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Toxic effects of zinc on the common mussel *Mytilus edulis* L. (Bivalvia) in brackish water. II. Accumulation studies

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Laboratory tests for accumulation of zinc were made in brackish water. Three weeks after a 24 h exposure to zinc (0–15 mg/l) the concentrations in common mussels (*Mytilus edulis*) varied from 233 to 340 µg/g DW. Six weeks after the exposure (24 h, 0–100 mg Zn/l) the concentrations varied between 302 and 550 µg/g DW. The concentrations of zinc increased during the 24 h exposure to 1 mg/l in the foot from 101 to 236 µg/g DW; in the mantle from 116 to 193 µg/g; in the visceral mass from 158 to 268 µg/g and in the gills from 171 to 390 µg/g. The total change, summed over these components, was from 150 to 250 µg/g DW. The greatest change occurred between the first and second hour of exposure. Small changes in the exposure concentration had no influence on accumulation, but could be observed in physiological tests.

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1. Introduction

Common mussels (*Mytilus edulis*) accumulate heavy metals and they are thus used as indicator organisms for heavy metal pollution (e.g. Goldberg 1975, Cunningham 1979, Goldberg 1980, Jensen et al. 1981, Elliot et al. 1984). They are suitable zinc indicators (Phillips 1976a).

The accumulation mechanisms of zinc are not known. According to Elliot et al. (1984), zinc accumulation takes place by passive diffusion. Most of the zinc is probably filtered with the food and is absorbed into the mussels through the digestive system, gills and mantle (Phillips 1977, George et al. 1978, George & Pirie 1980). Excess zinc stimulates metallothionein synthesis, at least that of Cu-Zn-thionein and Cd-Cu-Zn-thionein. Excess metallothionein is transferred to lysosomes in the digestive diverticula, where it produces insoluble polymers which are eliminated by exocytosis (Viarengo 1985). In hemolymph, 39% of the zinc is transferred inside amoebocytes, which actively intake metals by endocytosing high molecular weight proteins or membrane bound vesicles. Kidneys are the major site of accumulation and excretion of zinc, excretion taking place via exocytosis of lysosomal vesicles (Coombs & George 1978, George & Pirie 1980).

The basic levels of zinc in the mussels seem to be higher in the Baltic Sea than in the oceans (Goldberg et al. 1978, 1983, Gault et al. 1983). According to Kaitala (1981), the enrichment factor of zinc in the case of mussels in the Baltic is 110 000 (DW). The average concentration of zinc in the mussels in the coastal area of Finland is 178 µg/g DW. In the coastal area of Poland the concentrations of zinc in the mussels vary from 64 µg/g DW (large specimens) to 133 µg/g DW (small specimens) (Brzeźińska et al. 1984) and in Denmark the concentrations vary from Flensburg to Travemünde from 82 to 308 µg/g DW (Möller et al. 1983). The concentrations in oceanic mussels vary from 60 to 200 µg/g DW (Goldberg et al. 1978).

This paper presents the results of accumulation studies in brackish water in aquarium conditions. The results of physiological and histological tests of the same samples are presented in part I of this study (Hietanen et al. 1988). The results of both studies are compared: how high an accumulation must there be in the tissues before physiological or histological changes appear?

2. Material and methods

Mussels were collected by a diver near the island of Sundholmen (depth 5–8 m) and Granboskan (10–15 m), in the Gulf of Finland (59°50'N, 23°15'E) in June and at the beginning of July 1985. The mussels in all experiments were 3–4 cm long. Mussels were exposed to different concentrations of zinc for 24 h and then transferred to clean brackish water for 21 days (concentrations: control, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 15 mg/l). In another test, mussels were kept in clean water for 41 days after a 24h exposure (concentrations: control, 0.5, 1, 2, 4, 6, 8, 10, 15, 25, 50 and 100 mg/l). During the tests the animals were kept in aerated (20×20×25 cm) aquaria, with boards on the bottoms to which the mussels could attach themselves. At the beginning of the tests all the mussels that were not able to attach themselves to the boards within 24 h before the exposure were removed from the experiment. Dead animals were removed daily from the aquaria. In both tests the water in the aquaria was changed for clean brackish water every second day. Before determining the concentrations of zinc in each test the mussels were brushed clean and placed in filtered brackish water for 24 h (Baltic Sea Environmental Proceedings 12, 1984). The soft parts of 10 to 12 mussels (except for eight from the 50 mg/l and five from the 100 mg/l exposure group) were used for determination of the concentrations of zinc.

The accumulation of zinc in relation to time and different organs was tested with an exposure to 1 mg/l ($ZnCl_2$ analytical grade). The organs of the mussels were dissected after 0.5, 1, 2, 6, 12 and 24 hours, and tissue samples of ten mussels were pooled for each determination. The gills, mantle, visceral mass (= soft parts excluding gills, mantle, foot and kidneys) and foot were prepared separately for determination. The experiment was made in 20 l aerated aquaria (50×50×25 cm), where the mussels were kept individually in small plastic compartments. They were picked up and prepared one by one to provide an exact exposure time.

During preparation teflonated forceps and plastic knives were used. Zinc concentrations were measured by a Perkin-Elmer 305 atomic absorption spectrometer and the samples were prepared before determination by the method of Kotz et al. (1972).

3. Results

The zinc concentration in the mussels before the test was 204 µg/g DW. Three weeks after the exposure to 0 15 mg/l of zinc the concentrations were 233–450 µg/g DW (Fig. 1). Six weeks after the exposure to 0 100 mg/l of zinc the concentrations were 302–553 µg/g DW.

During a 24 h exposure to 1 mg/l the amount of zinc increased in the summed dry weight of gills, visceral mass, mantle and foot from 150 µg/g DW to 252 µg/g DW (Fig. 2). The concentration of zinc in the foot increased during the test from 101 µg/g DW to 236 µg/g; in the mantle from 116 to 199 µg/g; in the visceral mass from 158 to 268 µg/g and in the gills

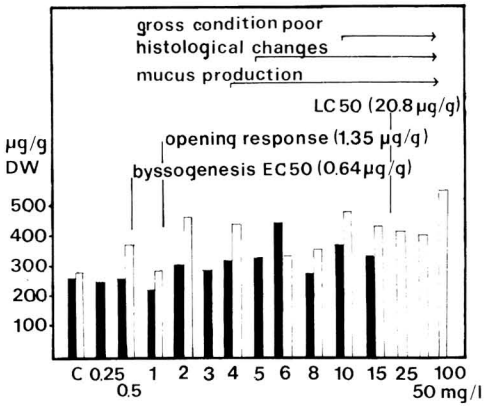

Fig. 1. Physiological responses of the mussels after a 24 hour exposure to different concentrations of zinc. Responses are recorded beside columns, which represent accumulation levels after an exposure to these concentrations. Black columns: the concentrations of zinc in mussels 20 days after exposure (µg/g DW). White columns: the concentrations of zinc in mussels 41 days after the exposure (µg/g DW).

from 171 to 390 µg/g DW (Fig. 3). During the test concentrations were significantly higher in the visceral mass and gills than in the mantle and foot (gills-foot t=7.15***, gills-mantle t=6.38***, visceral mass-foot t=6.76***, visceral mass-mantle t=5.51**, paired t-test). During the first hour of exposure the concentrations of zinc decreased in the mantle and foot and increased in the gills (Fig. 3). The greatest change in summed concentration occurred between the first and second hour (Fig. 2). After 24 hours the concentration of zinc was still increasing in the gills and foot but remained steady at the level attained after two hours in the visceral mass and mantle.

4. Discussion

The accumulation of zinc is affected by several factors and is very complicated. The concentrations of heavy metals vary annually in accordance with the reproductive cycle (Simpson 1979). Zinc concentration does not depend on either the size or the age of the mussel, but in polluted areas large specimens have accumulated more zinc than small specimens because they are older (Lobel et al. 1982, Lobel & Wright 1982a and b, Popham & D'Auria). The depth of water and seasons also affect the concentration of zinc in mussels (Phillips 1976b, Cunningham 1979).

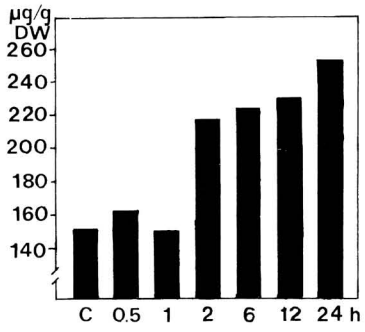

Fig. 2. The change in concentration of zinc in the total dry weight of the foot, mantle, visceral mass and gills during a 1 mg/l exposure.

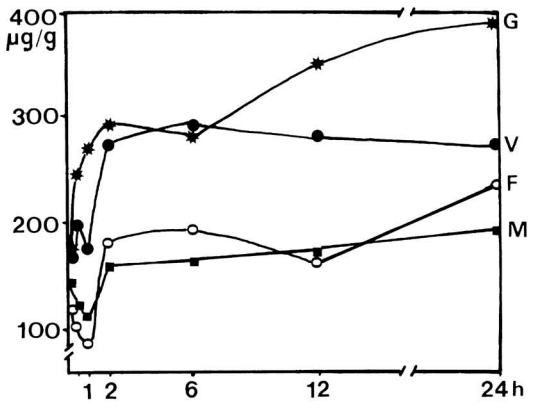

Fig. 3. The change in concentration of zinc during a 1 mg/l exposure in the foot (F), mantle (M), visceral mass (V) and gills (G).

Lobel et al. (1982) suggested that the common mussel may have two genetically differentiated detoxification systems for zinc, on the grounds that there are specimens which concentrate zinc effectively and store it and others which excrete zinc quickly or are impermeable to it. The distribution of its concentration in mussels is oblique towards smaller concentrations and the median and mean of zinc concentrations differ significantly. The concentration of zinc can vary from 10 to 14 times in specimens collected from exactly the same place and conditions. But the pollution of zinc can be indicated by using the zinc values of the specimens which have the highest values of concentration, or by determining the concentration of the combined biomass of several specimens.

The decrease in the concentration of zinc during the first hour of exposure in some organs in this study may be due to the activation of detoxification mechanisms. At a concentration of 1 mg/l the balance between intake and excretion was not attained in 24 h (Fig. 2 and 3). The accumulation rate of zinc slowed down after two hours. Accumulation of zinc was very fast, the concentrations doubling in 24 h in this study. George & Pirie (1980) found that the intake of zinc at a concentration of 1 mg/l continues linearly for weeks. The rapid intake by the gills and visceral mass and then the slower intake after two hours may indicate the transfer of zinc from these organs to the kidneys. The linearity of accumulation may be attained after the first day or the first few days. The concentration of zinc in the kidneys was not measured in this study because of the small size of the organ in brackish water mussels. The largest specimens found in the study area are 39 mm in length (Segerstråle 1942).

Pentreath (1973) found that the absorption of zinc occurs in the following order (over 28 days): stomach and digestive gland > gills > foot > mantle > gonads > pallial muscle. The results are in accordance with this study. During the first few days in Pentreath's studies the accumulation coefficients were exponential. After two weeks a balance was attained. In a polluted area, *Mytilus galloprovincialis* accumulated zinc mostly in the byssus threads, kidneys, hepatopancreas and gills, in that order (Martincic et al. 1984). The high content of metals in the byssus thread (highest concentrations of Zn, Pb and Cu) constituted, according to Martincic et al. (1984), evidence of their metal storage function.

The excretion of zinc is slow, taking up to six months after the mussels have been removed from a polluted area to clean water (Roesijadi et al. 1984). The accumulation test in this study proved that it is possible to detect elevated concentrations of zinc several weeks after the exposure.

The exposure concentration of zinc, which had an effect on the opening response and byssogenesis, was low (Hietanen et al. 1988). The accumulation of zinc following exposure to these concentrations did not differ from that of the controls (Fig. 1). Mortality, histological changes and the production of mucus occurred in animals with an elevated body loading.

The concentration of zinc was already high in mussels from the sampling stations (200 µg/g DW). Such a high initial level might well explain the low EC 50 values: mussels already exposed in the wild are exposed further in the aquaria, a small additional stress then being capable of producing a response.

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