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# Short-Term Ecotoxicity of a Mixture of Five Metals to the Zebra Mussel *Dreissena polymorpha*

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Mixture toxicity experiments reflect the actual hazard of contaminated environments better than experiments in which the effect of a single toxicant is tested. In most mixture toxicity experiments, however, only two toxicants have been tested, although in polluted ecosystems, the presence of hundreds of toxicants has been registered.

Könemann (1981) observed that a mixture of a large number of organic toxicants with the same mode of action, had an additive effect on survival of fish. However, it has been suggested that the potential for addition may be reduced when sublethal instead of lethal parameters are studied (Hermens *et al.* 1984; Deneer *et al.* 1988). Furthermore, it is likely that inorganic toxicants, like metals, have different modes or sites of action. Consequently, metals may affect a specific biological endpoint differently. Therefore, it is likely to expect deviations from concentration addition when sublethal effects of a mixture of metals are determined. Indeed, studies on the effects of binary sublethal metal mixtures found such deviations from additivity, either less or more than concentration addition (Mohan *et al.* 1986; Van Gestel and Hensbergen 1997; Weltje, 1998). Kraak et al. (1994a), studying the effects of binary metal mixtures on the filtration rate of the zebra mussel *(Dreissena polymorpha)*, also observed more as well as less than additive effects. However, a mixture of three metals (Cu, Zn and Cd) was concentration additive. Although this could be purely incidental, it could also be argued that the deviations from concentration addition were “averaged out” in a mixture consisting of more than two metals. Based on the latter assumption, it could be suggested that a mixture of several metals is not likely to deviate from additivity. However, when the number of compounds in a mixture increases, the concentration of the individual compounds decreases. This may imply that in such a mixture essential metals (Cu, Zn, Ni) do not, or to a lesser extent, contribute to the toxicity of the mixture, because low concentrations of essential metals are under metabolic control (Amiard

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et al. 1987; Rainbow and White 1989; Kraak *et al.* 1994a;b; Van Gestel *et al.* 1993; Van Gestel and Hensbergen 1997). Consequently, it could be hypothesised that a metal mixture, containing one or more essential metals, is likely to have a less than additive effect. To test this hypothesis, the effect of a mixture of Cu, Zn, Ni, Cd and Pb on the filtration rate of the zebra mussel *D. polymorpha* was determined. A concentration range of mixtures was tested, using the Toxic Unit concept, based on the previously determined $EC_{50}$ values for these five metals (Bleeker *et al.* 1992; Kraak *et al.* 1994b; Stuijfzand *et al.* 1995).

## MATERIALS AND METHODS

Zebra mussels *(Dreissena polymorpha)* 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 (25 pm) and kept in a storage barrel from which it was pumped continuously over a sand filter. The mussels were sorted by length (16-23 mm) and distributed over the experimental treatments, such that the average length of the mussels was equal for all treatments. An experimental treatment consisted of 25 mussels placed in a polystyrene aquarium (10 L), containing 3 L of particle free lake water. Evaporation of water was reduced by covering the aquaria with sheets of glass. Water temperature was kept at 15° C, hardness was 150 mg $CaCO_3$/L. The pH varied between 7.8 and 8.0, and a 16 : 8 hr light : dark regime was applied. The water was aerated and always oxygen saturated.

Mixtures of Cu, Zn, Ni, Cd and Pb were added to the water in the aquaria, the day after the mussels were collected. Stock solutions of 1000 mg metal/L were used; Cu as $CuC1_2$, Zn as $ZnC1_2$, Ni as $NiC1_2$, Cd as $CdC1_2$ and Pb as $Pb(NO_3)_2$. In these mixture experiments a range of toxic units was tested, based on individual metal toxicities, determined in previous experiments in our laboratory, using the same experimental set-up (Bleeker *et al.* 1992; Kraak *et al.* 1994b; Stuijfzand *et al.* 1995). In the mixture, the toxicant concentrations were added as fractions of their EC50 and the total toxic units (T.U.) of the mixture were the sum of the individual fractions. Equal fractions of the $EC_{50}$ of the toxicants were (Sprague 1970; Konemann 1981). If the EC50 of a mixture would be significantly lower than 1 T.U., then the toxicants are more than concentration additive. If the $EC_{50}$ would not differ significantly from 1 T.U., the toxicants are concentration additive and if the EC50 would be significantly higher than 1 T.U., the toxicants are less than concentration additive.

The experimental set-up allowed 7 trials to run simultaneously, one of them being a control. Mixtures of 0, 0.5, 0.75, 1, 1.5, 2 and 3 T.U. were tested. There were two replicates per treatment, except for 0.5 and the control which were tested in triplicate. A total number of 400 mussels were tested.

After 24 and 48 hours, water was renewed and metals were added again. At 1, 24, 25 and 48 hours water samples were taken and the concentrations of the metals were analysed by flame (Perkin Elmer 1100B) or furnace (Perkin Elmer 5100PC) AAS, in order to determine the actual concentrations of metals to which the animals were exposed during the experiments.

The filtration rate was measured after 48 hours of exposure to the metal mixtures, directly after the last renewal of water and metals. To determine filtration rate, the mussels were fed with the green alga *Scenedesmus acuminatus*. The algal concentration in the aquaria was approximately 20,000 cells / ml. The algal concentration decreased, due to the filtration activity of the mussels. The decrease in algal concentration was determined by taking water samples (5 ml) from each aquarium at 0, 10 and 20 minutes after addition of the algae. The algal concentrations in triplicate 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):

$$m = \frac{M}{nt} \ln \frac{C_0}{C_t} \qquad (1)$$

in which m = filtration rate in ml / mussel / hour,
M = volume of water in the aquaria (3 L),
n = number of mussels in each aquarium (25),
t = duration of filtration measurement (h),
$C_0$ = concentration of algae at the start of the filtration measurement,
$C_t$ = concentration of algae after t hours.

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 a dose-response relationship, from which the $EC_{50}$ value based on the average actual metal mixture concentration in the water with 95% confidence limits was calculated by curve fitting according to Haanstra *et al.* (1985), using the computer programme Kaleidagraph (Abelbeck Software).

After completing the experiments, the soft tissues without byssus threads of ten 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. The soft tissues were

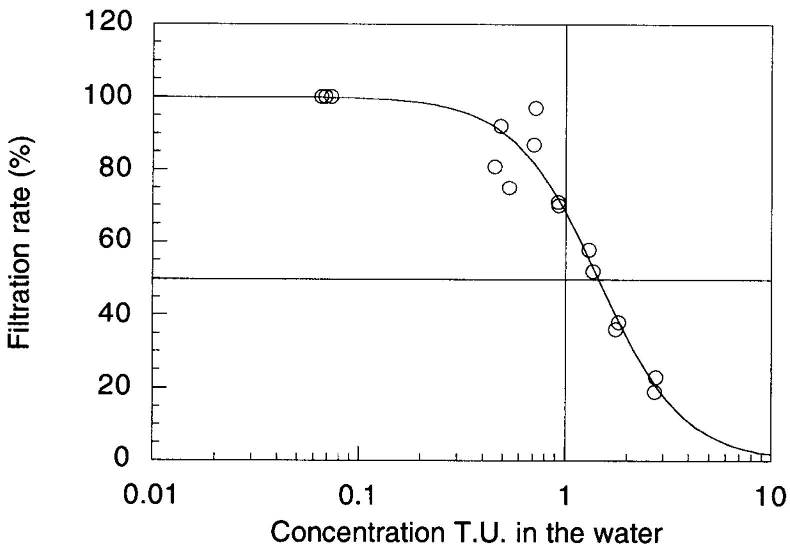


**Figure 1.** Filtration rates of *Dreissena polymorpha* after 48 hours exposure to mixtures of Cu, Zn, Ni, Cd and Pb; data are presented as percentages of the corresponding controls.

analysed for Cu, Zn, Ni, Cd and Pb by flame or furnace AAS. 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).

## RESULTS AND DISCUSSION

No mussels died during the experiment. Filtration rates in the controls varied between 132-155 ml/mussel/hour, which is normal to high (Sprung and Rose 1988; Hinz and Scheil 1972), indicating that the animals were in good condition. Filtration rates decreased with increasing metal mixture concentrations in the water (Figure 1). The EC50 of the mixture was determined as 1.46 T.U. (confidence limits: 1.32-1.60 T.U.), indicating a less than additive effect on the filtration rate of *D. polymorpha.*

The accumulation of metals by the mussels in the mixture toxicity experiment was compared with the accumulation in experiments in which the metals were tested individually (Bleeker *et al.* 1992; Kraak *et al.* 1994b; Stuijfzand *et al.* 1995)(Figure 2). This comparison

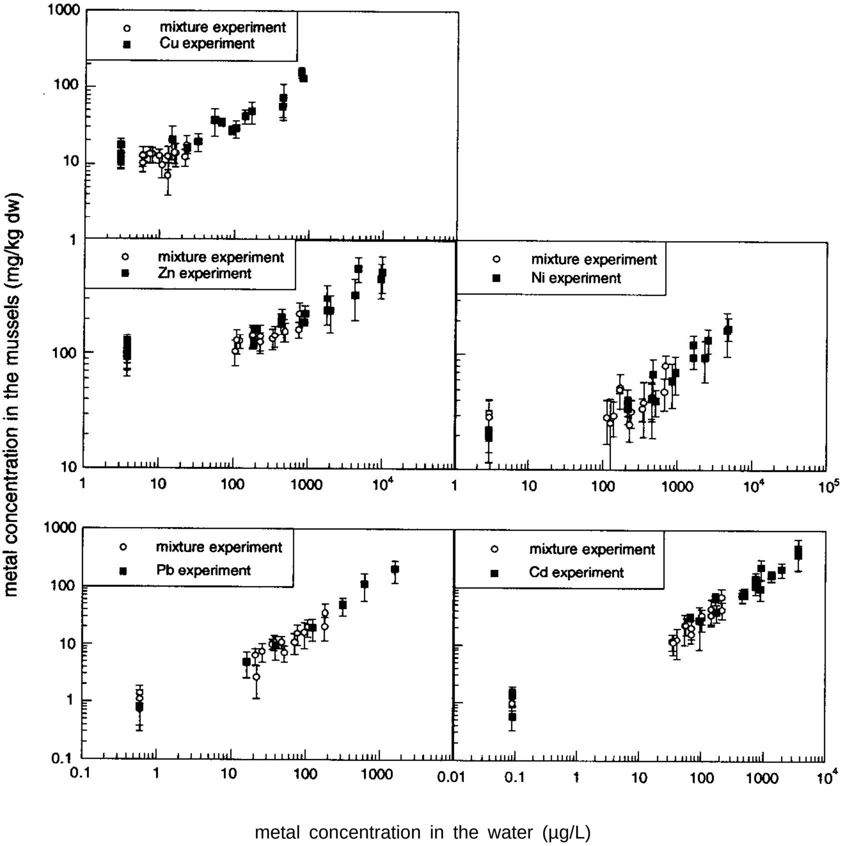


**Figure 2.** Metal concentration in *Dreissena polymorpha* (mg/kg dw) plotted against the metal concentration in the water (µg/L) for the mixture (open dots) and for the metals tested individually (filled squares). Data for accumulation of individually tested metals are from Kraak *et al.* (1994a)(Zn, Cu, Cd), Stuijfzand *et al.* (1995)(Ni) and Bleeker *et al.* (1992)(Pb).

demonstrates that the accumulation of each metal by the zebra mussel was not influenced by the presence of the four other metals. In addition it can be seen that at low but elevated concentrations of the essential metals Cu, Zn and Ni no increase of the concentration of these metals in the soft tissues of the mussels was observed. In contrast, the non-essential metals Cd and Pb were accumulated at all exposure concentrations. Hence, the present study showed that zebra mussels are able to regulate the body concentration of the essential metals (Cu, Zn and Ni) up to a certain metal concentration in the water, as has been found in previous studies using zebra mussels (Kraak *et al.* 1994a;

Stuijfzand *et al.* 1995). The ability to regulate the body concentration of the essential metal Zn is widespread over the different invertebrate taxa (Amiard *et al.* 1987; Rainbow and White 1989; Van Gestel *et al. 1993;* Van Gestel and Hensbergen 1997). However, not all bivalves are able to regulate the body concentration of Cu (Amiard *et al.* 1987). Experimental data on Ni regulation are scarce.

The accumulation of each metal by the zebra mussel was not influenced by the presence of the four other metals, as has been observed for a mixture consisting of Cu, Zn and Cd (Kraak *et al.* 1994a). Further comparisons are restricted to binary metal mixtures for which additive, antagonistic and synergistic effects on metal uptake have been observed, as discussed by Van Gestel and Hensbergen (1997).

The results of this study clearly showed that the effect of a mixture of five metals on a sublethal parameter is less than concentration additive. In agreement with these results, Enserink *et al.* (1991) demonstrated that a mixture of eight metals also had a less than additive effect on population growth of *Daphnia magna,* although the authors concluded otherwise. Our results and those of Enserink *et al.* (1991) confirm our hypothesis that sublethal concentrations of metal mixtures containing several essential metals have a less than additive effect. The effects of binary metal mixtures remain, however, unpredictable (Mohan *et al.* 1986; Kraak *et al.* 1994a; Van Gestel and Hensbergen 1997).

The question remains what causes this loss of potential for additivity. It may be partly explained by the observed threshold concentration for the essential metals (Cu, Zn and Ni). It is likely that the mussels were able to regulate these essential metals up to a certain metal concentration in the water. Individual metal concentrations were low, since a sublethal effect was determined, and at 1 T.U. mussels were exposed to 20% of the EC50 of each metal. As a result, the contribution of essential metals to the mixture toxicity may have been reduced. This holds especially for Cu, because even at the highest mixture concentration tested, the mussels contained the same Cu concentration in their soft tissues as under control conditions. When the T.U. added in this experiment were recalculated omitting Cu and the $EC_{50}$ of the mixture was recalculated, the effect did not deviate from additivity. This suggests that the less than additive effects of complex metal mixtures may indeed be explained by a decreasing contribution of essential metals to the toxicity of the mixture.

The results of the restricted number of studies on complex mixtures, including the present study, have strong implications for expectations of toxic effects in contaminated freshwater ecosystems. In large

European rivers, hundreds of toxicants are present in low concentrations (RIWA 1996). The present study demonstrated that metal mixtures containing low concentrations of essential metals have a less than additive effect. In addition, Deneer *et al.* (1988) demonstrated that the additivity of a mixture of organic chemicals at the $EC_{10}$ level was lower than at the $EC_{50}$ and $LC_{50}$ level. Consequently, in the field concentration addition may almost never take place.

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