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Toxicity of copper and cadmium to Mytilus edulis L. (Bivalvia) in brackish water

Inke Sunila

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The effects of short term (24 h) exposure to copper and cadmium on the survival and subsequent physiology of the common mussel *Mytilus edulis* were studied in brackish water in aquaria. The LC 50 (6 °C) for copper was 0.4 ppm and for cadmium 4.0 ppm. Activity was defined as the rate of opening of the valves under exposure (EC 50: Cu 0.06 ppm, Cd 12.5 ppm), the growing capacity of the byssal apparatus, byssogenesis (EC 50: Cu 0.2 ppm, Cd 0.5 ppm), and the beating of the cilia (EC 50: Cu 1.4 ppm, Cd 5.1 ppm). In copper solutions, but not in cadmium, the rate of opening of the valves showed a negative regression with the concentration. The proportion of mussels that opened their valves under cadmium exposure correlated with the survival rate. In copper solutions the correlation was not significant, since already very low concentrations resulted in closure. There were differences between the tolerance of different populations to these metals. Small individuals were more resistant to cadmium and more sensitive to copper than large ones. No changes in histological sections from the digestive glands of exposed bivalves could be observed in light microscope studies. Mucus accumulated on the surface of the siphons of mussels exposed to copper. Cadmium caused loosening of epithelial cells. Gill filaments parted from each other after breaking of the interfilamentar junction.

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1. Introduction

Half the copper and cadmium contents of the Baltic Sea enter by way of rain fall, the remainder entering via rivers. No clear distribution to different parts of the sea can be observed: the Gulf of Finland does not differ from the other parts of the Baltic. The copper content varies from 1.0 to 10 µg/l and cadmium from 0.1 to 2.0 µg/l (Jankovsky et al. 1978). High concentrations are found near the outlets of rivers into which industrial waste waters have been discharged (Sen Gupta 1972, Westernhagen et al. 1978).

In the surface layers heavy metal concentrations are usually low; metals are absorbed by organisms or precipitate, forming sediments (Kremling 1978, Nordberg 1974). In ground waters heavy metals concentrate and again move through sediment to water through diagenesis (Elderfield & Hepworth 1975, Brzezińska 1978). This return of heavy metals to the water via sediments causes fluctuations in concentrations (Helz et al. 1974).

In seawater heavy metals undergo chelation, forming organometallic complexes or salts with dissolved anions. Cadmium is absorbed mainly into humus, whereas copper precipitates as the carbonate, $CuCO_3$ (Bryan 1971).

Mytilus edulis readily accumulates heavy metals (Portmann 1971, Pentreath 1973, Eustace 1974, Schulz-Baldes 1974, Goldberg 1975, Alexander & Young 1976, Phillips 1976). The efficiency of accumulation can be shown as enrichment factors; e.g. the amount of copper in the mussel increases to 3 000 times and the amount of cadmium to 100 000 times higher than in seawater (Brooks & Rumsby 1965). Thus *Mytilus* is a useful indicator in heavy metal monitoring.

Bio-accumulation studies indicate contamination by a specific metal, but they do not alone indicate the condition of the organism and its surroundings. Information on the condition of the mussel can be obtained from measurements of mortality and activity.

Low salinity increases the toxicity of cadmium (George et al. 1977, Jackim et al. 1977) and

copper (MacInnes 1979). Higher concentrations are found in *Mytilus* in brackish waters, e.g. in the Gulf of Finland, than in the ocean (Phillips 1977a).

To study the toxicity of copper and cadmium on brackish water mussels, tests were performed in aquaria. Lethal concentrations (LC 50) were determined and the effects of these metals on activity were studied. Activity was defined as the rate of opening of the valves (the time interval between beginning of the exposure and opening of the valves), beating of the cilia and growing capacity of the byssal apparatus. In addition, samples of digestive glands, gills, and siphons were taken for histological examination.

2. Material and methods

Samples were collected from two mussel fields to study the differences between populations. The first field is located near Tvärminne Zoological Station in 6—7 $^0/_{00}$ seawater (depth 6—7 m). The other (Källvik) is located in the vicinity of the bay Pohjanpitäjänlahti. There is a vertical salinity gradient throughout the year as the result of rivers flowing into its head, the freshwater remaining in the surface layers (Niemi 1977), which limits the distribution of *Mytilus* in this area. The salinity near this sampling station varies from 5—6 $^0/_{00}$ at the bottom (6 m) to 1—4 $^0/_{00}$ in the surface layers (Halme 1944). During the sampling, salinity in Tvärminne was 6.5 $^0/_{00}$ and in Källvik 6.3 $^0/_{00}$.

Survival rate and opening of the valves

Two size classes were compared: 1.0—1.5 cm (age 2—4 years) and 2.0—2.5 cm (5—7 years); Källvik specimens varied from 2.0 to 2.7 cm. Before the aquaria experiments, mussels were tested by removing their byssi and using only those which attached themselves to boards with new byssi during the following 24 hours. The aquaria (20 × 20 × 25 cm) were aerated, each containing 100 individuals. The effects of copper were studied at concentrations of 0.1—5.0 ppm ($CuSO_4.3H_2O$ analytical grade) and cadmium at 0.5—25 ppm (3 $CdSO_4.8H_2O$ analytical grade) in natural brackish water (7 $^0/_{00}$, 6 °C). Mussels were fastened on boards at the bottom of the aquaria. The exposure time was 24 hours, during which the opening of the valves was observed. After exposure the mussels were moved with their boards into clean water. The opening of the valves was again observed for one hour. Thereafter the aquaria were checked daily for three weeks and dead bivalves removed. The water was changed every second day. To study the effects of the breeding cycle, concentrations of 0.3—0.4 ppm Cu and 3—4 ppm Cd were tested after spawning in August, and other concentrations during spawning in June. Brackish water used with copper concentrations of 0.2 and 2.0 ppm was filtered (Whatman 1). Experiments were always begun in the morning to avoid the effects of diurnal rhythm.

Byssogenesis

Byssal filaments were removed with scissors and the mussels placed on boards in the aquaria (17 °C). Concentrations of 0.2—2.0 ppm Cu and 0.2—10 ppm Cd were tested. Samples were taken only from the Tvärminne population. Size classes of 1.0—1.5 cm and 2.0—2.5 cm were compared. Attachment was observed for 24 hours and again after 48 hours.

Ciliary activity

Fragments of gills (7 × 4 mm) were placed on petri dishes containing natural brackish water (7 $^0/_{00}$) with different concentrations of copper or cadmium (6 °C). The beating of the terminal cilia was observed under a stereomicroscope and the activity classified as follows:

3 activity normal
2 activity reduced; movement of single cilium noticeable
1 activity greatly reduced; accumulation of mucus
0 activity ceased

Histological studies

Gills, digestive glands and mantle edges (siphons) were prepared and studied after exposure (24 h), then after 11 and 21 days. Samples were fixed in Bouin's fluid. Sections 10 μm thick were stained with Masson-Gomori (digestive glands) or Grossmon's Hematoxylin — Acid fuchsin — Orange — Light green (gills and siphons) (Romeis 1968).

3. Results

3.1. Survival rate and opening of the valves

Control animals opened in three minutes. However, exposure to copper or cadmium resulted in closing of the valves. At the beginning of exposure, mussels first opened but then closed the valves almost immediately. Then the valves were again opened, the time interval between opening and the beginning of exposure depending on the concentration of the metal.

Even very small concentrations of copper (e.g. 0.1 ppm) resulted in closing of the valves. Cadmium had no immediate effect: mussels did not close until a concentration of 3 ppm was attained. The rate of opening of the valves in copper solutions, but not in cadmium solutions, showed a negative regression with the concentration (Cu: $b = -0.71^{**}$, Cd: $b = -0.07$). The number of mussels that opened during cadmium exposure anticipated the survival rate (Fig. 1). Correlation between these variables is significant ($r = 0.71$), while in copper it is not ($r = 0.34$). Thus in cadmium solutions about as

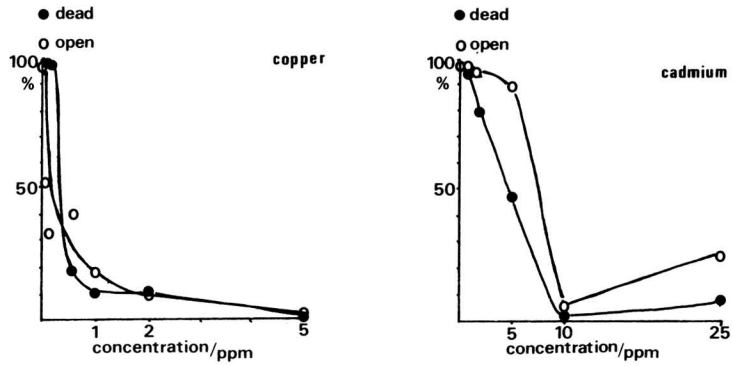


Fig. 1. The proportion of mussels that opened during 24 h exposure to copper or cadmium and the proportion of dead mussels after three weeks in clean sea water in relation to the exposure concentration (6 °C).

many died as opened under exposure. In copper solutions mussels closed their valves even in sublethal concentrations. The EC 50 (the concentration that causes reaction in 50 % of the test animals) for opening of the valves was 0.06 ppm for copper and 12.5 ppm for cadmium.

When moved to clean water, mussels exposed to copper opened immediately, independent of the exposure concentrations, and remained open like the control animals. After cadmium exposure the number of mussels that opened their valves remained the same as during exposure. Survival rates after copper and cadmium exposures during the following three weeks in clean sea water are illustrated in Fig. 2.

In copper solutions mortality increased after a lag of five to six days, but immediately in high cadmium concentrations. Small specimens were more sensitive to copper, but more tolerant to cadmium than large ones. Filtering of brackish water used with copper solutions increased the toxic effect. Mussels collected from Källvik were more tolerant to both copper and cadmium than mussels from Tvärminne (Fig. 3). The LC 50 values for Tvärminne bivalves did not effect the survival rate of Källvik mussels significantly. Spawned specimens were more sensitive to cadmium than specimens taken during spawning (Fig. 2).

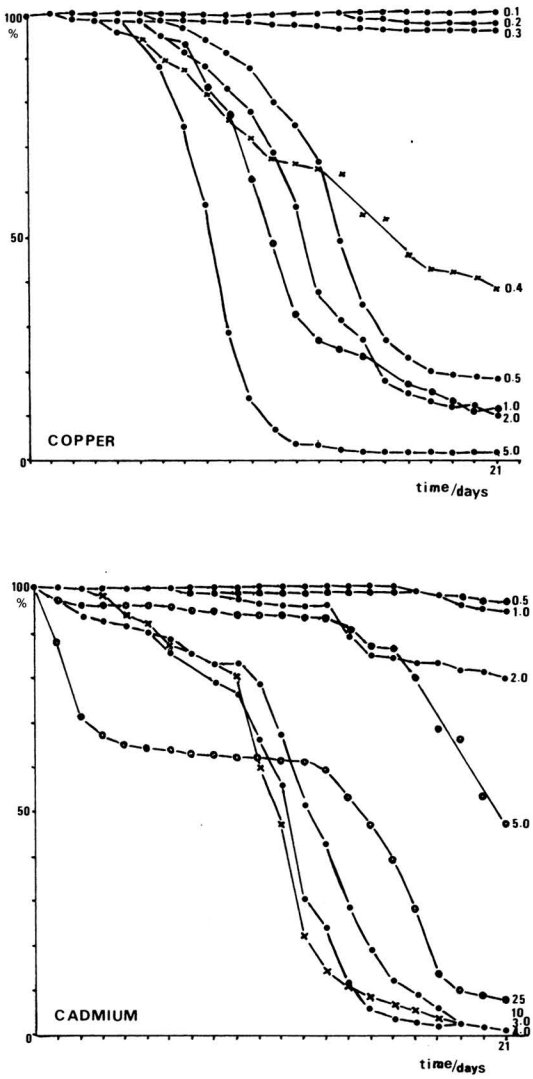
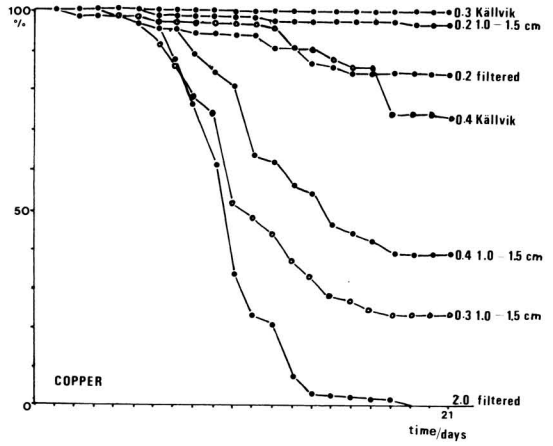


Fig. 2. Survival rates during the three weeks in clean sea water following exposure to copper and cadmium. Percentage of living mussels plotted against time (6 °C). 2.0—2.5 cm long specimens during breeding period, except mussels exposed to 3 and 4 ppm cadmium after spawning. Mussels were collected from Tvärminne.



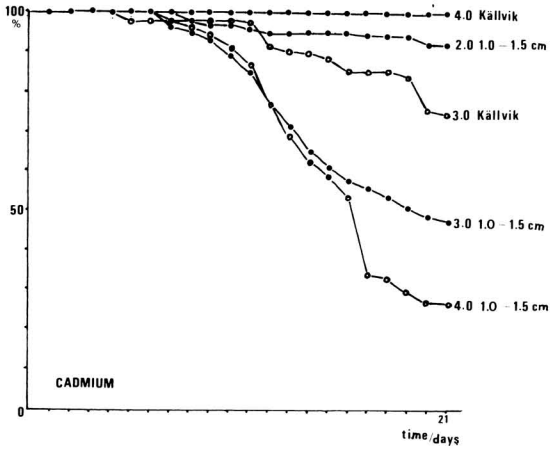


Fig. 3. Survival rates during the three weeks in clean sea water following exposure to copper and cadmium. Percentage of living mussels plotted against time (6 °C). Mussels were collected from Tvärminne, except those exposed to 0.3 and 0.4 ppm copper, and 3.0 and 4.0 ppm cadmium (Källvik). Källvik mussels were 2.0—2.7 cm long. Mussels exposed to 0.2 and 2.0 ppm copper in filtered sea water were 2.0—2.5 cm long and others 1.0—1.5 cm.

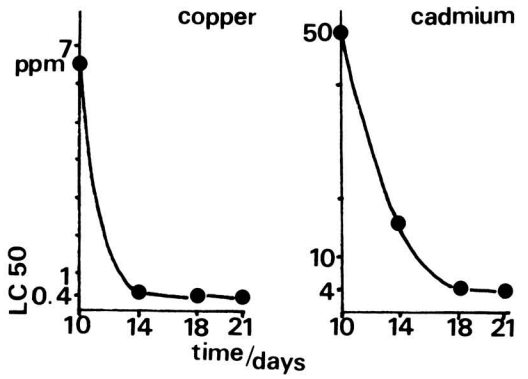


Fig. 4. Change in LC 50 values with time (6 °C) in clean sea water. Animals were exposed to heavy metals for 24 h.

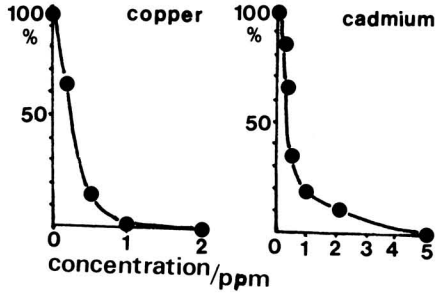


Fig. 5. Byssogenesis. Proportion of attached mussels in copper and cadmium solutions (17 °C).

The LC 50 values (6 °C) for the Tvärminne population were 0.4 ppm for copper and 4.0 ppm for cadmium. The LC 50 values in relation to time are illustrated in Fig. 4.

Specimens exposed to copper or cadmium secreted mucus. In copper solutions mucus was often blue and in cadmium solutions yellow.

Exposed mussels, unlike the control animals, did not move in the aquaria. After losing their byssal attachment, they could not fasten again. Some individuals exposed to cadmium did not withdraw their siphons when closing, but left them between the valves. In this compressed stage the siphons became everted and bleached, losing their normal brown or purple colour.

3.2. Byssogenesis

Most mussels grew byssi in two hours. Attachment began with opening of the valves, after which the foot was pushed out to feel the substrate. Small specimens grew byssi more quickly and a larger proportion of them became attached to the boards than did large ones. Copper and cadmium lowered the proportion of attached mussels, as shown in Fig. 5.

The EC 50 for copper was 0.2 ppm and for cadmium 0.5 ppm. Small individuals were more sensitive to copper and more resistant to cadmium than large ones. The shapes of curves illustrating attachment in copper and cadmium solutions differ (Fig. 6). In cadmium solutions most mussels

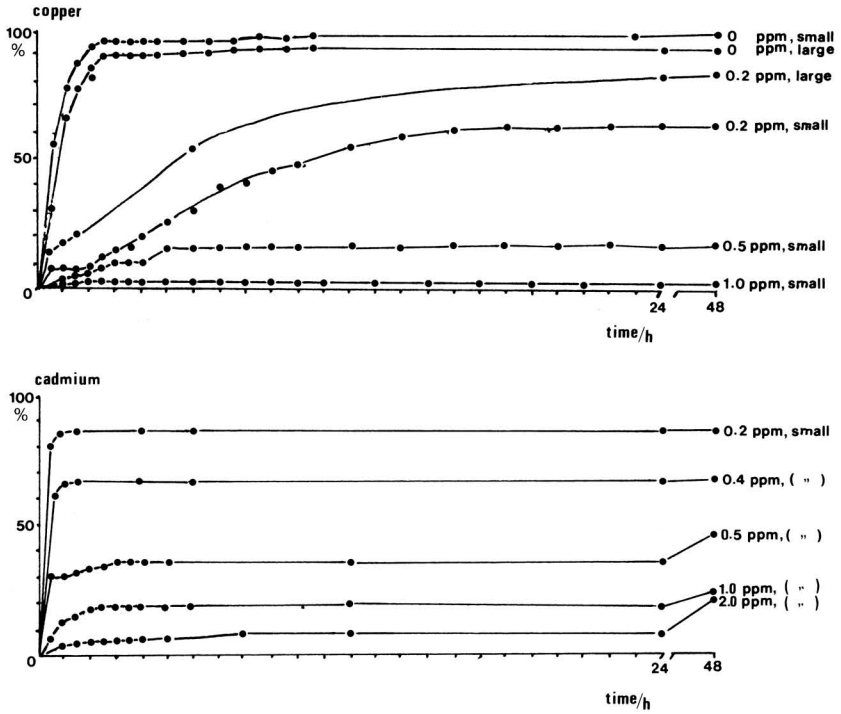


Fig. 6. Byssogenesis. Attachment during exposure to copper and cadmium solutions. Percentage of attached mussels plotted against time (17 °C).

became attached to the boards during the first two hours, while in copper solutions they attached themselves slowly, throughout the exposure.

3.3. Ciliary activity

The activity of the gill fragment cilia lasted 11 days in petri dishes containing clean seawater. Mean activity times and standard deviations in different concentrations are presented in Table 1.

Table 1. Ciliary activity times (h) in different concentrations (ppm) of copper and cadmium (mean ± *SD*).

Concentration	Activity time
Control	233.50 ± 32.54
0.2 Cu	162.15 ± 22.01
1.0 Cu	200.87 ± 15.03
5.0 Cu	10.80 ± 4.28
2.0 Cd	191.48 ± 18.61
5.0 Cd	78.10 ± 20.52
10 Cd	37.05 ± 12.75

Activity in relation to concentration and time is depicted in Fig. 7.

The amount of mucus on the gills increased with time. The cilia could not carry the mucus away, but remained beating underneath. Later mucus covered the terminal and frontal surfaces wholly. In gill fragments exposed to copper, the interfilamentar junctions broke; however, cilia remained beating on separate filaments. The LC 50 and EC 50 values of different tests are summarized in Table 2.

Table 2. LC 50 and EC 50 values for different activities in copper and cadmium solutions.

	LC 50 (6 °C)	EC 50 (17 °C) byssogenesis	EC 50 (6 °C) opening of the valves	EC 50 (6 °C) activity of the cilia
Copper	0.4	0.2	0.06	1.4
Cadmium	4.0	0.5	12.5	5.1

3.4. Histological studies

No changes in the digestive glands could be observed. The mantle edges, siphons, are covered

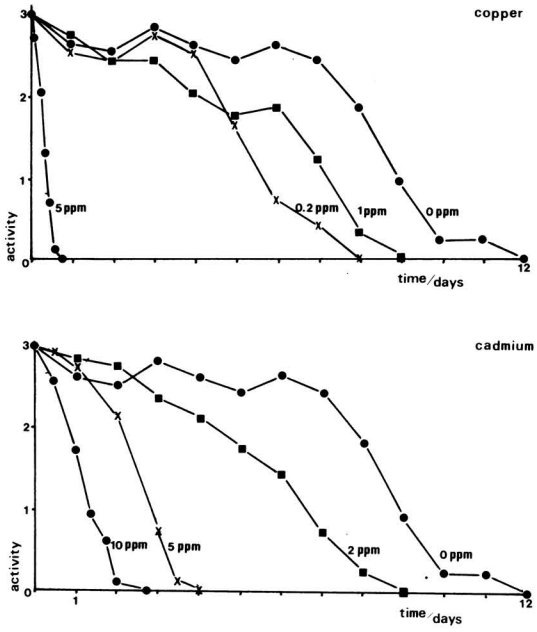


Fig. 7. The activity of the cilia of excised gills during exposure to heavy metal solutions in relation to the concentration and time (6 °C).

by folded, simple epithelium (Fig. 8a). Goblet cells appear between epithelial cells; in the tissue underneath there are glandular cells and muscle fibres. In 0.5 and 2 ppm copper the epithelium was covered during the first 24 hours by a mucous sheet that did not disappear for three weeks (Fig. 8b). In 5 ppm cadmium cilia lost their synchronization and were covered by particles. After 11 days they were covered by mucus and the epithelium was loose in places. In 10 ppm cadmium the epithelium broke during the first 24 hours. The folded configuration disappeared, epithelium loosened in large cell layers (Fig. 8d) or by one cell at a time (Fig. 8c). Where epithelium was present, cilia were disordered and covered by mucus.

There are two gills on each side of the visceral mass, each consisting of two lamellae which are further comprised of filaments joined by ciliary junctions (Fig. 8e and 8f). Lamellae are joined to each other by cellular junctions (Fig. 9a). Gills are covered by ciliary epithelium. Different types of cilia have been described, e.g. by Dral (1967) and White (1937) (Fig. 9b). In 0.5 ppm copper part of the interfilamentar junctions broke during exposure (Fig. 9c). At the same time cells became detached from the epithelium (Fig. 9d). In 2 ppm copper the inner parts of the filaments swelled due to vacuolization (Fig. 9e). In 5 ppm cadmium there was no effect. In 10 ppm cadmium the interfilamentar junctions of some individuals broke. Later the epithelium broke up and the inner parts of the filaments disappeared completely; the outer parts of the filaments became flattened (Fig. 9f).

4. Discussion

After 24 h exposures to copper and cadmium mortality is preceded by a lag period. Following this, the period of acute mortality lasts over two weeks, after which the LC 50 curves turn asymptotically with the time axis (Fig. 4). This point is the threshold concentration and reliable LC 50 values are not obtained until then (Bryan 1971). Thus long observation periods must be used, balancing the effects of laboratory conditions and those of exposure. Aquarium conditions cause lowering of the condition index and weight (Bayne & Thompson 1970).

The opening of the valves at the beginning of exposure cannot be considered an accurate method for determining toxicity, since different metals result in specific responses. In addition, the moment of opening of the valves also depends on factors other than the concentration, e.g. the amount of material available for fermentation in the anaerobic state. The opening of the valves after exposure likewise results in a specific reaction to each metal. Davenport (1977) has studied the opening of the valves under heavy metal stress using an automatic recording apparatus. On entering a copper solution a mussel first tests the quality of the water by alternately opening and closing the valves. Closing is obviously a behavioural mechanism for protection against poisoning. Finally breathing stress forces the mussel to open.

The present results are in agreement with those of Martin et al. (1975), who reported an EC 50 for byssogenesis under exposure to a copper concentration of 0.25 ppm (LC 50 0.3 ppm) and for cadmium 0.5 ppm (LC 50 2.5 ppm).

Byssogenesis tests are suitable for monitoring purposes; reliable results are obtained after a 24-hour observation period. Standardized conditions are required, since water movements, salinity, oxygen and temperature are among the factors which affect byssogenesis (Roberts 1975). Mussels produce more filaments when in groups than when alone, and more are produced at night than during the day (Martella 1974).

Preparation of the gills in ciliary activity tests

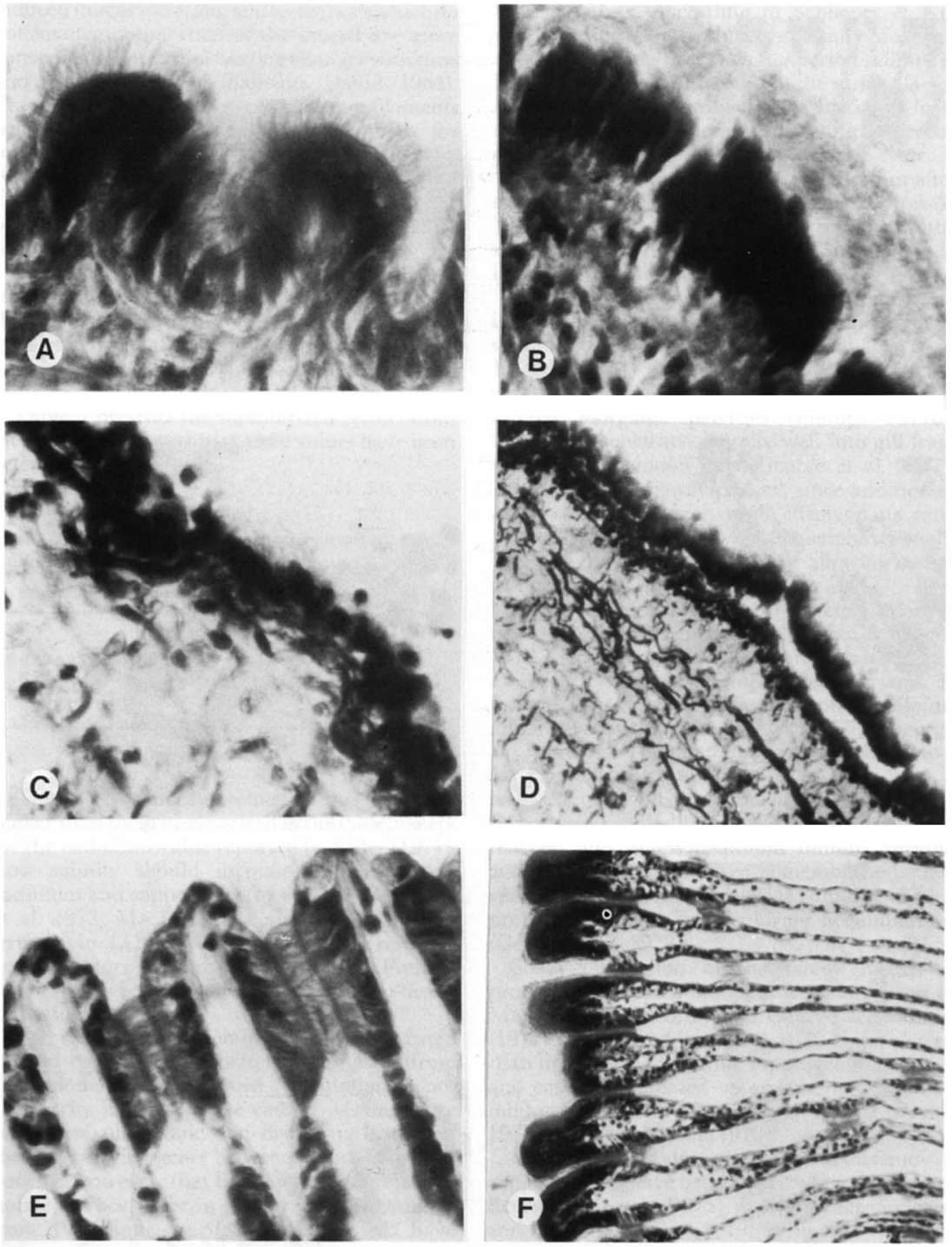


Fig. 8. Histological effects of copper and cadmium on the siphons and gills of *Mytilus edulis*. Sections are stained with Grossmon's Hematoxylin — Acid fuchsin — Orange — Light green. A. Siphons are covered by simple epithelium (× 1000). B. Siphons exposed to copper are covered by mucus (× 1000). C. Epithelium breaks in siphons exposed to cadmium (× 1000). D. In places epithelium breaks up as cell layers in siphons exposed to cadmium (× 250). E. Gill filaments are joined to each other by ciliary junctions (× 1000). F. A longitudinal section of the interfilamentar junctions (× 250).

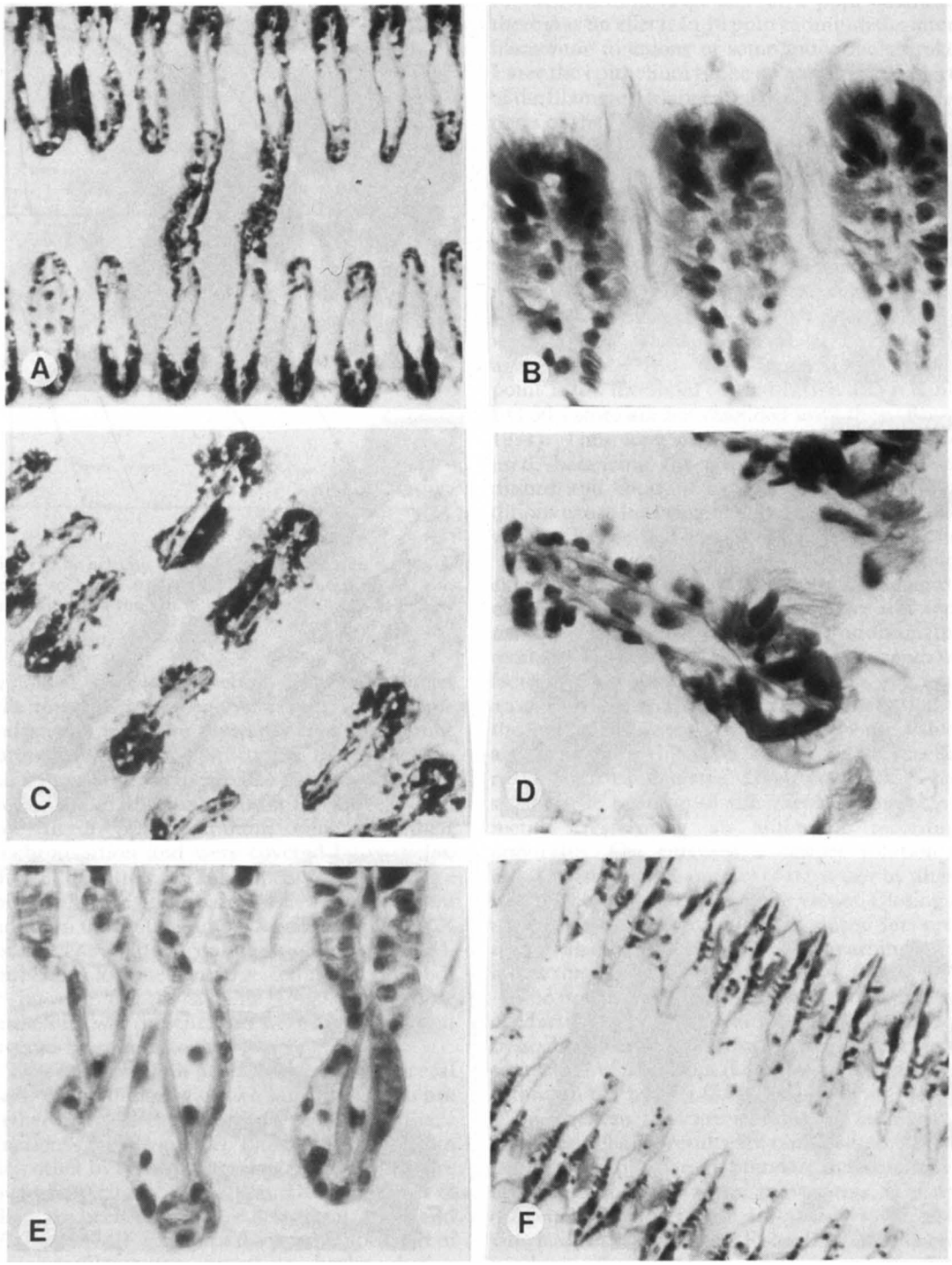


Fig. 9. Histological studies of copper and cadmium on the gills of *Mytilus edulis*. Sections are stained with Grossmon's Hematoxylin — Acid fuchsin — Orange — Light green. A. Gill lamellae are joined to each other by cellular junctions (× 400). B. There are different types of cilia in the epithelium (× 1000). C. Interfilamentar junctions break under exposure to copper (× 400). D. Epithelium breaks under exposure to copper (× 1000). E. Epithelial cells swell due to vacuolization under exposure to copper (× 1000). F. Epithelium breaks and outer parts of the filaments flattened under exposure to cadmium (× 400).

induced mucus secretion, and so methods which do not involve preparation of the mussel are more convenient. The rate of beating changes with time and also within single filaments (Davids 1964). Fragments of gills or even separate filaments behave as entire gills. Different methods for measuring the activity of the cilia are described by Nomura & Tomita (1933), Davids (1964), Theede & Lassig (1967), McDowell Capuzzo & Sasner (1977), Sturesson (1978), and Riisgård & Møhlenberg (1979).

In every test small individuals were more sensitive to copper and more tolerant to cadmium than large ones. More copper is accumulated per mass unit by small individuals, but there are no differences between size classes in the case of cadmium (Boyden 1974).

Table 3 presents the summarized results from those references in which LC 50 values have been determined.

Table 3. Reported LC 50 values (ppm) for copper and cadmium.

Copper	Cadmium	Reference
	1.62	Ahsanullah 1976
	1.5	Talbot & Magee 1976
0.2—0.3		Delhaye & Cornet 1975
0.3	2.5	Martin et al. 1975
	25	Eisler 1971
0.4	4.0	This study

The LC 50 values obtained in this study are higher than those summarized in the table, except for the cadmium value reported by Eisler (1971). Low salinity should increase the toxicity of cadmium and copper (George et al. 1977, Jackim et al. 1977, MacInnes & Calabrese 1979). Differences in LC 50 values might be a result of genetic factors. Mussels from the Gulf of Finland might have become adapted to higher heavy metal loads.

The mussel population from Källvik tolerated the test concentrations better than mussels from Tvärminne. Use of the word "population" is not necessarily justified in the case of *Mytilus,* since larvae are planktonic and drift long distances, thereby carrying genes between populations. It is possible, however, that these two populations are isolated. They differ in appearance: the mussels from Tvärminne are darker, flatter, and have thinner valves. According to Schlieper & Kowalski (1956) *Mytilus* requires a salinity of at least 4—6 ‰. During the spawning period salinity in Källvik varies from 1 to 4 ‰ in the surface layers (Halme 1944). Planktonic larvae drifting in from the sea might not tolerate this salinity, thereby encountering a reproduction barrier.

Mussels were more sensitive to cadmium after spawning than during spawning (Sturesson 1978). Delhaye & Cornet (1975) stated that the spawning period is the most sensitive, due to increased metabolism.

The intake of copper and cadmium by *Mytilus* is directly proportional to the concentration in solution (George et al. 1977, Westernhagen et al. 1978, d'Silva & Qasim 1979). Metals are absorbed from seawater through food or infiltered inorganic particles (Phillips 1977b). Cadmium penetrates equally well into gill fragments as into intact gills (George et al. 1977). Transport is obviously passive, since addition of metabolic inhibitors has no effect on its rate. Mucus, a complex carbohydrate-sulphate, might act as an ion exchanger, thereby allowing metals in through the gills (Cunningham 1979).

Copper and cadmium form a metallothionein in *Mytilus* (Noël-Lambot 1976, Talbot & Magee 1978, George et al. 1979). Cadmium also has an affinity for sulfhydryl- and imidazol groups. It reacts with polythiols, albumins, phospholipids, and nucleic acids (Vallee & Ulmer 1972). George & Coombs (1977) stated that cadmium binds to a carrier before transport to cytoplasm and is transferred to a metallothionein. Toxic effects are therefore prevented before the saturation point is reached and excess cadmium inhibits enzyme action. Copper is mobilized in membrane-bound vesicles in the oyster as sulphur complexes. This probably protects tissues during accumulation (George et al. 1978).

Some proportion of the heavy metals is probably eliminated in mucus secretion (Scott & Major 1972). According to George & Coombs (1977) secretion of cadmium is 18 times slower than its uptake. Metabolic suppression of copper and cadmium is based on enzyme inhibition or inhibition of ciliary activity by mucus (Bryan 1971, Brown & Newell 1972).

Effects similar to those described in this study of copper on gills have been described by others, e.g. Betzer & Yevich (1975) in *Busycon canaliculatum* and Engel & Fowler (1979) in oysters.

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