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SHORT-TERM EFFECTS OF METALS ON THE FILTRATION RATE OF THE ZEBRA MUSSEL *Dreissena polymorpha*

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Abstract

In order to study the short-term ecotoxicity of metals to the freshwater mussel Dreissena polymorpha, *the effects of Cu, Zn and Cd on the filtration rate of this mussel were determined in laboratory experiments. Filtration rate was chosen as the endpoint, because it is a sensitive sublethal parameter compared to mortality and it is an important parameter given the ecological role* D. polymorpha *fulfills. The filtration rate was calculated from the decrease in algal concentration, fed to mussels in aquaria, containing different metal concentrations. The* EC_{50} *for Cu (41 μg litre*$^{-1}$*) was lower than for Cd (388 μg litre*$^{-1}$*) and Zn (1350 μg litre*$^{-1}$*). The* $NOEC_{accumulation}$ *for the essential metal Zn was higher than for the essential metal Cu. Cadmium, a non-essential metal, was accumulated at all elevated water concentrations, so the* $NOEC_{accumulation}$ *was the concentration in the control water (<0·2 μg litre*$^{-1}$*). All (no)effect concentrations found in this study were above the quality criteria set for metal concentrations in Dutch surface water, suggesting that the zebra mussel is sufficiently protected by these quality criteria.*

Keywords: filtration rate, zebra mussel, *Dreissena polymorpha,* metal accumulation.

INTRODUCTION

The zebra mussel *Dreissena polymorpha* plays an important role in various freshwater ecosystems. It is the main source of food for benthivorous fish, such as roach (*Rutilus rutilus*), and for diving ducks (Stanczykowska, 1977; Suter, 1982) which winter in large quantities in the Netherlands. Zebra mussels are able to reduce high phytoplankton abundances by their high filtration activity (Stanczykowska *et al.*, 1975; Reeders *et al.*, 1989). *D.polymorpha* was chosen as a test organism for ecotoxicological laboratory experiments as adverse effects of toxicants on this mussel may affect the aquatic foodchain (Scholten *et al.*, 1989). *D. polymorpha* also plays an important role in the detection of pollutants: it has been used in early warning systems (Kramer *et al.*, 1989) and as a biomonitoring organism (Kraak *et al.*, 1991).

Metals are known to reduce the performance of bivalve molluscs, including *D. polymorpha*. In the presence of elevated metal concentrations, bivalve molluscs keep their shells closed for a longer period of time (Slooff *et al.*, 1983; Doherty *et al.*, 1987; Kramer *et al.*, 1989), produce fewer byssus threads (Martin *et al.*, 1975) and have reduced heart rates (Akberali & Black, 1980; Grace & Gainey, 1987). It has been demonstrated for some marine bivalves, that metals reduce their filtration rate (Watling, 1981; Grace & Gainey, 1987; Redpath & Davenport, 1988).

In these experiments, the authors determined the effects of Cu, Zn and Cd on the filtration rates of the freshwater mussel *D. polymorpha*. The EC_{50} values for filtration rate of marine bivalves are often considerably lower than the LC_{50} values (Abel, 1976; Watling, 1981). So, filtration rate is both a sensitive and relevant parameter considering the ecological role *D. polymorpha* fulfills.

Amiard *et al.* (1987) demonstrated that the marine mussels *Mytilus edulis* and *Scrobicularia plana* were capable of regulating the body concentration of the essential metal Zn. Copper, which is also an essential element, could be regulated by *M. edulis*, but not by *S. plana*. Cadmium, a non-essential element, could not be regulated at all. In our study metal accumulation in the mussels was determined in the same experiments in which the effects on filtration rate were determined.

MATERIALS AND METHODS

Zebra mussels (*Dreissena polymorpha* (Pallas)) and water were collected from Lake Markermeer (The Netherlands), a relatively clean location (Kraak *et al.*, 1991). The mussels were picked from the stones of the dike. The water was filtered (50 μm) and kept in a storage barrel from which it was pumped continuously over a sand filter. The mussels were sorted by length (1·6–2·0 cm) and distributed over the experimental treatments. The average mussel length did not differ between treatments. An experimental treatment consisted of 25 mussels placed

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in a plastic aquarium (12·5 litre), containing 3 litres of filtered (0·45 μm) lake water. Water temperature was kept at 15°C, hardness was 150 mg CaO litre^{-1} and pH was 7·9. The water was aerated and always air-saturated with oxygen. Evaporation of water was reduced by covering the aquaria with sheets of glass. The light/dark regime was synchronized with the actual natural day/night rhythm, but was kept constant during each experiment.

The following concentrations of metals were tested: Zn: 9 (control), 200, 500, 1000, 2000, 5000 and 10000 μg litre^{-1}; Cd: <0·2 (control), 100, 200, 500, 750, 1000, 2000 and 5000 μg litre^{-1}; and Cu: 3 (control), 10, 20, 50, 100, 200, 500 and 1000 μg litre^{-1}. Stock solutions of 1000 mg litre^{-1} $CuCl_2$, $ZnCl_2$ and $CdCl_2$ were applied. The experimental set-up allowed four trials to run simultaneously, one of them being a control. There were two replicates per treatment and a total number of 800 mussels were tested. The aquaria were randomized within each trial.

Metal was added to the aquaria on the day after collection of the mussels. After 24 and 48 h, water was renewed and metals were added again. After 48 h of exposure, algae were added and filtration rates were determined. Water samples were taken just before and after water renewal and analyzed for metal by flame or furnace AAS. The actual metal concentration to which the animals were exposed during the experiments were determined from these values, using integral calculus.

To determine the filtration rate, mussels were fed with the unicellular green algae *Chlamydomonas eugametos* (30 000 cells per ml) at time t = 48 h. The algal concentration decreased, due to the filtration activity of the mussels. Pilot experiments showed that in this experimental set-up sedimentation of algae and cell division played an insignificant role. The decrease was determined by taking three water samples (2ml) from each aquarium at 0, 10, 20, 30 and 40 min after addition of the algae. The algal concentrations in the water samples were measured using a Coulter Counter. The filtration rate was calculated from the decrease in algal concentration, according to Coughlans (1969) formula:

$$m = \frac{M}{nt} \ln \frac{C_0}{C_t}$$

in which

m = filtration rate (ml mussel^{-1} h^{-1}),
M = volume of the test solution (3000 ml),
n = number of animals per aquarium (25),
t = duration of the experiment (h),
C_0 = algal concentration at the beginning of the determination of the filtration rate, and
C_t = algal concentration at time t.

The filtration rates of the experimental treatments were expressed as a percentage of the filtration rates in the corresponding controls. The results are given as dose–response relationships, from which the EC_{50} values were calculated by probit analysis (Finney, 1971) and the NOEC values using Williams' test (Williams, 1971).

After completing the experiments, the soft tissues without byssus threads of five mussels from each aquarium were placed individually in 2·2-ml polyethylene tubes, freeze-dried, weighed and dissolved by wet digestion using nitric acid and hydrogen peroxide (Timmermans *et al.*, 1989). Finally, the soft tissues were analyzed for each metal by flame or furnace AAS following Timmermans *et al.*. (1989). Quality control of metal analysis was performed using digestion blanks and reference material (IAEA shrimp MA-A-3/TM and IAEA simulated freshwater W-4). The measured values were in good agreement with the certified values (<10% deviation). To test if the metal concentrations in exposed mussels differed from the metal concentrations in the controls, Bartlett's test for homogeneity of variances, one-way analysis of variance and Scheffe's test for *a posteriori* comparison of means, respectively, were used.

RESULTS

Mortality was low and independent of metal concentrations. Filtration rates in the controls were approximately 100 ml mussel^{-1} h^{-1} and sometimes values up to 200 ml mussel^{-1} h^{-1} were measured. In Figs 1–3 the filtration rate of *D. polymorpha*, expressed as a percentage of the corresponding control, is plotted against the actual metal concentration in the water. A clear dose-dependent response was observed for all three metals. At high concentrations almost all animals closed their valves and the filtration rate fell to zero. *D. polymorpha* appeared to be sensitive to Cu, while Zn and Cd affected the filtration rate only at relatively high concentrations. The $EC_{50filtration}$ values (48 h) for Cu, Zn and Cd were 41, 1350 and 388 μg litre^{-1}, respectively. The $NOEC_{filtration}$ values were 16, 191 and 175 μg litre^{-1}, respectively.

In Figs 4–6 the metal concentration in the mussels at the end of the exposure period is plotted against metal concentration in the water. At low Cu and Zn concentrations in the water, the Cu and Zn concentrations in the mussels did not differ from the controls. The

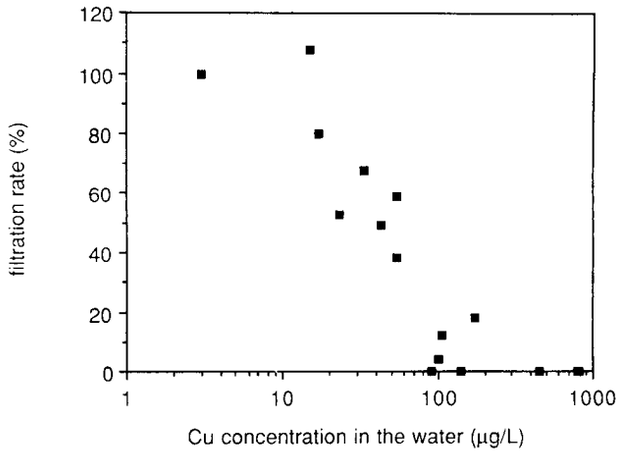

Fig. 1. Filtration rate of *Dreissena polymorpha* (%) at different Cu concentrations in the water (μg litre^{-1}).

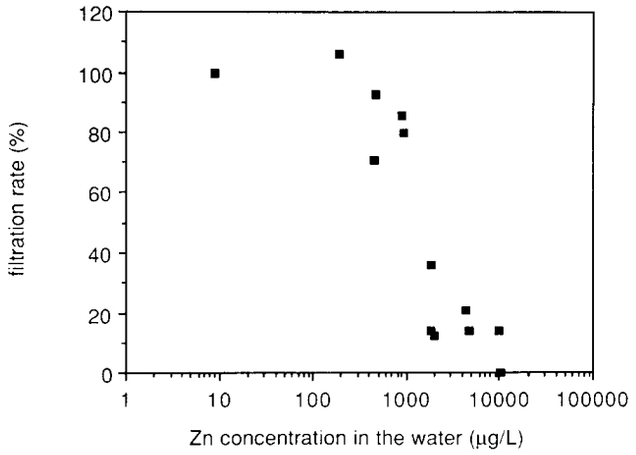

Fig. 2. Filtration rate of *Dreissena polymorpha* (%) at different Zn concentrations in the water (μg litre^{-1}).

$NOEC_{accumulation}$ can be defined as the highest metal concentration in the water which did not result in a significant ($p < 0.05$) increase of the metal concentration in the mussels. Above this concentration, accumulation of metal takes place in the mussels. In the case of Cu and Zn, the $NOEC_{accumulation}$ values were 28μg litre^{-1}, and 191μg litre^{-1}, respectively. The Cd concentration in all exposed mussels differed significantly ($p < 0.05$) from the controls, so the $NOEC_{accumulation}$ was the concentration in the control water (<0·2 μg litre^{-1}). Table 1 summarizes the main results of these experiments.

DISCUSSION

Experimental considerations

Several factors that influence the filtration rate of *D. polymorpha* were taken into account in the experimental set-up.

Filtration rate increases with increasing temperature (Morton, 1971; Hinz & Scheil, 1972), so temperature was kept constant at 15°C. This temperature guarantees relatively high activity in the mussel, but is still beneath the optimum temperature for reproduction of *D. polymorpha* (Sprung, 1987).

The type, size and concentration of food particles in the test solution influences the filtration rate. In these experiments *Chlamydomonas eugametos* was used as the food source. *Chlamydomonas* species are highly preferred by *D. polymorpha* (Morton, 1971; Sprung & Rose, 1988), leading to filtration rates of about 100 ml mussel^{-1} h^{-1} and sometimes up to 200 ml mussel^{-1} h^{-1} in the controls, which is at the top end of the range found in the literature: Benedens and Hinz (1980) reported only 7 ml mussel^{-1} h^{-1}; Hinz and Scheil (1972), 71 ml mussel^{-1} h^{-1}; Walz (1978), 77 ml mussel^{-1} h^{-1}. Morton (1971) reported 180 ml mussel^{-1} h^{-1} with *Chlamydomonas globosa* as a food source, which was much higher than with any other algal species. Sprung and Rose (1988) demonstrated a maximum of 230 ml mussel^{-1} h^{-1}, using *Chlamydomonas reinhardii* as the food source.

High food concentrations decrease the filtration rate (Morton, 1971; Walz, 1978; Sprung & Rose, 1988). A decrease to lower food concentrations during filtration can increase the filtration rate. This was demonstrated

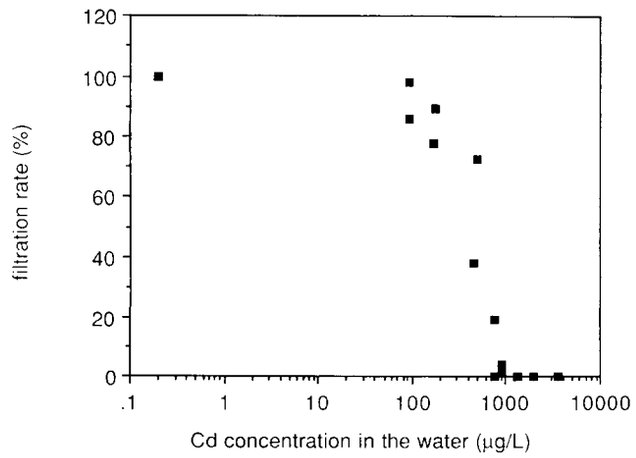

Fig. 3. Filtration rate of *Dreissena polymorpha* (%) at different Cd concentrations in the water (μg litre^{-1}).

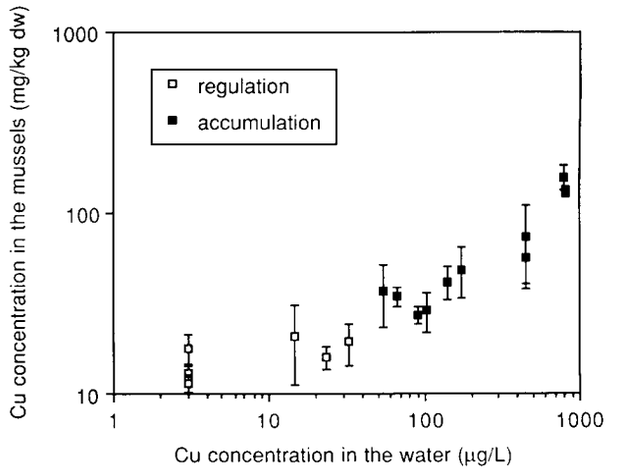

Fig. 4. Cu concentration in *Dreissena polymorpha* (mg kg^{-1} dw) at different Cu concentrations in the water (μg litre^{-1}). □, not significantly ($p > 0.05$) different from the controls; ■, significantly different ($p < 0.05$) from the controls.

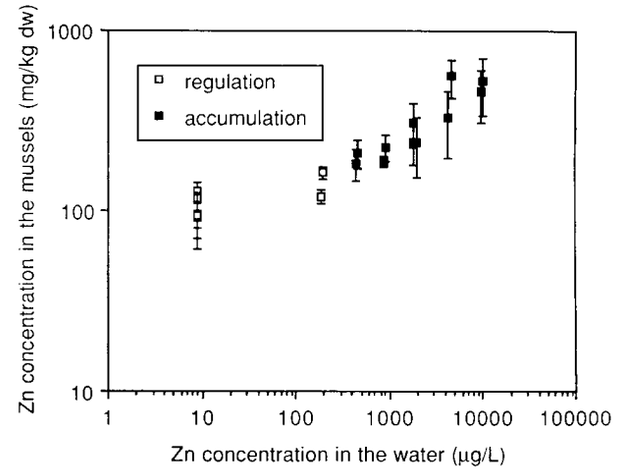

Fig. 5. Zn concentration in *Dreissena polymorpha* (mg kg^{-1} dw) at different Zn concentrations in the water (μg litre^{-1}): □, not significantly ($p > 0.05$) different from the controls; ■, significantly different ($p < 0.05$) from the controls.

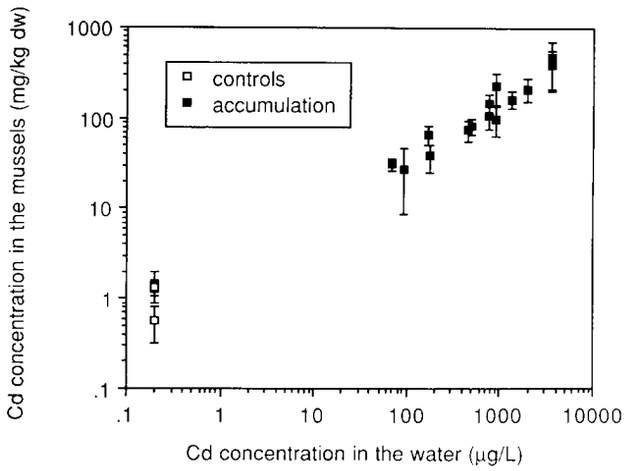

Fig. 6. Cd concentration in *Dreissena polymorpha* (mg kg^{-1} dw) at different Cd concentrations in the water (μg litre^{-1}). □, controls; ■, significantly different ($p < 0.05$) from the controls.

in pilot experiments and for *Mytilus edulis* by Davids (1964). In the experiments described here the algal concentration was about 30 000 cells ml^{-1}, resulting in a constant filtration rate in the controls for a minimum of 45 min.

In the experiments described here the metals were added to the water, while the algae came from a clean culture. This choice is supported by Borchardt (1983), Riisgård *et al.* (1987) and Pugsley *et al.* (1988). They demonstrated that metal uptake via food in both marine and freshwater mussels played an insignificant role. Moreover, algae were present for only 40 min at the end of the experiment.

In summary it can be seen that *D. polymorpha* appeared to be a suitable test organism for ecotoxicological laboratory experiments, using filtration rate as the endpoint. Very few deaths occurred and the filtration rate in the controls was relatively high.

Ecotoxicity and accumulation of metals

The results presented here demonstrate that filtration rate is a sensitive parameter for determining the effects of metals on *D. polymorpha*. In the case of Cu for example, even at the highest concentration tested, 1000 μg litre^{-1}, no mortality occurred after 48 h of exposure. So, the LC_{50} will be more than 25 times higher than the $EC_{50filtration}$ (41 μg litre^{-1}). Abel (1976) demonstrated for the marine mussel *Mytilus edulis* that the 96-h LC_{50} for Cu and Zn were 2 and 5 times higher than the $EC_{50filtration}$, immediately after addition of the metals. It may be concluded that sublethal parameters are far more realistic endpoints for ecotoxicological laboratory experiments than mortality.

Table 1. Comparison of the different effect criteria for Cu, Zn and Cd

	$NOEC_{accumulation}$ (μg litre^{-1})	$NOEC_{filtration}$ (μg litre^{-1})	$EC_{50filtration}$ (μg litre^{-1})
Cu	28	16	41
Zn	191	191	1350
Cd	<0·2	175	388

When the $EC_{50filtration}$ for Cu, Zn and Cd are compared (Table 1), Cu appears to affect filtration rates at low concentrations (EC_{50} 41 μg litre^{-1}). This is in agreement with results for marine bivalves (Abel, 1976; Watling, 1981; Redpath & Davenport, 1988). For marine bivalves, Zn often affects filtration rate at lower concentrations than Cd (Watling, 1981; Redpath & Davenport, 1988) which is in contrast with the results for *D. polymorpha* (EC_{50} 388 μg litre^{-1} Cd; 1350 μg litre^{-1} Zn).

In Figs 4 and 5 it can be seen that the concentration of Cu and Zn in *D. polymorpha* did not increase within a certain range of external concentrations. The Cd concentrations in all exposed mussels differed from the controls. Although the exposure period was only 48 h, it might be suggested that *D. polymorpha* is capable of regulating the body concentration of the essential metals Cu and Zn, whereas Cd, a non-essential element, cannot be regulated. The $NOEC_{accumulation}$ for Cu is 28 μg litre^{-1} and for Zn 191 μg litre^{-1} (Table 1), suggesting that Zn can be regulated up to higher external concentrations than Cu. This has been found for several other organisms, including *Mytilus edulis* (Amiard *et al.*, 1987; Rainbow & White, 1989). Rainbow & White (1989) showed that Zn regulation in the decapod *Palaemon elegans* is an active process: an increased rate of Zn uptake was matched by an increase of Zn excretion. Above the $NOEC_{accumulation}$ the excretion could no longer compensate for the uptake of the metal, resulting in a net accumulation of the metal (White & Rainbow, 1982).

When the (no)effect concentrations for Cu, Zn and Cd found in this study (Table 1) are compared with quality criteria for metal concentrations in surface water (3 μg litre^{-1} Cu, 30 μg litre^{-1} Zn and 0·2 μg litre^{-1} Cd (Ministerie van Verkeer en Waterstaat, 1988–1989)), it appears that all (no)effect concentrations are above these levels, suggesting that *D. polymorpha* is sufficiently protected by these quality criteria.

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