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Toxic effects of zinc on the common mussel *Mytilus edulis* L. (Bivalvia) in brackish water. I. Physiological and histopathological studies

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The toxicity of zinc to the common mussel, *Mytilus edulis* L. (Bivalvia), was tested in aquaria in brackish water (7‰). Elevated concentrations of zinc were put into the aquaria for an exposure period of 24 h (12°C). Survival rates and the effects of sublethal concentrations on the activity of the mussels were observed for six weeks. Samples were taken from the gills for histopathological analysis. The LC 50 (lethal concentration) was 20.8 mg Zn/l. The exposure affected the byssal attachment of the mussels. The EC 50 (effective concentration) for the byssogenesis test was 0.64 mg/l. The EC 50 for the opening response was 1.35 mg/l. The exposure resulted in an acute inflammatory reaction in the gills of the mussels. The branchial veins were dilated, and the postlateral cells were swollen. In high concentrations hydropic degeneration of mucus secretory cells and necrosis of hemocytes were found.

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1. Introduction

Zinc is a rare metal in nature but it is commercially one of the most important metals in the world. It contaminates the Baltic Sea mostly via rivers but also by way of rainfall. The total amount of zinc entering the Baltic Sea is from 15000 to 20000 t/a (Melvasalo et al. 1981). In the Gulf of Finland, the concentration of zinc varies from 2 to 120 µg/l. In sea water, vertical differences in the concentration of zinc can increase from 60 to 70 times towards the bottom as a result of the descent of organic detritus (Förstner 1981a). Thus, the concentration of zinc in sediment is higher than in sea water.

In addition to being an environmental pollutant, accumulating in high concentrations in bivalves, zinc is essential to the normal cell functions of these animals. It stabilizes membranes by becoming bound to their structural components, and in contrast to the other heavy metals, to lysosomal enzymes as well (Lowe & Moore 1979, Viarengo 1985). In oysters zinc may also become bound to ATP, ADP and taurin (Coombs 1974). Lipid peroxidation probably depends on the relation of zinc and other metals (Viarengo 1985).

Numerous accumulation studies on the common mussel, *Mytilus edulis*, have been carried out under true marine conditions. In addition, the condition of the mussels should be measured to determine if the accumulated amounts have any effects on the health of the mussel. As no data is available about the accumulation of zinc in the mussel *Mytilus edulis* in brackish water, accumulation studies were carried out at different concentrations of zinc in aquaria. In order to study the biological significance of these concentrations, physiological and histological measurements were made. The lethal concentration (LC 50) and effective concentration (EC 50) were determined. The byssogenesis test, opening response and production of mucus were used to detect the effects of sublethal concentrations on the mussels. The gills of the mussels were examined histologically. The results of the physiological and histological studies are presented in this paper. The results of the accumulation studies are presented in part II (Hietanen et al. 1988).

2. Material and methods

2.1. Sampling stations

Our sampling stations are located near the Tvärminne Zoological Station, in the Gulf of Finland (59°51'N, 23°15'E, salinity 7‰). Mussels for the LC 50 test were collected by a diver near the island of Granboskan (depth 10–16 m) and for the byssogenesis test and histological analysis near the island of Sundholmen (depth 5–8 m) at the end of June and the beginning of July 1985.

2.2. Survival rates

After sampling, the animals were placed in aerated aquaria (20×20×25 cm), about one hundred per aquarium, at the Tvärminne Zoological Station. Next day those individuals which had not attached themselves to the boards on the bottom of the aquaria were removed from the experiment because detachment is a sign of poor condition. Twelve different concentrations of zinc, from control to 100 mg/l ($ZnCl_2$ analytical grade), were applied in the aquaria for an exposure period of 24 hours. After the exposure the water in the aquaria was changed for clean brackish water. During the observation period (41 days) the water was changed every other day. The mussels were examined daily and dead animals removed. The production of mucus and detachment of the byssi were observed during the test. Opening response was observed at the end of the exposure. The water temperature in the aquaria was 12.2±1.5°C.

2.3. Production of mucus

After the exposure to zinc the mussels started to secrete light grayish brown mucus through their siphon. The proportion of the animals producing mucus was counted at first daily and later just before changing the water every second day for the ensuing three weeks.

2.4. Byssogenesis test

After determining the LC 50 value, suitable exposure concentrations for the byssogenesis test and histological studies were selected. The concentrations of zinc used in the byssogenesis test were control, 0.25, 0.5, 1, 2, and 4 mg/l ($ZnCl_2$ analytical grade). Byssal threads were removed from the mussels with scissors before the mussels were placed in the aquaria (40×24×12 cm), 50 mussels to each. Boards were placed on the bottom of the aquaria to serve as a base for attachment. The attachment of the mussels was observed for the first time 0.5 h after starting the exposure and then at hourly intervals for a period of nine hours. The number of mussels attached to the boards was checked again after 24 and 48 hours.

2.5. Histological studies

Gill preparations were made after the 24h exposure to zinc. The exposure concentrations were control, 0.5, 5, 25 and 50 mg/l ($ZnCl_2$ analytical grade). New samples were taken from mussels which had been placed in clean brackish water for two weeks after the exposure. There were 25 specimens in each sample. The middle third of the inner demibranch was removed and fixed in Helly's fixative for 6.5 h (Barszcz & Yevich 1975). 6 µm thick paraffin sections were stained with Hematoxylin-Eosin (Romeis 1968).

Morphometric studies on the histological preparations were made with a Wild M-20 microscope and a projection head, using a Mertz's lattice. Thirty fields from each individual (ten from each group) were counted. Point counting was carried out for the following components of the filament: frontal cells, lateral cells, endothelial and abfrontal cells, and the branchial vein. 1410 power magnification was used. The relative volumes of different cell groups and the branchial vein were compared in different groups. The diameter of abfrontal mucus secretory cells was measured using an ocular micrometer. Ten cells from ten specimens in each group were measured.

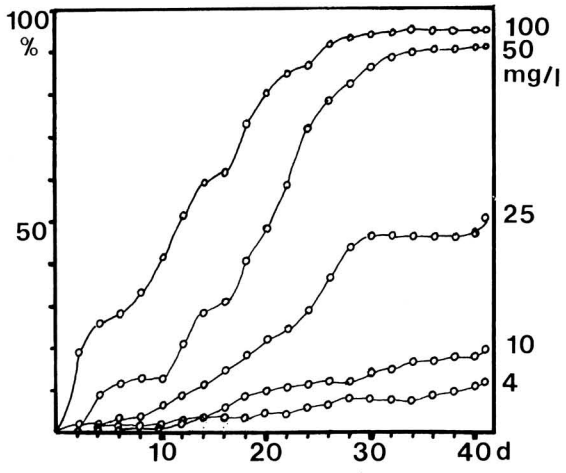

Fig. 1. Mortality rates. The mussels have been in clean brackish water for 41 days after a 24 h exposure to zinc (12°C). Mortality percentages plotted against time. In the control mortality was 4%.

3. Results

3.1. Survival rates

The LC 50 value of the common mussel in brackish water for zinc was 20.8 mg/l (95% confidence intervals were 18.6 and 23.3 mg/l, probit analysis). The mortality rates in different concentrations are illustrated in Fig. 1. The change in LC 50 value in relation to time is illustrated in Fig. 2. The test continued for 41 days, while 4% of the control animals died. After the exposure to high concentra-

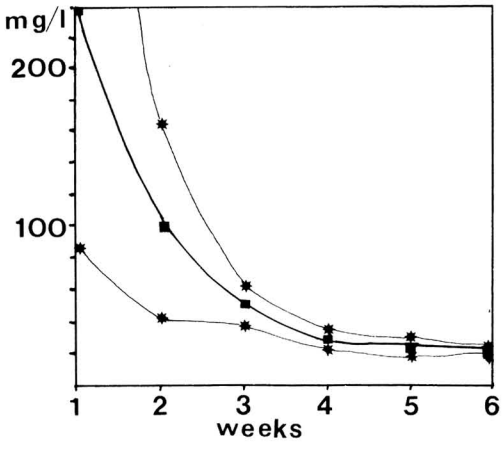

Fig. 2. Change in LC 50 values with time with confidence limits (5 and 95%). Mussels were kept in clean brackish water after a 24 h exposure to zinc.

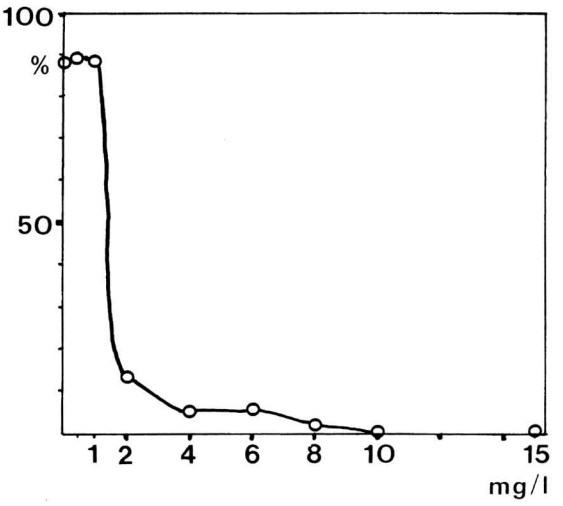

Fig. 3. The opening response. The percentages of mussels keeping their valves open after a 24 h exposure plotted against concentration of zinc.

tions (10 mg/l or more) recovery was very slow. The exposed mussels detached their byssi and secreted mucus. Reaction to irritation (closing of the valves, withdrawing of the foot and siphons) was slow or absent and mussels were immobile. The foot and siphon of some individuals were edematous and had lost their normal colour.

3.2. Opening response

During the exposure the mussels reacted to the presence of the heavy metal by not opening their valves. The EC 50 value (24 h) for the opening response was 1.35 mg/l (95% confidence intervals were 1.25 and 1.53 mg/l). After 24 h 90% of the mussels in the control group and 13% of the animals exposed to 2 mg/l were open (Fig. 3).

3.3. Production of mucus

Mucus secretion during the first two to four days after the exposure was copious. When the exposure concentration had been 4 mg/l or more, 20–50% of the mussels secreted mucus through their siphons. There was mucus also on the walls and bottoms of the aquaria. The higher the concentration of zinc the longer the mucus secretion lasted, in the highest concentrations (15–100 mg/l) for over three weeks.

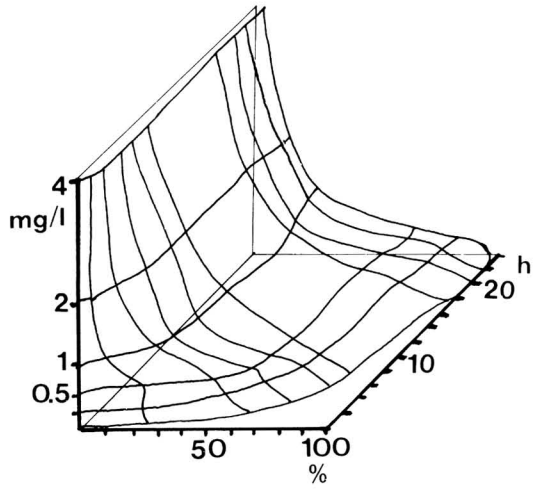

Fig. 4. The byssogenesis test. The percentage of attached mussels plotted against concentration of zinc and time.

3.4. Byssogenesis test

The EC 50 value for byssogenesis was 0.64 mg/l (95% confidence intervals 0.53 and 0.77 mg/l). In the control group all the animals attached themselves within 24 h. During the exposure to 4 mg/l the proportion of attached mussels was only 2.1%. The results of the byssogenesis test are illustrated in Fig. 4.

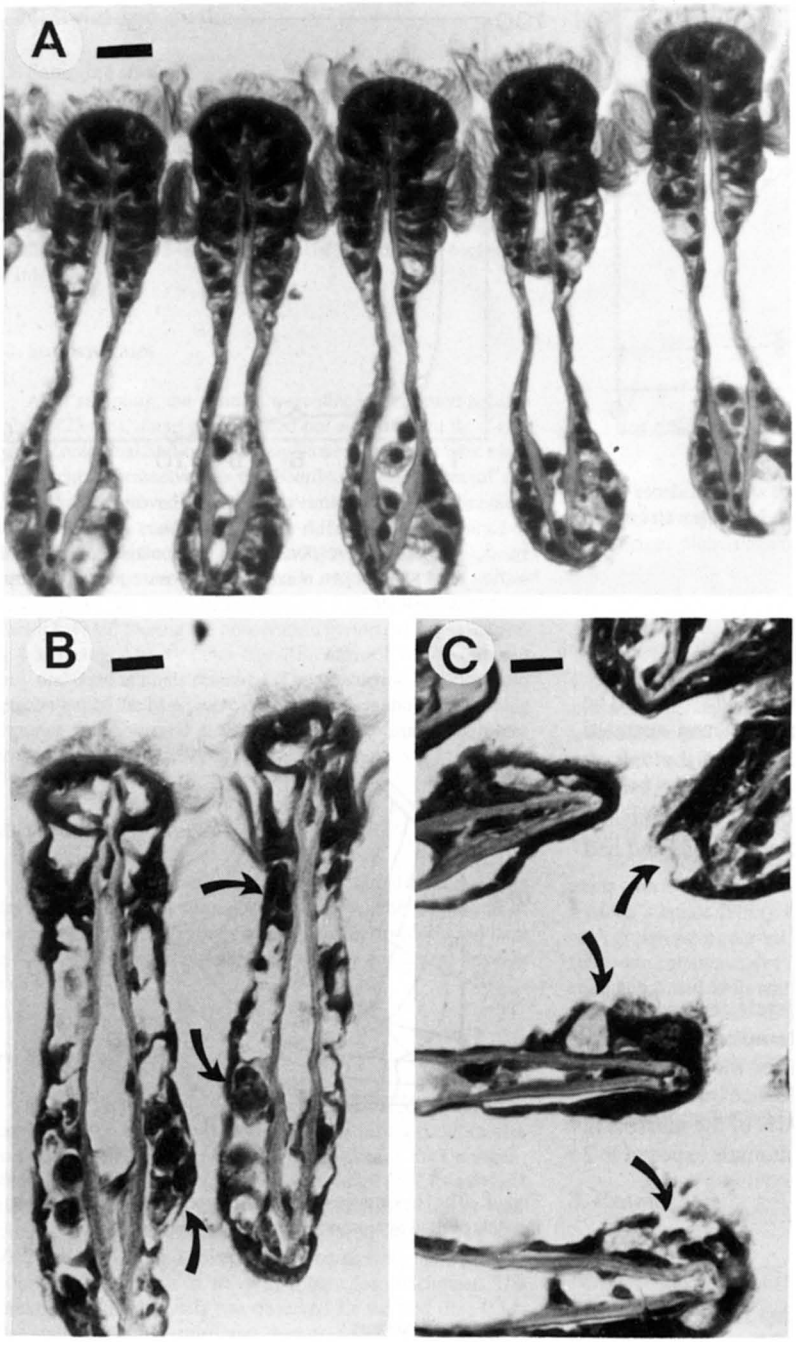

Fig. 5. Transverse section of a filament (scale bar 10 µm). — a) Control. — b) Swelling of the endothelium and inflammatory cells (arrows). 5 mg/l exposure. — c) Mucus secretory cells (arrows) appear to rupture outside the abfrontal end of the filaments. 25 mg/l exposure.

The shape of the curves demonstrates that most of the mussels attached themselves during the first two hours of the experiment. There were no differences between the proportions of attached mussels after 24 and 48 hours at any level of significance.

3.5. Histological studies

The structure of the gill is illustrated in Sunila (1986). The simple epithelium around the branchial vein consists of various types of cells (Lucas 1931).

Table 1. The incidence of reactions, and changes in secretory cell diameter (paired *t*-test), in mussels exposed to different Zn concentrations (mg/l).

	Control	0.5	5	25	50
Reaction					
acute inflammatory	–	–	+	+	+
degeneration	–	–	+	+	+
necrosis	–	–	(+)	+	+
Secretory cell diameter (μm)					
after exposure	11.6±0.55	14.1±0.38**	13.2±0.50	17.2±0.83***	15.3±0.64***
two weeks later		13.3±0.72**	14.4±0.49**	12.5±0.36	13.3±0.46*

Statistical significance: *** $P<0.001$, ** $P<0.01$, *$P<0.05$, ns $P>0.05$

Beneath the ciliated abfrontal cells there are mucus secretory cells (Fig. 5a).

Gills retained their gross structure well even at the highest concentrations (25 and 50 mg/l). The postlateral cells and abfrontal mucus secretory cells were swollen. There was an acute inflammatory reaction in the exposed mussels. The branchial veins were dilated and there were inflammatory cells present in the blood spaces as well as between epithelial cells (Fig. 5b). There were occasionally hydropic degeneration of mucus secretory cells and necrosis of hemocytes. Cells sloughed away from the chitinous rod. The incidence of these changes is shown in Table 1. There were no differences between the group prepared immediately after the exposure and the group prepared two weeks after the exposure.

The mucus secretory cells were often seen to rupture outside the gill filament (Fig. 5c). The differences between test groups and the control group in regard to mucus secretory cell diameter is shown in Table 1.

The volume of the branchial veins increased in the exposed gills. The volume of different cell groups (frontal, lateral, epithelial and abfrontal) did not change. A comparison (Mann-Whitney *U*-test) of the branchial vein volume in control and treated groups gave the following significances:

0.5 mg/l — U=32.5 ns 25 mg/l — U=8***
5 mg/l — U= 14*** 50 mg/l — U=5***.

4. Discussion

The period of acute mortality after zinc exposure lasted for four weeks before the LC 50 curve turned asymptotically with the time axis (Fig. 2). A reliable LC 50 value is obtained after this threshold point. Aquarium conditions cause lowering of the condition index and weight (Bayne & Thompson 1970), but since mortality in the control group was low, even this long observation period could be used. The LC 50 value in brackish water was significantly higher than the value measured in sea water. Ahsanullah (1976) reports LC 50 (96 h) values of below 5 mg/l for zinc in Australian waters. High LC 50 values have also been reported for copper and cadmium in brackish water (Sunila 1981). The availability of metals to filtering animals is lower in brackish water, because metals become bound e.g. to the surface of active humic acid complexes (Nelson & Donkin 1985). The toxicity of metals is dependent on the quality of metal complexes (Coombs & George 1978). However, heavy metals are more toxic to the molluscs of the Baltic Sea at lower salinities than at higher salinities (Förstner 1981b).

In this study, the sublethal effects of zinc could be detected in low concentrations (byssogenesis EC 50 0.64 mg/l, opening response EC 50 1.35 mg/l), but the lethal concentration was very high (LC 50 20.8 mg/l). Martin et al.(1975) found, by counting the number of byssal threads produced after cutting them away, that the EC 50 value for zinc after a seven day exposure in sea water was 1.8 mg/l. Martin et al. (1981) measured the development of valves in embryos and the EC 50 (48 h) was 0.175 mg/l. Embryos are more sensitive to heavy metals than adults (Calabrese et al. 1977). The EC 50 for the rate of growth was 0.06 mg/l (Strömgren 1982). the byssogenesis test is considered to be suitable for describing sublethal processes (Martin et al. 1975). The EC 50 values for copper and cadmium in brackish water were lower than that for zinc (Cu 0.2 mg/l, Cd 0.5 mg/l) (Sunila 1981). Sublethal concentrations of zinc in polluted waters

are probable (Wittmann 1981) and can in the long run alter the ability of animals to reproduce, catch food or attach themselves to the bottom. Concentrations as high as the LC 50 value have not been reported in natural waters, but values of the same magnitude as the EC 50 values of this study have been reported from polluted areas of the sea and brackish water.

The closure of the valves in response to foreign substances in the environment is a typical reaction of the mussels (Clarke 1947). The EC 50 value for this phenomenon seems to be dependent on the ability of the mussels to sense the foreign substance. For instance, the mussels did not recognize at low concentrations the presence of cadmium, which was considerably more poisonous to them than zinc (LC 50 value for Cd in brackish water 4.0 mg/l, EC 50 value for the opening response 12.5 mg/l, Sunila 1981).

The production of mucus in heavy metal poisoning is common in mussels (George & Pirie 1980, Sunila 1981, Lobel 1981). Lobel (1981) suggested that mucus production could be one way of inhibiting metal absorption into the mussel through the epithelia when iron is concerned. Since the production of mucus began only when the exposure to zinc was over, it appeared to be either a method of excretion or part of the inflammatory reaction.

The histological changes in the gills were mostly due to the inflammatory reaction. Swelling may be caused by disturbances in osmotic balance. Ion exchange and permeability to fluid are changed when zinc disturbs the function of the membranes (Viarengo 1985). Cadmium exposure also increases the volume of the branchial veins (Sunila 1986). Diapedesis is one of the defence mechanisms in molluscs. Phagocytic cells eliminate foreign material and cellular debris (Tripp 1974). The inflammatory reaction, detachment of cells and presence of a few necrotic mucus secretory and inflammatory cells were typical to acute zinc poisoning. The changes resemble those described after copper and cadmium exposure (Sunila 1981), but they appear in much higher concentrations. Secretory cells (postlateral and abfrontal) seemed to void their contents after the exposure. Morphometric studies showed that these cells were swollen after exposure. These cells must serve in defense or excretory mechanisms. Two weeks after the exposure to high concentrations (25 and 50 mg/l) the volume of these cells was smaller again, an intensive draining of the cells being noted prior to this.

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