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Short-Term Effects of Nickel on the Filtration Rate of the Zebra Mussel *Dreissena polymorpha*

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The zebra mussel, *Dreissena polymorpha*, is highly abundant in The Netherlands, other European countries and, more recently, in North America. The mussel is an important link in the aquatic food chain: it accelerates the circulation of organic compounds by filtering large amounts of phytoplankton from the water (Stanczykowska 1978). Zebra mussels are the main food source for diving ducks, coots (Suter 1982) and benthivorous fish such as roach (Prejs et al. 1990).

Different parameters can be chosen to quantify the effects of toxicants on organisms. Mortality is often used as such a parameter: however, effects on vital functions of organisms occur at lower concentrations (Kszos et al. 1992; Kraak et al. 1992). Therefore, determining the effects of sublethal contaminant concentrations is ecologically more relevant. Several studies describe the sublethal effects of metals on bivalves. Negative effects of copper and mercury on filtration rate and growth have been observed in the marine mussel *Perna viridis* (Krishnakumar et al. 1990). Copper exposure lowered the heart rate and filtration rate of *Mytilus edulis* (Grace and Gainey 1987). Effects of metals on the freshwater mussel *D. polymorpha* have often been observed. Exposure to copper, cadmium, zinc (Kraak et al. 1994) and lead (Bleeker et al. 1992) lowered the filtration rate of *D. polymorpha*. The toxicity of nickel has never been tested on *D. polymorpha* before, even though there are considerable amounts of this metal present in the environment (CBS 1986). In this study, short-term effects of nickel on the filtration rate of *D. polymorpha* was tested. In addition, accumulation of this metal in the mussels were determined after exposure to different nickel concentrations in the water.

MATERIALS & METHODS

Zebra mussels (*D. polymorpha*) were collected from Lake Markermeer

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(The Netherlands), a relatively clean site (Kraak et al. 1991). The experiments were carried out from the end of April until the middle of May (1993), a period in which the mussels filter at a high rate.

The mussels, 19.5 to 19.9 mm in length, were placed in plastic aquaria, with each aquarium containing 25 mussels. The average length of the mussels in each experiment was equal in all treatments. Lake Markermeer water was pumped through a sandfilter, in order to remove particles. The aquaria contained 3 L of filtered lake water, which was aerated and kept at 15°C. The aquaria were covered with glass plates to prevent evaporation. The hardness of the water was 150 mg CaO/L, the pH varied between 7.8 and 8.0, and a 16 : 8 hr light : dark regime was applied.

The animals were allowed to acclimatize to the experimental set-up for one day. At the start of the experiment, different concentrations of nickel were added to the water. A stock solution of 1000 mg Ni/L (as $NiCl_2$) was used. The concentrations tested were: 0, 250, 500, 1000, 1750, 2500, and 5000 µg/L. Each concentration was tested twice. A total of 350 mussels were tested. The water was renewed after 24 and 48 hr, and Ni was again added to the water. Just before and one hour after renewal, duplicate water samples (1 mL) were taken, and 20 µl HNO_3 (p.a.) was added, in order to determine the actual Ni concentration in the water. After 48 hr of Ni exposure, 60 mL of a culture of the green alga *Scenedesmus acuminatus* was added to the water to measure filtration rates. The density of the algal cells in the aquaria was approximately 20,000 cells/mL. When the mussels started filtering, 5 min after addition of the algae, three water samples were taken (5 mL). This was repeated 10 and 20 minutes after the first sampling. The decline in the number of algae in the water was measured using a Coulter Counter. The filtration rate (m) can be calculated using Coughlan's (1969) formula;

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

in which M = volume of water in the aquaria (3 L)
n = number of mussels in each aquarium (25)
t = duration of filtration measurement (hr)
C_0 = concentration of algae at the start of the filtration measurement
C_t = concentration of algae after t hours

The filtration rate (m) represents the volume of water filtered by *D. polymorpha* (mL/mussel/hr). Filtration rates of the treatments are presented as percentages of the controls. Directly after the filtration rate measurement, 10 animals were randomly selected from each aquarium to determine metal accumulation in their soft tissues.

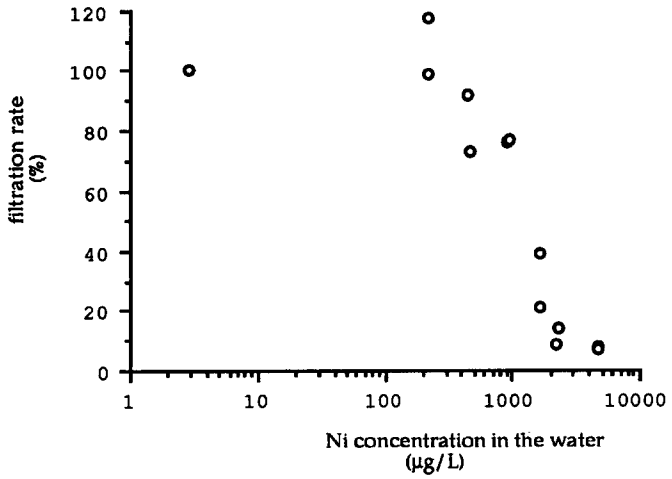

Figure 1. Filtration rates (%) of *D. polymorpha*, after 48 hr exposure to different Ni concentrations in the water.

The mussels, without byssus threads and shells, were placed in polyethyleen tubes (2.2 mL). After freeze-drying, the tissues were weighed and digested in 500 µL HNO_3 (Ultrex). After evaporation, 250 µL of HNO_3 was added. Finally, 250 µL of H_2O_2 was added and evaporated. Next, 1 mL of acidified demi water (pH 2) was added. Nickel concentrations in the tissues and in the water samples were analyzed using an Atomic Absorption Spectrophotometer (A.A.S.) furnace (Perkin Elmer 5100PC) and flame (Perkin Elmer 1100B), detection limits for Ni were 5 µg/L and 52 µg/L, respectively. Metal analysis proved to be accurate, since measured values for digestion blanks and reference material (IAEA shrimp MA-A-3/TM and IAEA simulated freshwater W-4) always deviated < 10% from certified values.

The NOEC was determined using the Williams' test, and the EC_{50} by probit analysis. In order to test differences between internal Ni levels, the Student's t-test was used to test differences between two groups, while an ANOVA was carried out to test differences between more than two groups, followed by a Sheffé contrast.

RESULTS & DISCUSSION

A clear dose-response relationship was observed in this experiment (Fig. 1). The EC_{50} after 48 hr of Ni exposure was 1126 µg/L (confidence limits: 900-1409 µg/L), whereas the NOEC was 455 µg/L ($p<0.05$).

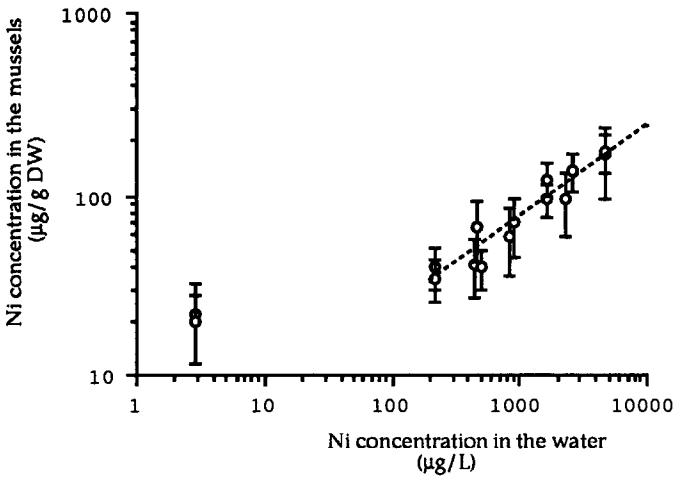

Figure 2. Internal Ni levels in soft tissues of *D. polymorpha* (mean ± SD), after 48 hr exposure to different Ni concentrations in the water. The dotted line represents the relationship between internal Ni levels and Ni exposure.

Filtration rates in the controls (115-184 mL/mussel/hr) were normal or even high for *D. polymorpha* of similar size (Sprung and Rose 1988; Hinz and Scheil 1972), suggesting the zebra mussels were in good condition. Only four out of 350 mussels died, and mortality was independent of the Ni concentration. However, mussels exposed to the highest Ni concentrations (5000 µg/L) showed indications of stress; the animals excreted mucus to reduce contact with the metal. Effects on filtration rate clearly occurred at lower levels than effects on mortality.

Little is known about the effects of Ni on organisms and comparisons of available data are difficult to make, since different parameters and exposure times were used in other studies. The LC_{50} after 96 hr of exposure to Ni was 238 µg/L for the freshwater snails *Juga plicifera* and *Physa gyrina* (Nebeker et al. 1986), suggesting that these snails are more sensitive than *D. polymorpha* to Ni. *Daphnia magna* showed decreased fertility after exposure to 40 µg Ni/L, while 21 days of exposure to the same Ni concentration had no effect on survival (Kszos et al. 1992). The LC_{50} after 48 hr of exposure to Ni of first instar larvae of *Chironomus riparius* was 80 mg/L (Powlesland and George 1986).

Figure 2 shows the Ni concentrations (µg Ni/g DW) in the soft tissues of *D. polymorpha*. There were no significant differences between similar treatments ($p>0.05$). Internal Ni concentrations of all

treatments differed from controls ($p<0.05$). Internal Ni levels of *D. polymorpha* in controls (22 µg Ni/g DW) were similar to concentrations found in zebra mussels (20 µg Ni/g DW) in Lake IJsselmeer (Del Castilho and Marquenie 1983), a lake connected to Lake Markermeer. Significant uptake of Ni occurs above 215 µg/L ($p<0.05$). The relation between internal Ni levels and external Ni concentrations (dotted line, Fig. 2) is described by the power function

$$C_m = aC_w^b$$

(Amiard et al. 1987), in which C_m = concentration in the mussels, C_w = concentration in the water, a = 2.16 and b = 0.51. Since the levels in the controls are not on the dotted line (Fig. 2), the internal Ni concentration may remain constant at low but elevated concentrations in the water. This may suggest regulation of the metal. However, external Ni concentrations between 3 and 215 µg/L need to be tested before firm conclusions can be drawn.

Regulation of metals has often been observed. *D. polymorpha* is able to regulate Cu and Zn (Kraak et al. 1994). The capability to regulate internal metal concentrations can vary considerably between different invertebrate species. The shrimp *Palaemon elegans* is also able to regulate these metals (White and Rainbow 1982). The amphipod *Echinogammarus pirloti* approached regulation of Zn, but accumulated Cu. The barnacle *Elminius modestus*, however, accumulated both metals (Rainbow and White 1989). Moreover, metal accumulation in the body can vary within one species. Lobel et al. (1982) observed that some individuals of *Mytilus edulis* could regulate Zn, but other individuals of the same species accumulated Zn to high concentrations. In this study, it's unknown whether the level of Ni will remain constant at low external Ni concentrations as a result of a dynamic equilibrium (uptake rates equal elimination rates) or as a result of impairment of metal uptake (Depledge and Rainbow 1990). Therefore, it might be useful to determine excretion rates. Obviously, more physiological research is needed to clarify accumulation mechanisms in organisms.

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