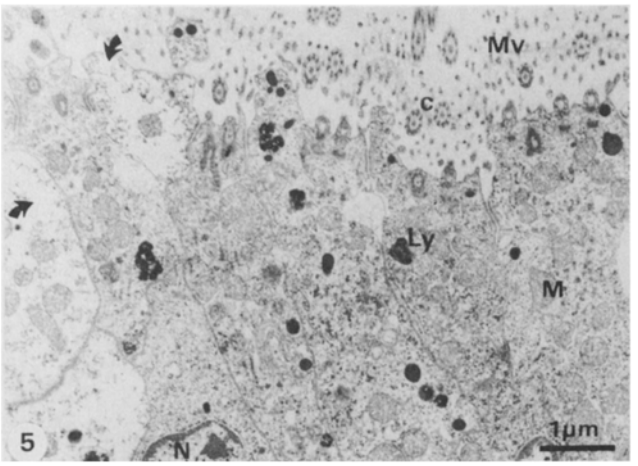


Figure 5. Gill epithelium of oyster exposed to sediment-bound lead. Some cells are well preserved, but in others the hyaloplasm appears, especially in the apical area (arrows). × 9240. C = cilia, Ly = lysosomes, M = mitochondria, Mv = microvilli, N = nucleus.

contained mitochondria partially or entirely deprived of cristae, and some nuclei exhibited a vacuolized nucleoplasm (Figures 3 and 4). The nuclear envelope was often swollen like the endomembraneous system, e.g. the rough endoplasmic reticulum (RER). In the well-preserved cells, in which the organelles (mitochondria, RER, nuclei) were similar to controls, numerous lysosomes contained electron-dense microgranules in which lead was detected (see Figure 1).

In oysters exposed to sediment-bound lead, the gill epithelium was generally more preserved than after contamination through water. Nevertheless, some cells were damaged (Figure 5), especially at the plasma membrane level, where the microvilli sometimes completely disappeared. Moreover, these cells showed apical extrusions of cytoplasmic areas devoided of organelles (Figure 5). The cytoplasm was lacunar. In spite of the high number of endosomes (Figure 6), endocytotic processes of particles were never observed. The quantity of lysosomes did not increase and their granulous content (Figure 6) was similar to that observed in controls (Figure 2).

Digestive tract. Compared with controls (Figure 7), in oysters exposed to lead through water, the intestinal epithelium showed a big swelling of apical areas of the cells, frequently extruded in the lumen (Figure 8). In the damaged cells, the cytoplasm was lacunar and many of the mitochondria had lost cristae and showed a clear matrix (Figure 9). Other cells were well preserved and exhibited numerous lysosomes with electron-dense content (Figure 8) in which significant amounts of lead were detected, as mentioned above (Figure 1).

In oysters exposed to sediment-bound lead, the intestinal cells were either well preserved or damaged. In the latter, the whole organelles were often extruded in the intestinal

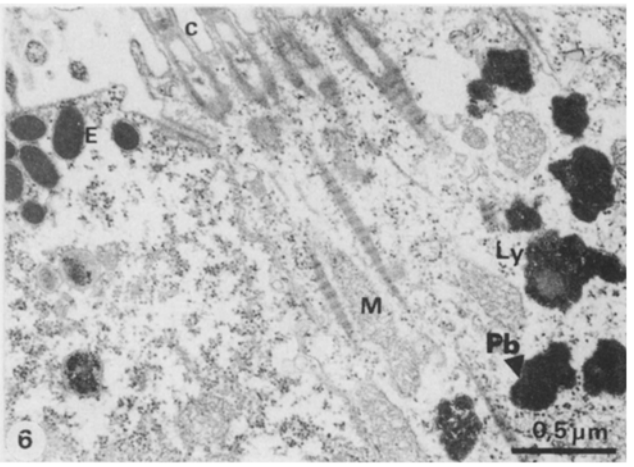


Figure 6. Apical area of an epithelium, gill cell of oyster exposed to sediment-bound lead. In the granulous content of the lysosomes (Ly), lead is scarcely detected. × 26 400. C = cilia, E = endosomes, M = mitochondria.

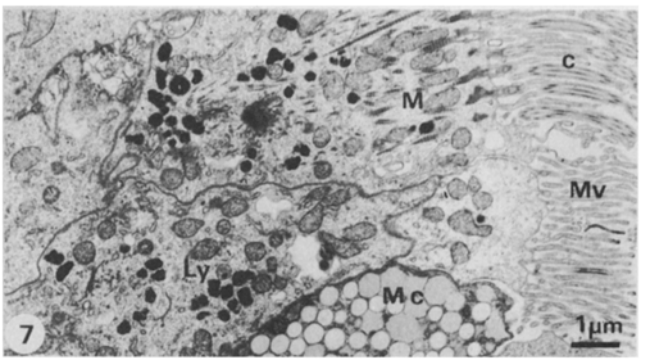


Figure 7. Cells of the digestive tract. Control oyster. The numerous lysosomes store various elements (Ca, Zn, Cu, Fe, P and S). × 5940. C = cilia, Ly = lysosomes, M = mitochondria, Mc = mucous cells, Mv = microvilli.

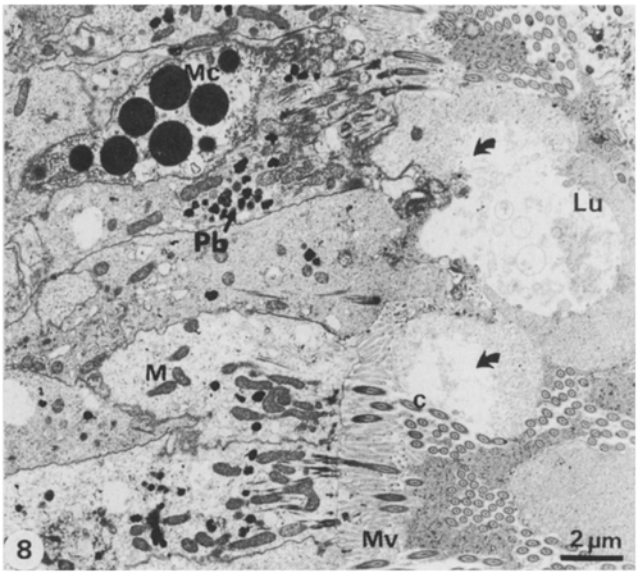


Figure 8. Intestinal epithelium. Oyster exposed to lead through water. Note the swelling of apical cells areas (arrows), extruded in the lumen (Lu). In the lysosomes, lead is detected significantly. × 3960. C = cilia, Mc = mucous cells, Mv = microvilli.

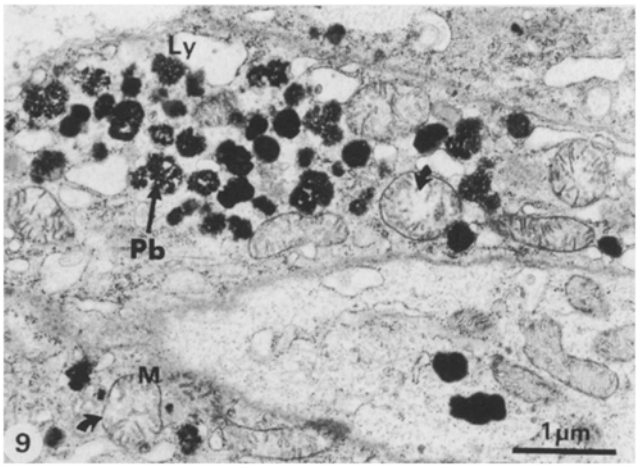


Figure 9. Apical area of an intestinal cell. Oyster exposed to lead through water. In the damaged cells, mitochondria loose cristae (arrows) and show a clear matrix. The lysosomes (Ly) store significant amounts of lead. × 13 200. M = mitochondria.

lumen and the cytoplasm looked empty (Figure 10). In other cells, mitochondria showed desorganized cristae in a clear matrix. Apical extrusions were scarce, in comparison with oysters exposed to soluble lead in water. EPMA detected lead in some lysosomes of the undamaged cells. No endocytotic processes involving sedimentary particles provided to oysters as food were noticed at the ultrastructural level of the intestinal epithelium.

Digestive gland. In oysters exposed directly to lead in water, the tubules of the digestive gland did not show ultrastructural damage compared with controls (Figure 11), except at the level of cellular apex which had lost microvilli (Figure 12). EPMA showed that the digestive vacuoles contained microgranules in which lead (Figure 1) was associated with the elements (Zn, Cu, Fe, Ni, Al, Si, Ca, P and S) usually stored in controls.

In oysters exposed to sediment-bound lead, the cytological aspect of the digestive tubules was the same as in oysters exposed to soluble lead in water. Nevertheless, the number of semi-empty digestive vacuoles was increased, as was the quantity of mucus secretion in the tubule lumen. Only lead traces were detected in digestive vacuoles (Figure 1).

Discussion and conclusion

The *in vitro* study suggests that during digestion the effect of pH changes is more important than the effect of enzymes upon lead release from the gut content of bivalves. Some

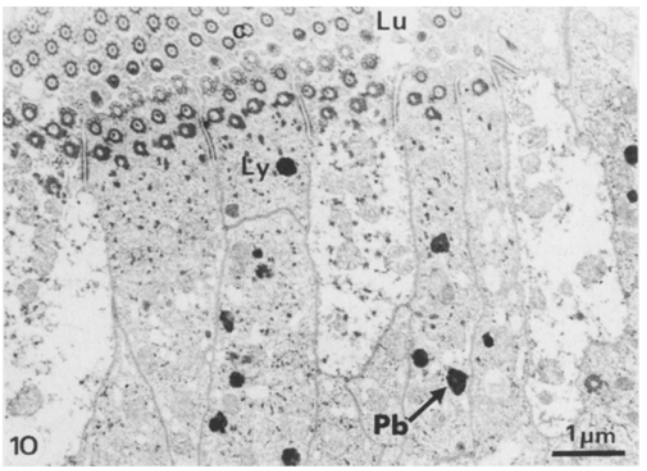


Figure 10. Digestive tract of oyster exposed to sediment-bound lead. Numerous cells are well preserved and contain lysosomes (Ly) in which lead is detected. In other cells, the hyaloplasm and the whole organelles are extruded in the intestinal lumen (Lu). × 9240. C = cilia.

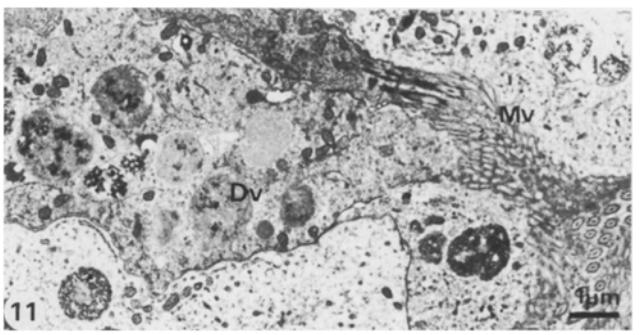


Figure 11. Digestive gland of control oyster. The digestive vacuoles contains various elements (Ca, Zn, Cu, Fe, Ni, Al, Si, P and S). × 6070. Dv = digestive vacuole, Mv = microvilli.

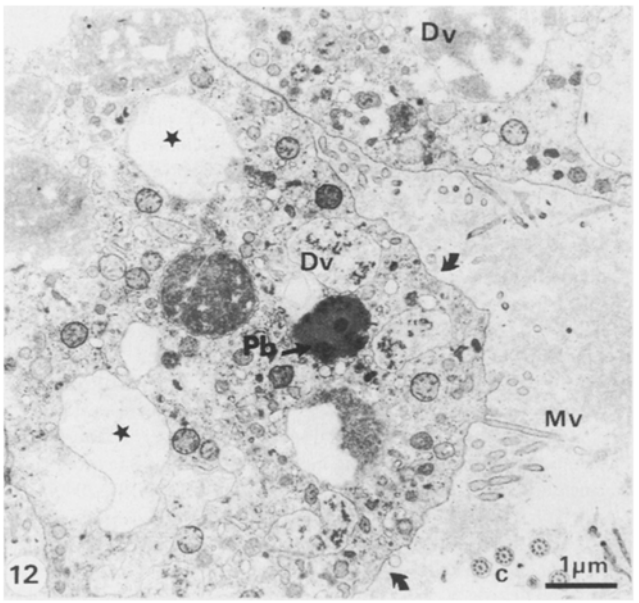


Figure 12. Digestive gland of oyster exposed to lead through water. Note the disappearance of microvilli at the cellular apex (arrows). The digestive vacuoles sometimes look empty (stars), but lead is detected in others. ×9240. C = cilia, Dv = digestive vacuole, Mv = microvilli.

cases of antagonism have been even noted between these factors. It may be hypothesized that the cleavage of some sediment constituents due to enzyme activities would offer new sites of binding to lead released as the consequence of pH changes.

When the sediment of the Bay of Bourgneuf was submitted to overloads of lead as high as 70 $\mu g l^{-1}$ of seawater, the subsequent release in 0.1 N HCl reached 87%. With lowest overloads, no significant desorption occurred (Table 3). In natural environments submitted to chronic pollution, lead concentrations in sediments were similar to those determined experimentally in the present study: under these conditions, important release was induced in the presence of 0.1 N HCl (Table 3). For copper, it has been shown previously that the higher the overloads, the easier was desorption, even by simple rinsing with clean seawater (Ettajani *et al.* 1992).

Even with 0.1 N HCl, desorption is considerably increased in comparison with release observed in experiments designed to mimic the conditions of digestion in bivalves. Thus, the biological significance of leaching with HCl solution as concentrated as 1 N is highly questionable, even if correlations between extractable lead and marine bivalves have been shown in some cases (e.g. Luoma & Bryan 1978, Bourgoin *et al.* 1991).

The results obtained with the experimental model of a food chain about lead transfer from sediment to oysters are in agreement with the data obtained *in vitro*. Neither enzymatic action nor leaching at low pHs (both aspects of digestion) induce the release of important amounts of lead from particles. Thus the retention of lead from the trophic source is relatively less important (1%) compared with direct contamination (5%), which is contrary to previous observations for copper (Ettajani *et al.* 1992). When the particulate phase consisted of phytoplankton, the relative accumulations of lead from both sources were approximately equal (Schultz-Baldes 1974).

The cytopathological data are in agreement with the results about lead accumulation at both the cellular and organism levels: in oysters exposed to sediment-bound lead, impairments are not so marked as in individuals contaminated directly by soluble lead in water; however, in both experimental populations, the same organelles are concerned. In the latter, both organelle impairments and cellular extrusions occurred in both gill and intestine epitheliums. In well-preserved cells, the storage of exogenous lead in the lysosomal system is particularly effective and probably insures the accumulation of an important part of the total lead detected in whole soft tissues. This is in accordance with previous studies showing the storage in the lysosomal system of both exogenous elements (cadmium and silver) and essential metals (copper, iron, nickel and zinc) (Jeantet *et al.* 1985, Martoja *et al.* 1988). Notably in the digestive tract, numerous lysosomes loaded with lead are often carried away on the occasion of cellular apex extrusion. This phenomenon could lead to a depuration of the toxicant and would explain the fact that no supplementary accumulation occurs in the whole soft tissues after 2 weeks of exposure. Since lead is absent in hemocytes, the mechanism of regulation of the body burden involving the rejection of loaded hemocytes which has been demonstrated for silver (Martoja *et al.* 1987, Berthet *et al.* 1990) is not functional for lead.

In a previous experiment, higher levels of soluble lead (200 compared to 70 $\mu g l^{-1}$ in the present study) associated with copper overloads had induced only low cytopathological effects. This apparent discrepancy could result from metal interactions such as the binding of lead by copper-thioneins (Nolan & Sheikh 1992), favoring the maintenance of cell integrity. However, the presence of lead-thioneins, which is known in vertebrates (Nolan & Sheikh 1992), has not been demonstrated in molluscs. Another source of changes in the biological response may be the origin of experimental populations of oysters exposed to lead. In the case of silver, it has been demonstrated that incorporation could vary by an order of magnitude according to this factor (Berthet *et al.* 1992).

The impairments observed in the present study are in agreement with previous observations in organisms exposed to lead, notably at the level of the endoplasmic reticulum, mitochondria (Bolognani-Fantin & Franchini, 1990) and nucleus (Russo *et al.* 1988). Mitochondrial impairments could result from a lead–calcium antagonism (Nolan & Sheikh 1992) or may be related to the effect of lead on cellular respiration processes (Bolognani-Fantin & Franchini 1990). It is known that low concentrations of lead may interact with enzymes of the intermediate metabolism and inhibit the oxidative phosphorylation (Bull 1980, Silbergeld 1984). Changes involving the granular endoplasmic reticulum may affect the level of protein synthesis (Viarengo *et al.* 1980).

The sedimentary particles provided to oysters have not

given rise to endocytosis either in the gills or in the digestive gland or digestive tract. The presence of silicon and aluminum in lysosomes shows that silicate endocytosis is possible. The size of particles selected in the present study with the aim to meet feeding requirements of oysters seems incompatible with endocytosis at the level of the plasma membrane. Consequently, lead measured in the whole soft tissues is properly incorporated and not only stored with inert particles. Cytological lesions observed in the digestive tract may be due to lead desorption from the sedimentary particles in the conditions (pH, action of enzymes) prevailing in the lumen. Gill lesions are not so easy to understand. It may be hypothesized that the release of acidic peptoglycans by mucocytes could induce changes in lead binding and lead to a local desorption of small quantities of metal (Denny 1983, Part & Lock 1983).

Whatever the way of exposure and the induced lead body burden, no acute biological effect was recorded. The condition index in both controls and contaminated oysters was comparable with those determined in environmental conditions over a 4 year period (Bodoy *et al.* 1986). However, ultrastructural studies have shown liminal effects of lead (even with low incorporation at the organism level), the consequences of which are unknown in the case of long-term exposure.

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