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# Chronic ecotoxicity of Zn and Pb to the zebra mussel *Dreissena polymorpha*

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## Abstract

Organisms in contaminated aquatic ecosystems are often exposed to toxicants for their entire lifetime. In order to evaluate the ecological consequences of long-term exposure to metals, we studied the relation between short-term and long-term effects of Zn and Pb on the filtration rate and survival of zebra mussels (*Dreissena polymorpha*) in laboratory experiments. The results indicated that the effects of Zn and Pb on the filtration rate of *D. polymorpha* increased when the exposure time was increased, but it remained unclear whether the decrease in filtration rate is caused by accumulation of metals by the mussels or by the metal concentration in the water, or both. The capacity of *D. polymorpha* to regulate the body concentration of the essential metal Zn decreased when the exposure time was increased, whereas the non-essential metal Pb could not be regulated by the zebra mussel. Not only the concentration of metal accumulated is of importance for survival, but also the rate of accumulation. It was concluded that the relation between short-term and long-term toxicity was different for each metal and could not be predicted from the results of the short-term experiments.

*Key words:* Zinc; Lead; *Dreissena polymorpha*; Mussel; Filtration rate; Metal accumulation

## 1. Introduction

Organisms in contaminated aquatic ecosystems are often exposed to toxicants for their entire lifetime. Consequently, chronic experiments will give a better reflection of the field situation than acute experiments. It has been demonstrated that effective concentrations decrease during chronic exposure; e.g. for the freshwater mussel *Corbicula manilensis* the $LC_{50}$ for Cu decreased with increasing exposure time (Harrison et al., 1984). Kenaga (1982) calculated acute-chronic ratios (ACR; the acute $EC_{50}$

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divided by the chronic $EC_{50}$) for a number of substances and organisms and demonstrated that for *Daphnia magna* the ACRs for Pb and Cd were much higher than for Zn and Cu, possibly because Cu and Zn are essential metals, whereas Pb and Cd are not known to have any biological function. Kraak et al. (1992) observed that the $EC_{50}$ for Cd for filtration rate of the zebra mussel *Dreissena polymorpha* decreased when exposure time was increased from 48 h to 10 weeks, whereas the effects of Cu did not increase during prolonged exposure. This seems to support the idea that during chronic exposure the effects of non-essential metals increase more than the effects of essential metals. In the present study, the chronic effects of Zn (essential) and Pb (non-essential) on the filtration rate of *D. polymorpha* were analysed to determine whether this concept can be generalised. This provided the possibility to compare the relationships between the acute (48 h) and chronic (10 weeks) responses of the same sublethal parameter of the same organism for four metals. The zebra mussel *Dreissena poymorpha* was chosen as test organism because it plays an important role in many freshwater ecosystems. Lake IJsselmeer contains approximately $10^{11}$ zebra mussels. It is the main source of food for benthivorous fish and for diving ducks (Suter, 1982; Prejs et al., 1990). Zebra mussels are able to reduce high phytoplankton abundances by their high filtration activity (Stanczykowska et al., 1975; Reeders et al., 1989) and, therefore, the filtration rate was chosen as the effect parameter.

To study the influence of differences in metal accumulation on the relation between short-term and long-term toxicity, the effects of exposure to metals on the filtration rate of the mussels and on the accumulation of the metals were determined in the same experiments. It may be expected that the non-essential metal Pb will be accumulated at every Pb concentration in the water (Bleeker et al., 1992). In contrast, it has been shown that during short-term exposure (48 h) zebra mussels could regulate the body concentration of the essential metal Zn at low external Zn concentrations (up to 191 $\mu$g $L^{-1}$) (Kraak et al., 1994).

## 2. Materials and Methods

Mussels and water were collected from Lake Markermeer (The Netherlands), a relatively clean location (Kraak et al., 1991). The water was sieved (25 $\mu$m) and kept in a storage barrel from which it was pumped continuously over a sand filter. The mussels were sorted by length (1.6–2.2 cm) and distributed over the experimental treatments such that the average length did not differ between treatments. An experimental treatment consisted of 25 mussels placed in a plastic aquarium (10 L), containing 3 L of filtered lake water. Approximately 90% of the mussels re-attached within 24–48 h, indicating a good condition. The water temperature was kept at 15°C, the hardness was 150 mg CaO $L^{-1}$, and the pH was 7.9. The water was aerated and always saturated with oxygen. The experimental set-up is discussed in more detail by Kraak et al. (1994).

Filtration rate and mortality were scored within one experiment. In the first experiment 0, 40, 100, 400, 1400, and 3000 $\mu$g Zn $L^{-1}$ were tested. 1400 $\mu$g Zn $L^{-1}$ is slightly above the 48-h $EC_{50}$ (1355 $\mu$g $L^{-1}$) (Kraak et al., 1994). To get insight in the variation

between control treatments, a second control (control-B) was run simultaneously. Since high mortality was expected in the 3000 $\mu$g Zn L$^{-1}$ treatment, also this treatment was duplicated (3000-B). In this treatment, the filtration rate was not measured and mortality was the only effect parameter. In the second experiment the following Pb concentrations were tested: 0, 4, 10, 40, 100 and 400 $\mu$g L$^{-1}$. 400 $\mu$g Pb L$^{-1}$ is slightly above the 48-h EC$_{50}$ (370 $\mu$g L$^{-1}$). In this experiment also the control and the highest concentration were tested twice (control-B and 400-B). In the treatments control-B and 400-B, the filtration rate was not measured and mortality was the only effect parameter. The experiments lasted for 10 weeks and a total of 400 mussels were used. Metals were added to the water in the aquaria the day after the mussels were collected, using stock solutions of 1000 mg L$^{-1}$ (ZnCl$_2$ and Pb(NO$_3$)$_2$). Water was renewed and metals were re-added each working day. Just before renewal and one hour after renewal water samples were taken, which were analysed for metals by flame or furnace AAS. The average actual concentrations of metals to which the mussels were exposed during the experiments were determined from these values. The actual concentrations were: 3, 38, 101, 382, 1266, 2758 and 2739 $\mu$g Zn L$^{-1}$ (0.05, 0.58, 1.55, 5.84, 19.42, 42.20 and 41.90 $\mu$mol Zn L$^{-1}$) and 0.5, 4, 10, 38, 85, 358 and 369 $\mu$g Pb L$^{-1}$ (2.4, 19.3, 48.26, 173.75, 410.25 nmol Pb L$^{-1}$, 1.73 and 1.78 $\mu$mol Pb L$^{-1}$). The mussels were fed with *Chlamydomonas eugametos* (20 000 cells per mL lake water) each Monday and Friday and with *Scenedesmus acuminatus* (20 000 cells per mL lake water) each Wednesday.

The filtration rate was measured after 48 h and subsequently every Wednesday until the end of the experiment. To determine the filtration rate, the mussels were fed with *Scenedesmus acuminatus* (20 000 cells per mL). 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 (5 ml) from each aquarium at 0, 15, and 30 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 Coughlan's formula (1969):

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

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

The measurements of filtration rate failed in week 9 of the Zn experiment, because of an infection of the algae culture; in week 8 in 1400 $\mu$g Zn L$^{-1}$ and in week 10 in control-A of the Zn experiment, because of technical problems with the coulter counter. EC$_{50}$ and LC$_{50}$ values were calculated by probit analysis (Finney, 1971). In the case of 1400 $\mu$g Zn L$^{-1}$, 3000 $\mu$g Zn L$^{-1}$ (A and B) and 400 $\mu$g Pb L$^{-1}$ (A) enough mussels died to determine the LT$_{50}$ (the time it takes to kill 50% of the animals) by probit analysis.

The aquaria were checked every day for dead mussels, which were removed. A mussels was considered to be dead when during gaping it did not react on a mechanical stimulus. Their length was measured, after which the soft tissues were freeze-dried, weighed and digested by wet destruction with ultrex $HNO_3$ and $H_2O_2$. The samples were analyzed for metals by flame or furnace AAS following Timmermans et al. (1989). Quality control of metal analysis was performed, using destruction blanks and reference materials (IAEA shrimp MA-A-3/TM and NIST water SRM 1643c). The measured values were in good agreement with the certified values (<10% deviation). After the experiments were finished, the dw/length ratio of the soft tissues of the remaining mussels was determined and the samples were analyzed for metals. The results were tested successively using Bartlett's test for homogeneity of variances, analysis of variance (one way ANOVA) and Scheffé's test for a posteriori comparison of means. When two groups were compared the $t$-test was used.

## 3. Results

The control mussels remained in a good condition in all experiments; the mussels continued filtering throughout the entire experiment and mortality in the controls was low (average 8%). The mussels for the Zn experiment were collected in April 1993, when the temperature of the lake water was rising rapidly. This resulted in a high filtration rate (max. 216 $mL^{-1}$ per mussel) at the beginning of the experiment. The filtration rate decreased later on, but in the lake this happens as well (Kraak, unpublished results). At the end of the Zn experiment, the filtration rate in the controls was still higher than the filtration rate in the controls of the Pb experiment, which was carried out in the autumn of 1992. In both experiments, the control mussels filtered at a level similar to the higher values reported in the literature (Morton, 1971; Sprung and Rose, 1988), as discussed by Kraak et al. (1994).

The filtration rate of mussels exposed to 38 and 101 $\mu$g Zn $L^{-1}$ followed the pattern in the controls and no effect on the filtration rate was observed (Fig. 1). Expressed as percentages of the controls, the average filtration rates of mussels in these treatments were 104% and 99%. The effect of exposure to 382 $\mu$g Zn $L^{-1}$ increased strongly during the experiment: the filtration rate decreased from an initial 67% to 23% at the end of the experiment. In the two highest treatments (1266 $\mu$g and 2758 $\mu$g Zn $L^{-1}$) the filtration rate was very low, and after 6 weeks of exposure to 2758 $\mu$g Zn $L^{-1}$ so many mussels had died that a reliable estimation of the filtration rate could no longer be performed. The effects of Zn on the filtration rate of *D. polymorpha* increased when the exposure time was increased, caused mainly by the increasing effects in the 382 $\mu$g Zn $L^{-1}$ treatment and the mortality in the 2758 $\mu$g Zn $L^{-1}$ treatment.

The three lowest Pb concentrations tested (4, 10 and 38 $\mu$g Pb $L^{-1}$) had no significant ($p < 0.05$) effect on the filtration rate (104, 95 and 94%, respectively) and the trends of the filtration rate in these treatments followed those in the controls (Fig. 2). The filtration rate of mussels exposed to 85 $\mu$g Pb $L^{-1}$ also followed the pattern in the controls, but was reduced to an average of 81% (Fig. 3). Exposure to 358 $\mu$g Pb $L^{-1}$, approximately the 48-h $EC_{50}$ (370 $\mu$g Pb $L^{-1}$), resulted in a filtration rate of 60% after
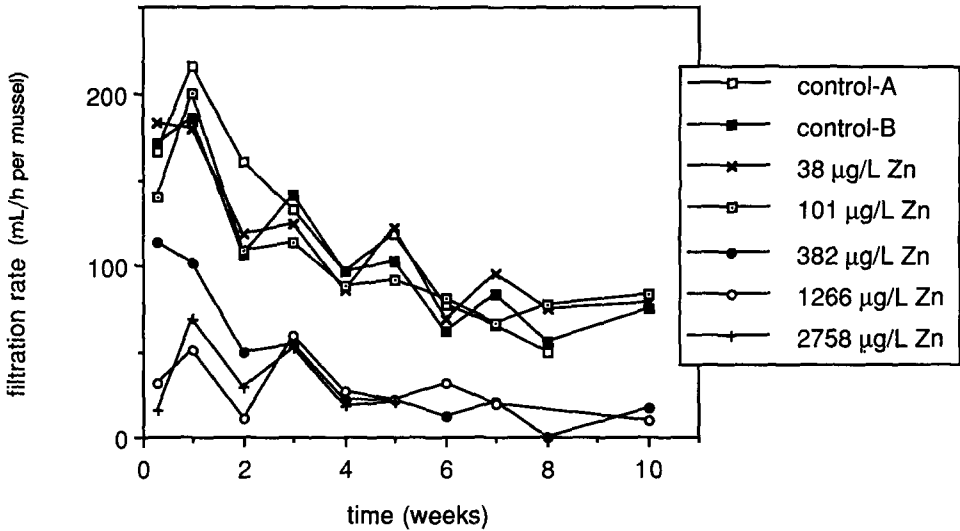
Fig. 1. Filtration rates of control *D. polymorpha* (A and B) and mussels exposed to 38, 101, 382, 1266, and 2758 $\mu$g Zn L$^{-1}$ for 10 weeks.
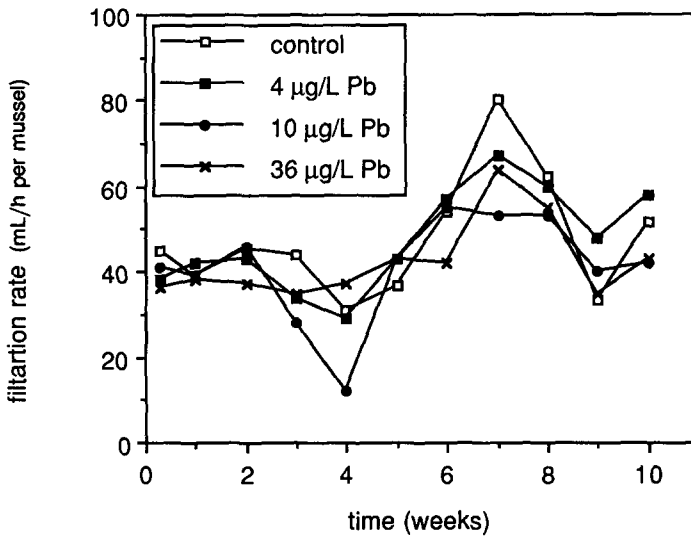


Fig. 2. Filtration rates of control *D. polymorpha* and mussels exposed to 4, 10 and 38 $\mu$g Pb L$^{-1}$ for 10 weeks.

48 h, but after one week the filtration rate dropped to zero (Fig. 3). Subsequently, a recovery to about 50% was observed until mass mortality started to occur (from week 7 onwards) and filtration rate dropped definitely to zero. The same remarkable pattern as in the 358 $\mu$g Pb L$^{-1}$ treatment has been observed previously for Cd (Fig. 4).
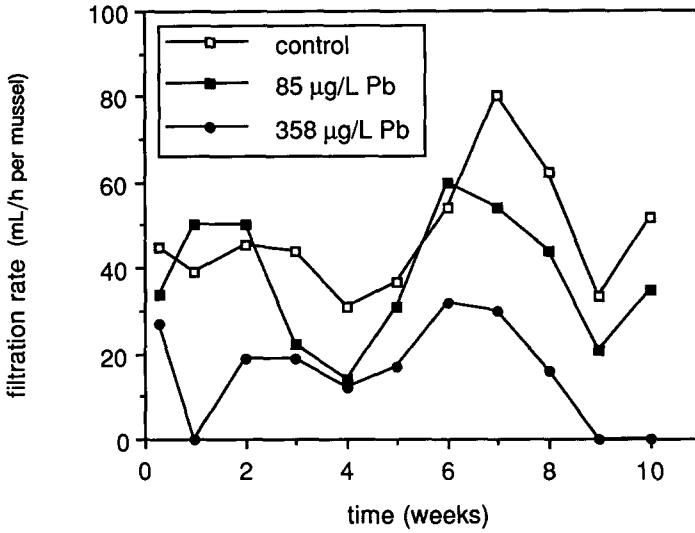
Fig. 3. Filtration rates of control *D. polymorpha* and mussels exposed to 85 and 358 $\mu$g Pb L$^{-1}$ for 10 weeks.
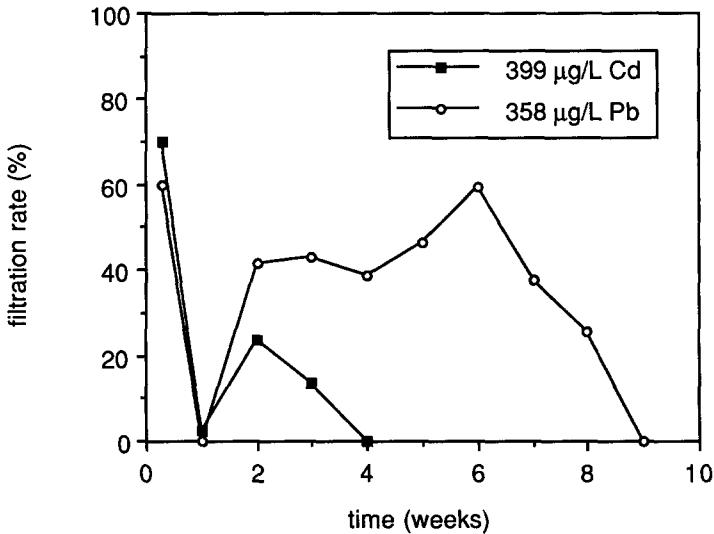


Fig. 4. Percentage filtration rates of *D. polymorpha* exposed to 399 $\mu$g Cd L$^{-1}$ and 358 $\mu$g Pb L$^{-1}$ (approximately the acute EC50's) for 10 weeks. Data on Cd from Kraak et al. (1992).

In the latter case, however, full recovery was not observed, since mortality already started after 2 weeks.

The increasing effects of Zn and Pb during prolonged exposure can be demonstrated by comparing the acute and chronic EC$_{50}$ values and by calculating the ACR

Table 1
Acute and chronic EC50's and ACR's for Cu, Zn, Pb, and Cd

| Metal | $EC_{50}$ 48 h | $EC_{50}$ 10 weeks | ACR |
|-------|-----------|--------------|-----|
| Cu | 41 | 43 | 1.0 |
| Zn | 560 | 131 | 4.3 |
| Pb | 370 | 91 | 4.1 |
| Cd | 388 | 27 | 14.4 |

(Table 1). The $EC_{50}$'s for Zn and Pb decreased by a factor 4 during 10 weeks of exposure, whereas the toxicity of Cu did not increase and the effects of Cd increased strongly during the same period of time. Consequently, a low ACR was observed for Cu (1.0) and a high ACR for Cd (14.4).

Mortality occurred at higher concentrations than effects on the filtration rate. Exposure to 38, 101 and 382 $\mu$g Zn L$^{-1}$ did not affect survival, while exposure to 1266, 2758 and 2739 $\mu$g Zn L$^{-1}$ caused substantial mortality (Table 2). In 2758 $\mu$g Zn L$^{-1}$ (3000-A) only one mussel survived the experiment, while in 2739 $\mu$g Zn L$^{-1}$ (3000-B) all the mussels died. The $LT_{50}$ in the 1266 $\mu$g Zn L$^{-1}$ treatment was significantly ($p < 0.05$) longer (67 days) than in the 2758 Zn L$^{-1}$ (3000-A) treatment (26 days), which was in turn significantly ($p < 0.05$) longer than in the 2739 $\mu$g Zn L$^{-1}$ (3000-B) treatment (17 days). Exposure to 4, 10, 38 and 85 $\mu$g Pb L$^{-1}$ did not affect mortality (Table 3). An increased mortality was observed in 358 and 369 $\mu$g Pb L$^{-1}$. From these results the $LT_{50}$ for 358 $\mu$g Pb L$^{-1}$ (400-A) was calculated as 72 days. The $LC_{50}$ for Zn could be determined from week 3 onwards and decreased from 4293 $\mu$g L$^{-1}$ to 1065 $\mu$g L$^{-1}$. The $LC_{50}$ for Pb could be determined from week 8 onwards and decreased from 2330 to 497 $\mu$g L$^{-1}$ at the end of the experiment.

Table 2
Cumulative percentage mortality of *D. polymorpha* exposed for 10 weeks to different Zn concentrations in the water

| Time (weeks) | Control A 3 $\mu$g L$^{-1}$ | Control B | | | | | 3000 A | 3000 B |
|---|---|---|---|---|---|---|---|---|
| | | 3 $\mu$g L$^{-1}$ | 38 $\mu$g L$^{-1}$ | 101 $\mu$g L$^{-1}$ | 382 $\mu$g L$^{-1}$ | 1266 $\mu$g L$^{-1}$ | 2758 $\mu$g L$^{-1}$ | 2739 $\mu$g L$^{-1}$ |
| 0.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 12 |
| 3 | 0 | 4 | 0 | 0 | 0 | 8 | 16 | 44 |
| 4 | 0 | 8 | 0 | 0 | 0 | 20 | 40 | 60 |
| 5 | 0 | 8 | 0 | 0 | 0 | 24 | 48 | 84 |
| 6 | 0 | 8 | 0 | 0 | 0 | 24 | 68 | 92 |
| 7 | 0 | 8 | 0 | 0 | 0 | 28 | 88 | 100 |
| 8 | 0 | 8 | 0 | 0 | 0 | 28 | 92 | 100 |
| 9 | 0 | 8 | 0 | 0 | 4 | 40 | 96 | 100 |
| 10 | 0 | 8 | 0 | 0 | 4 | 56 | 96 | 100 |

Table 3
Cumulative percentage mortality of D. polymorpha exposed for 10 weeks to different Pb concentrations in the water during

| Time (weeks) | Control A 0.5 $\mu$g L$^{-1}$ | Control B | | | | | 400 A | 400 B |
|---|---|---|---|---|---|---|---|---|
| | | 0.5 $\mu$g L$^{-1}$ | 4 $\mu$g L$^{-1}$ | 10 $\mu$g L$^{-1}$ | 36 $\mu$g L$^{-1}$ | 85 $\mu$g L$^{-1}$ | 358 $\mu$g L$^{-1}$ | 369 $\mu$g L$^{-1}$ |
| 0.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 4 | 0 | 0 | 0 | 8 | 4 | 8 |
| 2 | 0 | 4 | 0 | 0 | 0 | 16 | 4 | 8 |
| 3 | 0 | 8 | 0 | 4 | 0 | 16 | 4 | 8 |
| 4 | 4 | 8 | 4 | 4 | 0 | 16 | 8 | 8 |
| 5 | 4 | 8 | 8 | 4 | 0 | 16 | 8 | 8 |
| 6 | 8 | 8 | 12 | 4 | 4 | 16 | 12 | 12 |
| 7 | 8 | 8 | 12 | 4 | 8 | 16 | 16 | 20 |
| 8 | 12 | 8 | 12 | 4 | 8 | 16 | 24 | 28 |
| 9 | 16 | 8 | 12 | 4 | 12 | 16 | 40 | 32 |
| 10 | 16 | 8 | 12 | 4 | 16 | 16 | 52 | 32 |

All Zn and Pb concentrations in the water resulted in a significant ($p < 0.05$) increase of the metal concentration in the surviving mussels (Figs. 5 and 6), indicating that D. polymorpha was incapable of regulating the body concentration of Zn during exposure to 38 $\mu$g L$^{-1}$ and the body concentration of Pb during exposure to 4 $\mu$g L$^{-1}$.

No significant ($p > 0.05$) difference was observed between the dry weights of mussels which survived the Zn treatments and those of the controls. For 3000 $\mu$g Zn L$^{-1}$ (A and B) this could not be tested, since no or only one mussel survived. For Pb, only the mussels which survived exposure to 400 $\mu$g Pb L$^{-1}$ (A and B) had a significantly ($p < 0.05$) lower dry weight than the controls. Thus, only in the highest metal concentrations tested was a clear loss of condition and subsequent mortality observed.

## 4. Discussion

The results clearly demonstrate the importance of chronic ecotoxicity experiments. For both Zn and Pb, the effects increased by a factor 4 when the exposure period was increased from 48 h to 10 weeks. Kenaga (1982) calculated acute-to-chronic ratio's (ACR) based on LC$_{50}$'s for several toxicants. The few data available for invertebrates, mainly Daphnia, suggest that these ACR's are much higher for non-essential metals than for essential metals. The results on the sublethal parameter filtration rate presented here (Table 1) partly support this idea. Relatively low ACR's were observed for Cu and Zn, indicating that the effects of these metals increase with time, but not dramatically. The ACR for Cd was high, caused by a strong decrease of the EC$_{50}$ with time, as reported by Kraak et al. (1992). Our results for Pb deviate from those of Kenaga (1982), since the ACR for this metal is as low as the one for the essential metal Zn. It is concluded that the relation between short-term and long-term ecotoxicity
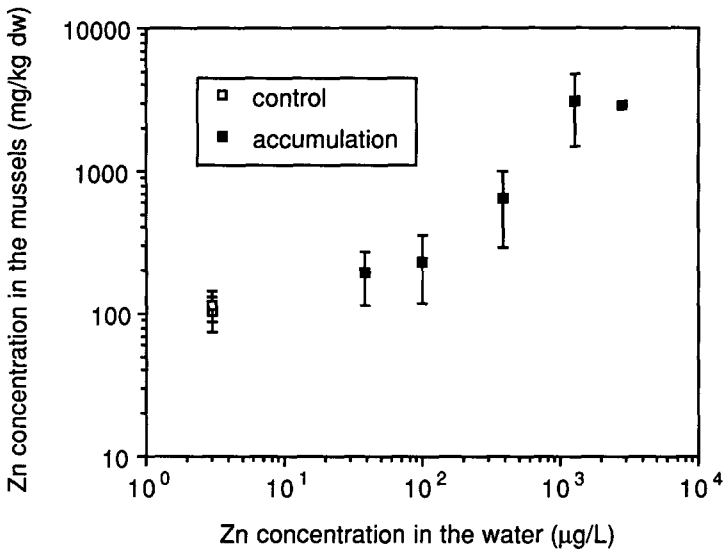
Fig. 5. Zn concentrations ± SD in *D. polymorpha* for different Zn concentrations in the water. Open squares indicate the Zn concentrations in the control and filled squares indicate the Zn concentrations in exposed mussels which differed significantly ($p < 0.05$) from the controls.
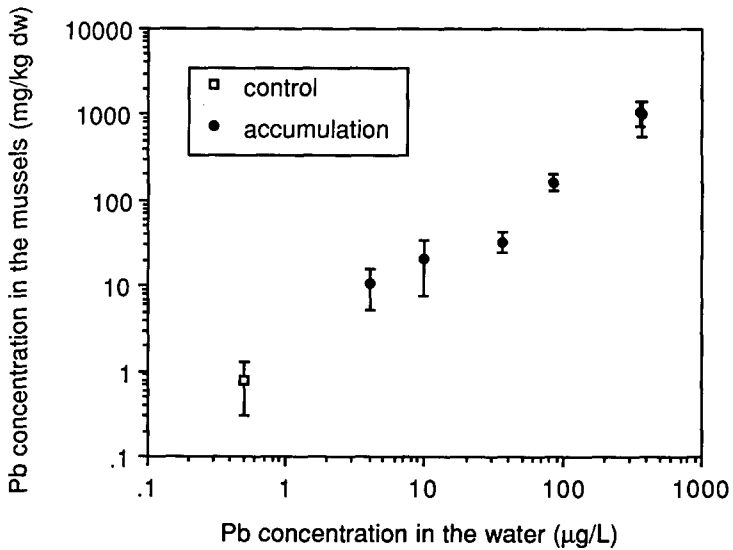


Fig. 6. Pb concentrations ±SD in *D. polymorpha* for different Pb concentrations in the water. Open squares indicate the Pb concentrations in the controls and filled squares indicate the Pb concentrations in exposed mussels which differed significantly ($p < 0.05$) from the controls.

was different for each metal and could not be predicted from the results of the short-term experiments (Bleeker et al., 1992; Kraak et al., 1994).

In the present experiments the 48-h EC50 for Zn was determined as 560 $\mu$g L$^{-1}$, lower than the EC50 for Zn found in 1989 (1355 $\mu$g L$^{-1}$, Kraak et al., 1994). Routine checks of the sensitivity of zebra mussels from Lake Markermeer for Cu showed a remarkable stability and hence reproducibility of the acute EC50. Apparently, the zebra mussels in Lake Markermeer have become more sensitive to Zn.

The chronic EC$_{50}$ for Zn (131 $\mu$g L$^{-1}$) is in good agreement with results obtained for other bivalve species. Doherty et al. (1987) studied valve movement of the Asiatic clam *Corbicula fluminea*. They observed that the concentration needed to decrease the effect parameters (mean time to first closure and mean time of valve being parted) was around 200 $\mu$g L$^{-1}$. Redpath and Davenport (1988) demonstrated that concentrations of 230–470 $\mu$g L$^{-1}$ reduced the pumping of the marine mussel *Mytilus edulis*. Abel (1976) found higher effect concentrations (1.5 mg L$^{-1}$) for the filtration rate of *Mytilus edulis*, but this concerned acute experiments. Salanki and V.-Balogh (1989) observed that exposure to 50 $\mu$g Pb L$^{-1}$ significantly reduced the active periods of the freshwater mussel *Anodonta cygnea*. This concentration is close to the chronic EC$_{50}$ in our study (91 $\mu$g L$^{-1}$).

A striking feature was the complete breakdown of the filtration rate after one week and the subsequent recovery after 2 weeks of exposure to Cd and Pb. This phenomenon occurred at concentrations which resulted in approximately 50% reduction after 48 h of exposure (Fig. 4). This pattern was not observed for the essential metals Cu (Kraak et al., 1992) and Zn (Fig. 1). The reason for this pattern remains unclear, but it suggests that the zebra mussel reacts differently to essential and to non-essential metals, which could possibly be related to differences in regulation/accumulation of the metals. At low, but elevated, Zn (38 and 101 $\mu$g L$^{-1}$) and Pb (4, 10 and 38 $\mu$g L$^{-1}$) concentrations in the water, the filtration rate was not affected, no mortality occurred, but a significant accumulation took place. This may indicate that the amounts of metals accumulated were too low to cause an effect on the filtration rate or that accumulation and effects on filtration are not related. The latter suggestion is supported by the treatment at 85 $\mu$g Pb L$^{-1}$. The filtration rate of these mussels was lower than in the control, but did not decrease with time, in spite of a continuing accumulation. However, in the 382 $\mu$g Zn L$^{-1}$ treatment a different response was observed: a gradually decreasing filtration rate coincided with a continuing accumulation. For Cu and Cd examples for both possibilities were found (Kraak et al., 1992). So it remains unclear whether the decrease in filtration rate is caused by accumulation of metals by the mussels, or by the metal concentration in the water, or both. For mixtures of metals indications were found that the filtration rate was determined by the metal mixture concentration in the water and was not related to the increasing metal concentrations in the mussels (Kraak et al., 1993). Consequently, differences in accumulation/regulation between essential and non-essential metals cannot explain the differences in reaction of the mussels to these metals.

In the present chronic experiments, the mussels accumulated Zn during exposure to 38 $\mu$g L$^{-1}$, while in acute experiments (48 h) exposure to 191 $\mu$g Zn L$^{-1}$ did not result in an increase of the Zn concentration in the mussels (Kraak et al., 1994). This

suggests that the capacity to regulate the body concentration of this essential metal decreased from 191 $\mu$g L$^{-1}$ after 48 h to lower than 38 $\mu$g L$^{-1}$ after 10 weeks. These findings are in accord with those of Amiard et al. (1987), who observed that the concentration at which the marine mussel *Mytilus edulis* could regulate the body concentration of Zn decreased from 100 $\mu$g L$^{-1}$ after 4 days to 50 $\mu$g L$^{-1}$ after 16 days of exposure. The ability to regulate the body concentration of Zn was observed for all bivalves studied (Lakshmanan and Nambisan, 1989), for amphipods (Ahsanullah and Williams, 1991; Borgmann et al., 1993) and other invertebrate taxa studied (Amiard et al., 1987; Rainbow and White, 1989). Pb was accumulated at all Pb concentrations in the water, indicating that this non-essential metal could not be regulated by *D. polymorpha*. This is in agreement with the results of acute experiments on Pb (Bleeker et al., 1992) and holds for all bivalves (Amiard et al., 1987; Lakshmanan and Nambisan, 1989; Salanki and V.-Balogh, 1989), as well as for all other invertebrate taxa studied (Amiard et al., 1987; Borgmann et al., 1993).

From the present experiments, it appeared that not only the amount of metal accumulated is of importance, but also the rate of metal accumulation. Only one mussel survived the total exposure period of 10 weeks in the 2739 and the 2758 $\mu$g Zn L$^{-1}$ treatments and this mussel contained 2949 mg Zn/kg dw. In the 1266 $\mu$g Zn L$^{-1}$ treatment about half of the mussels survived the experiment, but they contained approximately the same Zn concentrations (3105 mg/kg dw) as the mussel that survived the higher treatments. This can be explained by the lower rate of metal accumulation in the 1266 $\mu$g L$^{-1}$ treatment compared to the 2758 and 2739 $\mu$g L$^{-1}$ treatments. Bivalves are capable of detoxifying metals by the synthesis of metallothioneins (Doherty et al., 1988, Bebianno and Langston, 1991). As long as metal accumulation is matched by metallothionein synthesis, the mussels can survive. When accumulation increases, metallothionein synthesis probably reaches a maximum. It is possible, that in the 1266 $\mu$g L$^{-1}$ treatment a higher percentage of the accumulated metal was detoxified than in the 2758 and 2739 $\mu$g L$^{-1}$ treatments, resulting in lower mortality in the 1266 $\mu$g L$^{-1}$ treatment. In addition to the rate of accumulation, the individual sensitivity matters as well: within one treatment, metal concentrations in dead and surviving mussels did not differ. This suggests that the dead individuals had a lower lethal body burden than the surviving mussels.

## 5. Conclusions

The relation between short-term and long-term toxicities was different for each metal (Cu, Zn, Pb and Cd) and could not be predicted from the results of the short-term experiments. It remained uncertain whether the decrease in filtration rate is caused by accumulation of metals by the mussels, or by the metal concentration in the water, or both. The differences between responses of zebra mussels to essential and non-essential metals cannot be explained by differences in regulation/accumulation of these metals. The capacity of *D. polymorpha* to regulate the body concentration of the essential metal Zn decreased when the exposure time was increased. The non-essential

metal Pb could not be regulated by the zebra mussel. Not only the concentration of metal accumulated is of importance for survival, but the rate of accumulation as well.

## Acknowledgements

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