Influence of Chronic Exposure to Silver and Mercury in the Field on the Bioaccumulation Potential of the Bivalve Macoma balthica

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ABSTRACT

In order to assess the adaptation to metals previously observed in the bioindicator organism, Macoma balthica, subjected to chronic contamination by silver and mercury in the French Loire estuary, the bioaccumulation potential of individual organisms originating from the contaminated Loire estuary and a relatively uncontaminated control estuary (Somme) was evaluated using both radiotracers and stable isotopes of Ag (80 μg Ag litre⁻¹) and Hg (100 μg Hg litre⁻¹). Clams from the contaminated estuary were more sensitive to Ag (LT₅₀ = 9 d) than those originating from the Somme estuary (LT₅₀ > 15 d), even though the former bioaccumulated Ag to a significantly lower degree. This is attributed to a consequence of the chronic stress induced by Ag while clams were living in their natural environment. Therefore, past history of trace metal contamination should be considered when evaluating the susceptibility of M. balthica to heavy metal exposure. Lower uptake rates obtained for Hg (during the initial uptake phase only) and for Ag in clams from the polluted estuary suggest the presence of an adaptive trait for survival in contaminated areas. However, the lower degree of bioconcentration observed for Ag was not sufficiently low to reduce the sensitivity of the organisms to Ag and allow them to resist the toxic stress. Clams that survived Ag or Hg exposure at LT₅₀ did not protect themselves against metal toxicity by accumulating a significantly lesser amount of these metals than clams which did not survive metal stress. The results suggest that the bioaccumulation

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potential of each individual was not a factor which can explain the survival ability of M. balthica exposed to chronic Ag and Hg contamination in estuaries. In this case, cellular, biochemical and genetic levels of adaptation are presumed to be of greater importance. © 1998 Elsevier Science Ltd. All rights reserved

**Keywords:** adaptation, bioaccumulation, mercury, silver, *Macoma balthica*, toxicity.

**INTRODUCTION**

Apart from natural continental runoff (Bruland, 1983), many cases of elevated trace metal concentrations can be directly attributed to industrial and municipal waste discharges, especially in estuaries (Bryan, 1984; Bryan and Langston, 1992). Salinity is one of the environmental factors that can have a wide variety of effects on the chemical speciation of river-borne trace metals such as desorption of particle-bound metals, organic complexation and removal of trace metals from solution (Li *et al*., 1984). Therefore, in euryhaline environments such as estuaries, sediments often serve as a sink for trace metals. Furthermore, bioturbation and current-induced resuspension of sediment particles can cause trace metals to be reintroduced to the system, leading to locally enhanced trace metal concentrations. For these reasons, benthic organisms living in close contact with the sediments are frequently used as bioindicators for trace contaminants in pollution monitoring programmes (Cain and Luoma, 1985, 1990).

Silver and mercury originating from sewage discharge or industrial activities are two elements of concern in the context of managing the environmental impact of heavy metals (Cain and Luoma, 1985; Martin *et al*., 1988; Bryan and Langston, 1992; Sanudo-Willhelmy and Flegal, 1992). These two elements, together with Cu, are the most toxic metals to invertebrates in the estuarine environment (Bryan, 1984). Tolerance to metallic pollutants has been demonstrated in different species chronically exposed to metal contamination in their environment (Klerks and Weis, 1987). For example, in flat oysters (*Ostrea edulis*), individuals originating from a clean area were able to accumulate cadmium twice as fast as oysters living in a cadmium-contaminated area, a fact which could result from an increased resistance in oysters chronically exposed to this metal in their natural environment (Frazier and Georges, 1983). For *Scrobularia plana* (Bivalve, Tellinidae), the mean zinc concentration in soft tissues of clams from a clean area and exposed to zinc levels approaching LC$_{50}$, reached 650 mg kg$^{-1}$ dry wt whereas concentrations as high as 4900 mg kg$^{-1}$ dry wt were established in *S. plana* living in the highly polluted Restronguet Creek (Bryan *et al*., 1980; Amiard *et al*., 1987). In another clam, *Macoma balthica*, the LC$_{50}$ obtained for Cu was higher in individuals from the polluted San Francisco Bay than in those collected in two clean areas (Luoma *et al*., 1983).

Tolerance is often due to the ability of organisms to detoxify metallic pollutants. The major pathways of detoxification are through metal-binding proteins, namely metallothioneins (Carpene, 1993), or the sequestration of metals as insoluble mineral concretions (Brown, 1982). Some organs act as sinks for detoxified metals, essential or non-essential, leading to high concentrations in the organism. In connection with this process, variations have been observed between American and European populations of *M. balthica* concerning their ability to synthesize metallothionein-like proteins (Cain and
Luoma, 1985; Johansson et al., 1986; Langston and Mingjiang Zhou, 1987; Bordin et al., 1994). On the other hand in the closely related species Scrobicularia plana, silver storage as sulphide has been noted in the haemocytes and basement membrane of the outer fold of the mantle edge (Truchet et al., 1990).

To examine the basis for a potential adaptation to metals, a multidisciplinary study was undertaken to compare populations of M. balthica originating from the comparatively clean area of the Baie de Somme and from the mouth of the industrialized and urbanized Loire estuary. During water quality monitoring surveys of the French marine environment (RNO, 1995), higher concentrations of Hg, Cd, Cu, Pb, and Zn were observed in mussels collected in the Loire estuary compared to those from the Baie de Somme. Previous studies with M. balthica have also shown higher levels of Ag, Cd, and Cu in individuals from the Loire estuary (Rainlet, personal communication; Hummel et al., 1996). The aim of the present experiments, which used Ag and Hg at levels higher than natural dissolved concentrations found in the most polluted estuaries (25 μg Ag litre⁻¹, Smith and Flegel, 1993; 100 ng Hg litre⁻¹, Sorensen and Bjerregaard, 1991), was to deliberately stress the organisms in order to demonstrate the presence of any adaptive response of M. balthica previously subjected to chronic metal contamination in their natural environment. The bioaccumulation potential of the benthic marine bivalve was evaluated prior to carrying out selective biochemical analyses. In this approach a non-destructive method was required to measure bioaccumulation potential. Therefore, radioactive isotopes of silver (¹¹⁰ᵐAg) and mercury (²⁰³Hg) were used together with their stable isotopes in order to: (1) evaluate the bioaccumulation potential of the two populations experimentally stressed by the stable metal, (2) identify the relative distribution of the radionuclides in the organism tissues, and (3) characterise each organism as a high or low metal accumulator.

MATERIALS AND METHODS

Choice of species

Macoma balthica is a sediment dwelling bivalve widely distributed in temperate estuarine communities (Wolf and de Wolf, 1977; Nichols and Thompson, 1982), and is currently used as a bioindicator of trace metal contamination (Luoma et al., 1985; Cain and Luoma, 1990; Absil, 1993). In the context of the present study, this clam species was selected especially for large-scale (spatial and temporal) comparisons since its populations in French coastal waters appear to be much more stable in the long term than other species (Desprez et al., 1986, 1991). This clam is classified as a facultative deposit feeder, which means that it can feed on either suspended or deposited material depending on food bioavailability; however, most of the time it behaves as a deposit feeder (Hummel, 1985). Its main sources of food are benthic and pelagic diatoms, bacteria, and detrital organic material associated with fine-grained (<100 μm) superficial sediments (Tunnicliffe and Risk, 1977).

Sample collection and handling

Macoma balthica were collected from the tidal mudflat in the appropriate estuary 10 d before starting each experiment. To obtain an homogenous set of organisms as an aide
when comparing the two different areas, and to eliminate variable size dependence correlations, shown by Strong and Luoma (1981), clams were successively sieved through 12- and 4-mm mesh-size nets. In the pre-selected fraction of 5–12 mm range (2–3 y old), only the individuals with a size of approximately 10 mm were used for the experiments. Clams were transported under refrigerated wet conditions. Then, they were adapted to laboratory conditions during 1 week prior to the experiment: 32‰ salinity, 12°C, 12:12 light–dark regime. The clams were fed Isochrysis galbana \(10^3\) cells ml\(^{-1}\) during the acclimation period.

**Radiotracer experiments**

Polyvinylchloride aquaria were soaked in 10% HNO\(_3\) and rinsed with deionized water. High-purity deionized water \( (> 17.5 \text{ Mohm cm}^{-1})\) was used when preparing stock solutions of trace metals or adjusting salinity of the Mediterranean seawater to 32‰.

Four experiments were carried out to evaluate and compare the two groups of clams: (1) bioaccumulation of Hg in clams originating from the contaminated Loire estuary (Exp. I, March) and from the non-contaminated Somme estuary (Exp. II, April); (2) bioaccumulation of Ag in clams originating from the contaminated Loire estuary (Exp. III, May) and from the non-contaminated Somme estuary (Exp. IV, May).

For each of the estuaries, four or five aquaria of 2.5 litre each were filled with 2 litres of 0.22 \(\mu\)m-filtered Mediterranean seawater at 32‰ salinity, 12°C, and allowed to equilibrate for 3 d. The initial seawater was replaced with new seawater just before starting the experiment. One aquarium was used as a control for mortality and the others (replicates) were spiked successively with: (1) microlitre quantities of a solution of AgNO\(_3\) or HgCl\(_2\) in 0.1 M HNO\(_3\) to reach a final concentration of 80 \(\mu\)g Ag litre\(^{-1}\) (746–7 nm Ag) or 100 \(\mu\)g Hg litre\(^{-1}\) (497–3 nm Hg); (2) 7 \(\mu\)l of \(^{110m}\)Ag (Amersham, 18–110 MBq mg\(^{-1}\) Ag) in 0.1 M HNO\(_3\) or 15 \(\mu\)l of \(^{203}\)Hg (Amersham, 11–74 MBq mg\(^{-1}\) Hg) in 0.1 M HCl to give an activity of 14 Bq/ml and 5 Bq/ml in the experimental medium, respectively. The maximum amount of stable metal added with the radiotracers was less than 1 \(\mu\)g litre\(^{-1}\); therefore, tracer addition did not significantly perturbate the added stable metal concentration.

Previous experiments allowed determination of the stable metal concentrations necessary to achieve a lethal time 50 (LT\(_{50}\)) of roughly 10 d. Specific activities of each radiotracer and typical ambient levels of the metals in the Mediterranean surface seawater used in this study (100 ng Ag litre\(^{-1}\), Fukai and Huynh-Ngoc, 1971; 1-29 ng Hg litre\(^{-1}\), M. Horvat, personal communication) were considered when calculating the concentration of total stable metal in the experimental solution. The change in pH measured after these additions was negligible. The experimental medium was replaced every 2 d in order to maintain a relatively constant level of both radionuclide and stable metal concentrations. Radionuclide adsorption on walls was previously checked and evaluated to be less than 2 and 5% after 13 d of uptake for Ag and Hg, respectively. Evaporation of Hg was approximately 10% in 2 d.

For each experiment, 20 *M. balthica* were placed in each covered replicate aquarium \(n = 80\) ind. for the Loire estuary and \(n = 60\) ind. for the Somme estuary) and exposed to both the radioactive and the stable isotope of each metal, or kept free of contaminants (control aquarium). The uptake kinetics of Ag or Hg for *M. balthica* were followed in each live individual until the time was reached at which 50% of the animals were dead (LT\(_{50}\)). The \(\gamma\) emissions of \(^{110m}\)Ag (658 KeV) and \(^{203}\)Hg (280 KeV) in clams and seawater
were measured using a Packard NaI detector with automatic sample changer. Clams were briefly rinsed and blotted dry on absorbent paper before counting to eliminate any adhering dissolved radioisotopes. The detector was previously calibrated for volume and sample geometry with internal reference standards. Counting times ranged from 1 (for live animals) to 10 min (for tissues) in order to attain individual propagated counting errors of < 2% at 1 SD level. Counts were corrected for geometry, counting efficiency and radioactive decay. Concentration factors (CFs) over time were calculated from the uptake experiments as:

Activity in the whole clam (Bq/g wet wt)/activity in the seawater (Bq/ml).

CFs were computed for the whole clam because shell can contribute to enhance the level of contaminants transferred to higher trophic levels of the marine food chain. This is particularly true for small bivalves like those used in our experiments because they can be eaten whole by fish.

To reduce the influence of temporal fluctuations of the activity level in the labelled seawater, the radioactivity of the seawater used for each calculation was obtained by computing a running mean of the activities in seawater measured at each sampling day and after each new spike.

RESULTS

For all the experiments, there was no significant difference between replicate aquaria ($p = 0.05$, ANOVA) allowing the grouping of data into one set of $n = 60$ ind. for the Somme estuary and $n = 80$ ind. for the Loire estuary.

Toxicity

The toxicity of Ag and Hg was assessed using the LT$_{50}$, the time for which 50% of the population of clams exposed to a contaminant have died. The results obtained are presented in Table 1. LT$_{50}$ values observed for Ag were significantly lower ($p = 0.05$, Student's $t$-test) for the Loire than for the Somme estuary. Half of the clams collected in the polluted Loire estuary and exposed in the laboratory to 80 $\mu$g Ag litre$^{-1}$ died in 9 d. Under the same experimental conditions, for clams collected in the Somme estuary only 30% mortality was observed after 15 d exposure to Ag. In the case of $M$. balthica exposed

| TABLE 1 |
| LT$_{50}$ Obtained for *Macoma balthica* from a Polluted Estuary (Loire) and a Non-Polluted Estuary (Somme) |
| Estuary | Ag | Hg |
| Loire | 9.3 $\pm$ 0.9 d | 11.0 $\pm$ 0.8 d |
| Somme | > 15 d $^{a}$ | 12.0 $\pm$ 0.5 d |

Exposed to 80 $\mu$g litre$^{-1}$ Ag or 100 $\mu$g litre$^{-1}$ Hg (error bars correspond to 1 SD).

$^{a}$ Thirty per cent mortality after 15 d exposure.
to 100 µg Hg litre⁻¹, 50% of the clams from the Loire (collected in March) and the Somme (collected in April) estuaries died in 11 and 12 d, respectively. The sensitivity to Hg exposure did not appear to be significantly different (p = 0.05; Student's t-test) between the two populations.

Comparison of bioaccumulation potential

Accumulation of silver and mercury in M. balthica was also compared for clams from the Loire estuary and from the less polluted Somme estuary. Concentration factors for ¹¹⁰ᵐAg and ²⁰³Hg were followed over the time of exposure until LT₅₀ was obtained (Fig. 1). At this time, steady state was not reached. The level of metal accumulated at LT₅₀ expressed as final CFs, for the Loire and the Somme estuaries were, respectively, 108 ± 13 (9 d) and 193 ± 29 (15 d) for Ag, and 133 ± 27 (11 d) and 149 ± 21 (12 d) for Hg. Although during the experiment there was a tendency for somewhat higher Hg CF in clams from the Somme, there was no significant difference (p = 0.05 level) in the CF obtained after 11 d exposure to Hg for M. balthica from the two estuaries. On the other hand, after 9 d exposure, clams from the non-contaminated Somme estuary had accumulated significantly more Ag (p = 0.0001) than individuals from the Loire estuary.

Mean net uptake rates (Bq g⁻¹ wet wt d⁻¹) were evaluated for Ag and Hg from linear regressions computed for each individual from each treatment over the first 6 d of experiment. This time frame was chosen because it corresponded to the linear portion of the uptake curves and to a period during which all clams were still alive. Since it can be assumed that organisms accumulate the radioactive and the stable isotope at the same rate, the percentage of activity accumulated from the total pool of radioactive isotope was applied to the quantity of stable metal added to the experimental medium. The average values obtained for the Ag and Hg net uptake rates (nm g⁻¹ wet wt d⁻¹) in clams from the Loire and Somme estuaries are presented in Table 2.

Considering the whole population of clams studied, the influence of the estuary from where the clams were collected on the uptake of Ag and Hg by M. balthica was tested in a one-way variance analysis (Sokal and Rohlf, 1981). The net uptake rates evaluated from the linear regressions computed for n = 80 ind. (Loire) or 60 ind. (Somme) for the first 6 d

| TABLE 2 |
|---|---|---|---|
| | Loire | | Somme |
| | Ag | Hg | Ag | Hg |
| Whole population | 4.2 ± 0.7 | 3.8 ± 0.8 | 6.6 ± 1.1 | 5.6 ± 1.2 |
| Resistant clams | 4.1 ± 0.7 | 3.9 ± 0.7 | 6.6 ± 1.1 | 5.7 ± 1.0 |
| Sensitive clams | 4.3 ± 0.7 | 3.7 ± 0.8 | 6.8 ± 1.1 | 5.4 ± 1.3 |

Net uptake rates for all clams, or those that survived the metal exposure (resistant) or were dead at LT₅₀ (sensitive), were computed from significant linear regressions obtained for the figures plotted in Bq g⁻¹ wet wt d⁻¹ for 0–6 d exposure, and assuming that organisms accumulate the radioactive and stable isotope at the same rate. Average values were obtained using the uptake rate of n = 80 or n = 60 ind. for the Loire and Somme estuaries, respectively (errors correspond to 1 SD).
Fig. 1. Concentration factors in the clam *Macoma balthica* from the Loire and the Somme estuaries, exposed to 80 µg litre⁻¹ Ag (a) or 100 µg litre⁻¹ Hg (b). Error bars represent 1 SD.
exposure to Ag contamination (no dead clams), were significantly higher \( (p = 0.0001) \) for
*M. balthica* originating from the clean Somme estuary than for those previously living in
the polluted Loire estuary. For Hg, the uptake rates obtained between 0 and 6 d exposure
for clams from the Somme were significantly higher \( (p = 0.05) \) than the uptake rates
obtained under the same conditions for Loire estuary clams, even if the final CFs were not
significantly different at the \( p = 0.05 \) level (Fig. 1(b)). Therefore, the potential influence of
time of exposure on the accumulation of Hg by clams from a contaminated or clean
estuary was tested in a two-way variance analysis (Sokal and Rohlf, 1981). Exposure time
affected in a significantly different way \( (p = 0.0001) \) the bioconcentration of Hg for clams
from the Loire and Somme estuaries.

**Ag and Hg bioaccumulation potential in resistant and sensitive clams**

Concentration factors obtained over exposure time for resistant and sensitive clams from
the Loire and the Somme estuaries were compared for both Ag and Hg contamination.
Resistant and sensitive clams from the Loire estuary concentrated Ag to a similar level
(Fig. 2(a)), even at the end of the experiment when the LT\(_{50}\) was reached. The same results
were obtained for Ag in resistant or sensitive clams from the Somme estuary (Fig. 2(b)),
and also for Hg in both estuaries (Fig. 3(a, b)).

In order to evaluate a potential adaptation of individual clams from a given estuary to
prior chronic Ag and Hg contamination, uptake rates were compared for Ag and Hg in
surviving (resistant) and dead (sensitive) clams at LT\(_{50}\). Net uptake rates \( (\text{Bq g}^{-1} \text{ wet}
\text{ wt d}^{-1}) \) were evaluated from the linear regressions computed for each individual of the
sensitive or resistant group of clams over the first 6 d of uptake to assess their bioaccumu-
lation potential during the period before they began to die from metal toxicity. Sur-
viving clams and those that were dead at LT\(_{50}\) accumulated Ag at very similar rates
during the first 6 d of exposure: 4.1 ± 0.7 and 4.3 ± 0.7 \( \text{nm g}^{-1} \text{ d}^{-1} \) for the Loire estuary and
6.6 ± 1.1 and 6.8 ± 1.1 \( \text{nm g}^{-1} \text{ d}^{-1} \) for the Somme estuary. The corresponding uptake rates
obtained for Hg were for resistant and sensitive clams, respectively: 3.9 ± 0.7 and
3.7 ± 0.8 \( \text{nm g}^{-1} \text{ d}^{-1} \) (Loire estuary), and 5.7 ± 1.0 and 5.4 ± 1.3 \( \text{nm g}^{-1} \text{ d}^{-1} \) (Somme estu-
ary). Uptake rates for the clams in each estuary (at LT\(_{50}\)) were compared in a one-way
variance analysis \( (n = 80 \text{ ind. in the Loire estuary and } n = 60 \text{ ind. in the Somme estuary})\).
There was no significant difference \( (p = 0.05) \) in the uptake rates obtained for clams that
survived the Ag and Hg exposure or for those that died due to metallic stress. This result
was observed for both estuaries.

**Tissue distribution of Ag and Hg**

In order to evaluate the distribution of Ag and Hg in *M. balthica* and to discern the
fraction of metal accumulated in the soft tissues from that adsorbed onto the shell, indi-
viduals were periodically dissected during the experiments. The pallial fluid was experi-
mentally determined to represent approximately 15% of the total Ag and Hg accumulated
by the clams. In all cases, the heavy metal concentration in the shell and soft parts of
*M. balthica* increased during the experiment (not shown). After 1 d exposure to seawater
contaminated with Ag, Loire clams had approximately 70% of Ag associated with shell
and 30% in the soft tissues (Table 3). After 5 d exposure, the relative distribution between
shell and soft tissues had shifted to roughly 88 and 12%, respectively. A similar distribution
Fig. 2. Concentration factors of Ag in *Macaoma balthica* from the contaminated Loire estuary (a) and from the non-polluted Somme estuary (b) exposed to 80 μg litre\(^{-1}\) Ag. Values were computed for clams which survived the metal exposure (resistant) or were dead at the LT\(_{50}\) (sensitive). Error bars represent 1 SD.
**Fig. 3.** Concentration factors of Hg in *Macoma balthica* from the contaminated Loire estuary (a) or from the non-polluted Somme estuary (b) and exposed to 100 μg litre⁻¹ Hg. Values were computed for clams which survived the metal exposure (resistant) or were dead at the LT₅₀ (sensitive). Error bars represent 1 SD.
TABLE 3
Percentage Distributions of Accumulated Metals in Shell and Soft Parts of *Macoma balthica* (Excluding the Pallial Fluid which Represents Approximately 15% of the Total Activity Accumulated by the Organism) Expressed as Percentage of Whole Body Concentration

<table>
<thead>
<tr>
<th>Days</th>
<th>Location</th>
<th>Ag</th>
<th></th>
<th>Hg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Loire</td>
<td>Somme</td>
<td>Loire</td>
<td>Somme</td>
</tr>
<tr>
<td>1</td>
<td>Shell</td>
<td>71.8 ± 5.9</td>
<td>81.2 ± 4.6</td>
<td>81.0 ± 15.6</td>
<td>55.0 ± 9.9</td>
</tr>
<tr>
<td></td>
<td>Soft parts</td>
<td>28.2 ± 5.9</td>
<td>18.8 ± 4.6</td>
<td>19.0 ± 15.6</td>
<td>45.0 ± 9.9</td>
</tr>
<tr>
<td>5</td>
<td>Shell</td>
<td>88.4 ± 10.6</td>
<td>88.0 ± 5.1</td>
<td>59.1 ± 8.3</td>
<td>49.8 ± 13.1</td>
</tr>
<tr>
<td></td>
<td>Soft parts</td>
<td>11.6 ± 10.6</td>
<td>12.0 ± 5.1</td>
<td>40.9 ± 8.3</td>
<td>50.2 ± 13.1</td>
</tr>
<tr>
<td>LT&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Shell</td>
<td>82.2 ± 1.5</td>
<td>89.7 ± 6.3</td>
<td>62.5 ± 20</td>
<td>62.4 ± 13.0</td>
</tr>
<tr>
<td></td>
<td>Soft parts</td>
<td>17.8 ± 1.5</td>
<td>10.3 ± 6.3</td>
<td>37.5 ± 20</td>
<td>37.6 ± 13.0</td>
</tr>
</tbody>
</table>

LT<sub>50</sub> corresponds to 9 and 11 d for Ag and Hg, respectively (errors correspond to 1 SD).

was observed at LT<sub>50</sub>. For the Ag distribution in clams from the Somme estuary, an approximately constant relative tissue distribution, similar to that noted for the Loire after 5 d exposure, was observed after only 1 d exposure to the contaminant (Table 3).

In the first day of Hg exposure, the relative distribution of mercury in *M. balthica* originating from the Loire estuary was approximately 81% Hg adsorbed onto shell and 19% accumulated in the soft tissues (Table 3). After 5 d exposure, Hg distribution in the soft tissues increased to reach a final fraction of 41%. The same relative distribution of Hg in Somme clams, i.e. approximately 45% in the soft tissues and 55% in shell, was obtained after only 1 d of exposure to Hg.

**DISCUSSION AND CONCLUSION**

**Sensitivity of *Macoma balthica* to Ag and Hg exposure**

Ag and Hg are two of the most toxic heavy metals to invertebrates that have been documented in marine and estuarine environments (Bryan, 1984). No evident difference was observed in the response to the toxic effect of Hg for *Macoma balthica* naturally exposed to chronic contamination and for the same species originating from a non-polluted estuary. The LT<sub>50</sub>s obtained were similar for both populations of clams. In contrast, *M. balthica* from the Loire estuary, which is known to be contaminated by Ag (RNO, 1995; Hummel *et al.*, 1996; Rainglet, personal communication), were more sensitive to Ag exposure in terms of lethal time than similar clams from the Somme estuary. This difference between estuaries in LT<sub>50</sub>s for Ag can not be attributed to handling procedures, because there was no mortality observed in the control clams treated in the same way as the exposed organisms. Stable Ag concentrations measured in soft tissues of control clams were significantly different (*p = 0.05*) for the Loire and the Somme estuaries: 0.31 ± 0.14 mg kg<sup>-1</sup> wet wt and 0.07 ± 0.02 mg kg<sup>-1</sup> wet wt, respectively. Therefore, the higher sensitivity of clams from the Loire estuary to experimental Ag levels, may be the consequence of their previous exposure to trace contaminants in the field. In the case of mercury, concentrations (0.08 and 0.12 mg kg<sup>-1</sup> dry wt for the Somme and the Loire estuaries, respectively) observed in bivalves during a monitoring survey performed in these estuaries between 1979 and 1993.
(RNO, 1995) may not have been sufficiently higher in the Loire estuary to increase the susceptibility of *M. balthica* to this metal compared to control organisms.

Thus, the exposure history of environmental trace metal contamination of the population of clams considered should be evaluated to assess the variability of the toxicity threshold in marine bivalves. Furthermore, it has been reported in the literature that the variability of Ag toxicity also depends upon the species (Bryan et al., 1985; Berthet et al., 1992; Metayer et al., 1990), the time of collection (Martin et al., 1988) and the life stage of the organisms (Bryan and Langston, 1992).

**Bioconcentration of Ag and Hg by *Macoma balthica***

Steady state was not observed for Ag and Hg uptake by *M. balthica* when the LT$_{50}$ was reached and the final CFs evaluated. In the case of Ag bioconcentration by clams from the polluted Loire estuary, the following factor should be considered. CFs obtained from preliminary experiments carried out under the same conditions but without stable silver additions (CF = 212 ± 51 after 9 d exposure), were significantly higher (*p* = 0.05), suggesting a potential competition between stable and radioactive isotope which, in turn, leads to a decrease of the CF of the radioisotope when stable metal is added to the experimental medium (Amiard-Triquet and Amiard, 1980). This competition may be explained by the fact that Loire estuary clams, having been previously exposed to chronic Ag contamination, may have already accumulated stable Ag and are thus less able to concentrate Ag when exposed later to experimental contaminant concentrations. Such a hypothesis is supported by the environmental Ag concentrations obtained by Rainglet (1994) in soft tissues of *M. balthica* collected in the control Somme estuary (0.47 ± 0.10–0.68 ± 0.12 µg Ag g$^{-1}$ dry wt) which are much lower than those measured in individuals from the Loire estuary (3.20 ± 1.04 µg Ag g$^{-1}$ dry wt).

Relatively low percentages of $^{110m}$Ag and $^{203}$Hg were detected in the soft tissues of *M. balthica*. The largest proportion of radionuclide, and presumably stable metals, was located in the shell, 88% for Ag and 55% for Hg. An equilibrium in the distribution of Ag and Hg between shell and soft tissues was reached during the early stages of the metal uptake. The distribution in the clam may be partially explained by the fact that the shell, which is in continual contact with the contaminated medium, readily adsorbs trace metal. To our knowledge, there are relatively few data for Ag and virtually none for tissue distribution of Hg in *M. balthica*. Our radiotracer results concerning Ag distribution are consistent with the work of Luoma and Jenne (1975). The authors reported that approximately 60% of $^{110m}$Ag taken up from seawater was located in the shell of *M. balthica* after a 14-d exposure. Furthermore, Ag is rapidly partitioned in the shell of *M. balthica*, and in nature it may occur in high concentrations in mollusc shell (Luoma and Jenne, 1975). Some comparisons can be made with similar bivalve species such as *Scrobicularia plana* (Tellinidae) in which bioconcentration of Ag from seawater occurs mainly in shell (Amiard, 1978). In the case of *Mytilus edulis*, Fisher et al. (1996) reported that after 16 h exposure, 10% of Ag was located in the shell, 55% in the soft parts and 35% in the pallial fluid. The higher relative distribution of Ag in the soft tissues of mussels compared with results obtained for *M. balthica* after 1 d exposure in both estuaries, may be the consequence of a far higher ventilation rate for mussels: 48 l(g tissue)$^{-1}$ d$^{-1}$ (Hawkins and Bayne, 1992), than for *M. balthica*: 2.0 l(g tissue)$^{-1}$ d$^{-1}$ (Luoma *et al.*, 1992), and/or may be due to a different behaviour of metals in mussel tissues.
Bioaccumulation potential of Macoma

Mercury and Ag were concentrated to the same level from seawater by *M. balthica*: \(1.1 \times 10^2\) for Ag and Hg (after 9 d), \(1.6 \times 10^2\) for Ag and \(1.4 \times 10^2\) for Hg (after 11 d), for the Loire and the Somme estuaries, respectively. However, the relative distribution of the accumulated metals was different. The proportion of Hg located in the soft tissues of the clam was higher than that observed for Ag (Table 3). The higher LT\(_{50}\) observed for *M. balthica* from the unpolluted estuary and exposed to Ag (\(>15\) d), compared to Hg exposure (LT\(_{50}\) = 12 d), probably reflects the difference in the resultant tissue distribution for the two metals.

**Adaptation of a *Macoma balthica* population**

By comparing the metal uptake behavior of *M. balthica* previously exposed in the field to Ag and at a lesser degree to Hg contamination, with clams originating from an area outside the influence of any major metallic pollution, our results point to a different behavior of Ag and Hg in the two estuaries, suggesting a potential adaptation of clams previously living in a polluted estuary. As reported by Strong and Luoma (1981), body size is an important factor that can affect metal accumulation by *M. balthica* and thus confound the comparisons between different populations. In our work, this parameter was not a variable since difference in clam size in the two populations was not statistically different \((p=0.05)\): \(0.44 \pm 0.09\) g and \(0.64 \pm 0.15\) g wet wt for the Loire and the Somme estuaries, respectively.

In our study it was observed that, compared to the control clams (Somme estuary), *M. balthica* from a contaminated estuary concentrated significantly less Ag \((p=0.0001)\). Based on the relative distribution of Hg between shell and soft tissues early in the uptake phase (Table 3) and the fact that adsorption onto the shell was comparable between organisms from the two estuaries (results not shown), the total accumulation of Hg in soft tissues was initially at a relatively lower level in clams from the Loire estuary. Slow uptake of Hg during the initial phase, and of Ag during the entire experiment, could be viewed as an adaptative strategy for enhanced survival in contaminated areas. Similar results were obtained by Langston and Mingjiang Zhou (1987) for *M. balthica* exposed to Cd. However, the lower degree of bioconcentration observed for Ag (Fig. 1) was not sufficiently low to reduce the sensitivity of the organisms to Ag and allow them to resist the toxic stress (i.e. lower LT\(_{50}\) than in the control clams).

**Individual adaptation in a population of *Macoma balthica***

The possibility that metal adaptation developed in a *M. balthica* population was studied by comparing the metal accumulation kinetics in clams exposed experimentally to silver or mercury. For a given population of *M. balthica*, uptake rates in clams that died due to the metallic stress were not significantly different from those observed for clams that survived exposure to Ag and Hg. Resistant individuals did not protect themselves against Ag or Hg toxicity by accumulating these metals to a lesser degree (or by excreting them more rapidly) than the group that did not survive. The release of Ag is also part of the net accumulation process. In mussels, release is principally a result of excretion when Ag is accumulated from the dissolved phase (Wang *et al.*, 1996). Mechanisms other than the bioaccumulation potential must be invoked to explain the different behavior between the two groups of clams exposed experimentally to high Ag and Hg contamination in the
medium. One possible process could occur at the physiological level through complexation (and presumably detoxification) of the metals with proteins or metallothionein-like proteins as described by Johansson et al. (1986) for *M. balthica* exposed to Ag, and by Roesijadi (1982) for Hg accumulation in *Mytilus edulis*. Another process involved could be a genetic adaptation in *M. balthica* previously subjected to chronic exposure to elevated levels of Ag and Hg in the Loire estuary. Genetic variations have been found by Hummel et al. (1995) in *M. balthica* following exposure to low levels of copper. Hummel et al. (1996) also observed that when these clams live at the limit of their geographic distribution (e.g. the Loire estuary is close to the southern limit), they appear to be more sensitive to any stress, and especially to metallic impacts.

In order to assess how stress exerted by the metallic pollutants Ag and Hg may act as a selective agent between two populations of *M. balthica* or within a given population, the bioaccumulation behaviour of this species was investigated. From our results, the past history of Ag exposure of a population of *M. balthica* appears to have a major influence on the susceptibility and on the overall bioaccumulation potential of the clam population. Furthermore, when considering a given population of clams, the bioaccumulation potential of each organism does not appear to be the factor which can explain why some individuals are able to survive Ag and Hg contamination and others not. To answer this question, a better understanding of the cellular, biochemical and genetic levels of adaptation is required.

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REFERENCES


