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Simulating a heavy metal spill under estuarine conditions: Effects on the clam *Scrobicularia plana*

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Abstract

We describe the effect of heavy metals Zn, Cd, Pb and Cu on the induction of methallothioneins on the clam *Scrobicularia plana* along a salinity gradient simulated under laboratory conditions. The clams were exposed to constant heavy metal concentrations in a dynamic estuary simulator during a 15-day assay to investigate possible induction of metal-binding proteins in them. The concentration of heavy metals in water was analysed. Clams were analysed for methallothionein concentrations. The speciation of Zn, Cd, Pb and Cu along the salinity gradient was modelled. Zn showed the highest concentrations and its prevalent species was the free ion. Intersite differences have been observed in methallothionein concentration and related to the salinity gradient. It seems that synthesis of methallothioneins is the result of physiological forces acting in concert with the changes in the chemical speciation of metals, owing to the trace metals uptake is controlled by means of an interaction of physiology and physicochemistry.

Keywords: Bioassay; Chemical speciation; Clams; Effects-biochemical; Estuaries; Heavy metals; Simulation

Effects of heavy metals Zn, Cd, Pb and Cu related to an accidental spill on individuals of the estuarine clam *Scrobicularia plana* have been studied using a hydrodynamic simulator of estuarine conditions. The effect of the Aznalcóllar

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mining spill in the Guadalquivir estuary has been simulated (Gómez-Parra, Forja, DelValls, Sáenz, & Riba, 2000). The simulator system consists of 8 tanks (Plexiglas, cylindrical, and of about 10 L capacity) interconnected under a hydrodynamic regime. The upper tank is supplied with unfiltered fresh water taken from the Guadalquivir River. The lower tank is supplied with seawater from a clean coastal area (Bay of Cádiz). From the lower to the upper tanks, there is a forced flow of water controlled by peristaltic pumps. In the inverse direction, filling the containers in series with fresh water generates a down flow. This permits a constant volume of 10 L to be maintained in each tank. The flow and temperature are regulated by a Personal Computer. A more detailed description of this system can be found in García-Luque, Forja, DelValls, and Gómez-Parra (2003).

Specimens of the clam *S. plana* (10 per aquarium; average length: 4.4 cm; average wet weight: 2.5 g) were placed in each tank after two weeks of acclimatization under controlled conditions. Then a solution with known concentration of the four metals was injected into the lowest salinity tank of the system in a continuous flow by a peristaltic pump. (The measured heavy metal concentrations in this solution were Zn:Cd:Pb:Cu, 5:0.2:1:2 $mg\,L^{-1}$, respectively). This metal injection was diluted as it reached succeeding tanks by the hydrodynamic regime and the inherent reactivity of heavy metals along a salinity gradient. The exposure period of the bioassay was 15 days. The methallothionein concentration was analysed in the total body of each specimen using the procedure outlined by Olafson and Olsson (1991). Total content of proteins determination was based on the Bradford method (1976). The total concentration of heavy metals (Cd, Pb, Zn, Cu) in each tank was analysed by differential pulse anodic stripping voltametry (DPASV) (Gómez-Parra et al., 2000). The analytical procedure for dissolved metals was checked using reference material CASS3 with an accuracy of ±10%. All samples were measured at the end of the experiment. Nevertheless, the system had reached stationary state when the injection of the heavy metal solution to the system was carried out. Therefore, measured concentrations at the end of the assay are similar to those during the experiment. Table 1 shows the metal concentrations in the water measured in each tank as well as the methallothionein concentrations (normalized to total protein) in the soft body of the specimens in all tanks. Parallel to the experiment, 10 clams were maintained in a reference aquarium (absence of introduced metals). The background level of methallothioneins was about 33.6 μg per mg of total proteins.

It is accepted that the total concentration of heavy metals measured in water cannot explain alone all the effects measured under estuarine conditions (Riba, García-Luque, Blasco, & DelValls, 2004). The chemical speciation of metals Zn, Cd, Pb and Cu along the simulated salinity gradient has been derived following the model proposed by Turner, Whitfield, and Dickson (1981). Fig. 1 shows results of this chemical speciation for these metals. The model derives the chemical speciation based on thermodynamic considerations and expresses as percentages each free ion as well as the different associations of the heavy metals with the ligands CO_3^{2-}, Cl^-, SO_4^{2-} and OH^-.

Table 1
Variation of the average concentration of dissolved Zn, Cd, Pb and Cu (in $\mu g\,L^{-1}$) with their standard deviations along the salinity gradient tank-by-tank

Tanks	Salinity	[Zn]	[Cd]	[Pb]	[Cu]	[MT]
Tank 1	17.81	237.3 ± 9.4	12.0 ± 2.5	31.8 ± 4.9	69.1 ± 5.1	58.1 ± 17.4
Tank 2	19.15	146.9 ± 8.2	6.7 ± 0.7	19.6 ± 0.3	54.9 ± 0.3	48.1 ± 1.1
Tank 3	20.61	106.3 ± 13.8	7.8 ± 3.0	14.4 ± 0.3	51.1 ± 9.7	40.2 ± 13
Tank 4	22.38	107.7 ± 17.4	5.9 ± 0.9	13.4 ± 0.7	37.4 ± 1.5	35.4 ± 2.7
Tank 5	24.51	156.3 ± 5.3	5.8 ± 1.5	15.5 ± 0.4	44.3 ± 8.4	62.8 ± 15
Tank 6	26.62	104.8 ± 16.5	7.0 ± 2.3	10.0 ± 1.7	31.3 ± 1.0	71.6 ± 4.1
Tank 7	28.96	87.2 ± 17.2	4.2 ± 0.5	6.0 ± 0.4	28.7 ± 0.1	105.6 ± 8.9
Tank 8	31.93	71.9 ± 9.2	3.4 ± 0.2	4.5 ± 0.2	23.5 ± 0.2	73.1 ± 8.3

The last column shows the concentration of methallothioneins normalized to the total content of proteins ($\mu g\,mg^{-1}$) with their standard deviations measured in the soft body of the specimens. (Experimentally measured background level of methallothioneins was 33.6 $\mu g\,mg^{-1}$.)

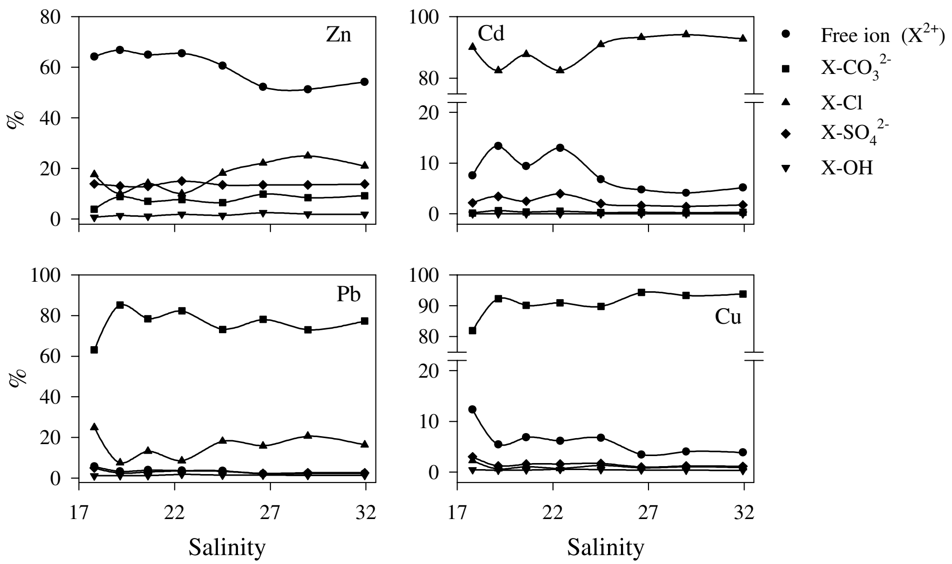

Fig. 1. Chemical speciation for Zn, Cd, Pb and Cu (expressed as percentage) along the salinity gradient simulated in the bioassay. "X" represents any of the four heavy metals. Each curve shows all the chemical species associated with the same ligand.

Results show that Zn had the highest concentration of all metals (twice that of the others combined; Table 1), and its prevalent species was the free ion. The free metal ion represents the bioavailable form (Rainbow, 1997). Therefore, free Zn ion appears to play a central role.

Changes in salinity cause not only physicochemical changes in metal speciation in the medium, but also an effect on the physiology of an aquatic invertebrate (Rainbow & Black, 2002). In that paper, the effects of salinity change on the rate of Zn uptake varied interspecifically in three species of crabs. Thus, in the case of *Carcinus maenas*, the Zn uptake rate decreased with salinity decrease. These results are consistent with a physiological response to low salinity values and determine that Zn uptake rate does not show an increase (in the reduced salinity area) as expected if only the physicochemical processes were considered. Similarly, we could assume that in our assay there were physiological forces acting in concert with the changes in speciation, which produced physicochemically non-expected results.

Thus, in the low salinity area (17–23‰) clams could reduce the rate of metal uptake (via physiological responses) to avoid excess input of free Zn ion. In Table 1 it is shown that synthesis of methallothioneins at 17–20‰ decreases to background level at 23‰, where physiological processes appear to control Zn uptake.

Above 24‰, the chloride concentration of the medium increases, producing an automatic increase of the chlorocomplexation of metal ions (Fig. 1). Thus, part of the free Zn ion is removed. Then, physiological responses are attenuated and an increase of Zn uptake occurs. Therefore, synthesis of methallothioneins is higher than that at low salinity values (Table 1). In fact, methallothionein concentration increased smoothly in the last four tanks of the system following a behaviour similar to that of free Zn ion in this area of the salinity gradient (Fig. 1).

In summary, the results of this bioassay show different patterns in the induction of methallothioneins along the salinity gradient. This result is a consequence of the interaction of physiological and physicochemical processes in controlling the effects of trace metals on a marine invertebrate species with versatility to occupy littoral and estuarine habitats.

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