Bull. Environ. Contam. Toxicol. (1992) 49:593–599

Environmental Contamination and Toxicology

Effects of Chromium, Copper, Nickel, and Zinc on Survival and Feeding of the Cladoceran *Moina macrocopa*

C. K. Wong

Department of Biology, The Chinese University of Hong Kong, Shatin, N. T., Hong Kong

Heavy metals are widely recognized as highly toxic and dangerous (Mance 1987). Past research activities on heavy metal pollution in Hong Kong have emphasized coastal environments (Chan et al. 1974; Phillips 1989; Chu et al. 1990). Since the main sources of heavy metals are the discharge and spillage of wastewater from electroplating factories, concentrations of heavy metals in streams and pools near industrial areas may be higher than those in coastal waters. Electroplating wastewater in Hong Kong contains high levels of chromium, copper, nickel and zinc. The toxicity of these heavy metals to aquatic organisms has been extensively reviewed (Mance 1987). Toxicity information for invertebrates shows that crustaceans are among the most sensitive organisms (Mance 1987). Of the crustacean species tested, cladocerans appear to be the most susceptible. Cladocerans are important components of many aquatic ecosystems. Despite their importance in many freshwater communities and their sensitivity to heavy metal toxicity, information on the toxicity of heavy metals to cladocerans is limited except for several Daphnia species (Biesinger and Christensen 1972; Winner and Farrell 1976; Ingersoll and Winner 1982). In Hong Kong the freshwater cladoceran Moina macrocopa occurs in small ponds and rice paddies and is mass cultured by some farmers as a high quality fish food. The objectives of this study are to determine the effects of various heavy metals on the survival and feeding of M. macrocopa.

MATERIALS AND METHODS

The freshwater cladoceran Moina macrocopa came from a continuous laboratory culture raised from a single parthenogenetic female. Experiments were carried out

Send reprint requests to C.K.Wong at the above address

with cohorts of newborn (< 24 hr) neonates obtained by isolating egg-bearing adult females from the stock culture.

Stock solutions (1.0 g/L) of metal ions were prepared by dissolving the metallic salts $K_2Cr_2O_7$, $CuSO_4.5H_2O$, $NiCl_2.6H_2O$ and $ZnSO_4.7H_2O$ in distilled water. Solutions for acute toxicity experiments and feeding experiments were prepared by adding aliquots of stock solution to filtered (Whatman GF/C) uncontaminated water from a large (300 L) aquarium.

Acute (48 hr) lethality experiments were carried out in 50 mL beakers containing 40 mL of test solution (pH 6.5) and 10 animals. Food consisted of a mixture of the algae Spirulina sp. and Chlorella pyrenoidosa. Food concentration was determined fluorometrically by measuring the amount of chlorophyll a in the water with a Turner Model 112 fluorometer. Initial food concentration was fixed at 10 ug Chla/L because results of an earlier study (Wong 1989) showed that the gut fullness of M. macrocopa feeding on C. pyrenoidosa increased with food concentration up to about 10 ug chla/L and then remained unchanged. Each metal ion was tested at 6 to 7 concentrations. Four replicates were used for each concentration. The beakers were weakly illuminated during day time by natural light. Temperature during experiments ranged from 24 to 27° C. Test solutions were renewed after 24 hr. Newborn animals produced at 24 hr were removed. The number of surviving animals was counted after 24 and 48 hr of exposure. Moribund animals were examined under a dissecting microscope. Individuals without heartbeats were considered dead.

The effects of various metals on feeding of M. macrocopa were studied at an exposure equivalent to the 48 hr LC50 of each metal. For each experiment, 20 animals were exposed in a 100 mL beaker containing 60 mL of test solution. Animals maintained in uncontaminated water were used as controls. Four to 6 replicates involving a total of > 100 animals were used for each metal. Food in the form of the alga C. pyrenoidosa was initially provided at a concentration of about 10 ug Chla/L. Feeding of the animals was measured after 0.5, 24 and 48 hr of metal exposure. Prior to the start of an experiment, the contents of the beaker were examined and dead animals were removed. Feeding experiments began when small aliquots of C. pyrenoidosa were added to the beakers to raise the food concentration to 50 ug Chla/L. After 15 to 20 min of feeding, animals were collected on a 0.125-mm mesh screen and inactivated by freezing. Gut fullness (chlorophyll + phaeopigments) was used as

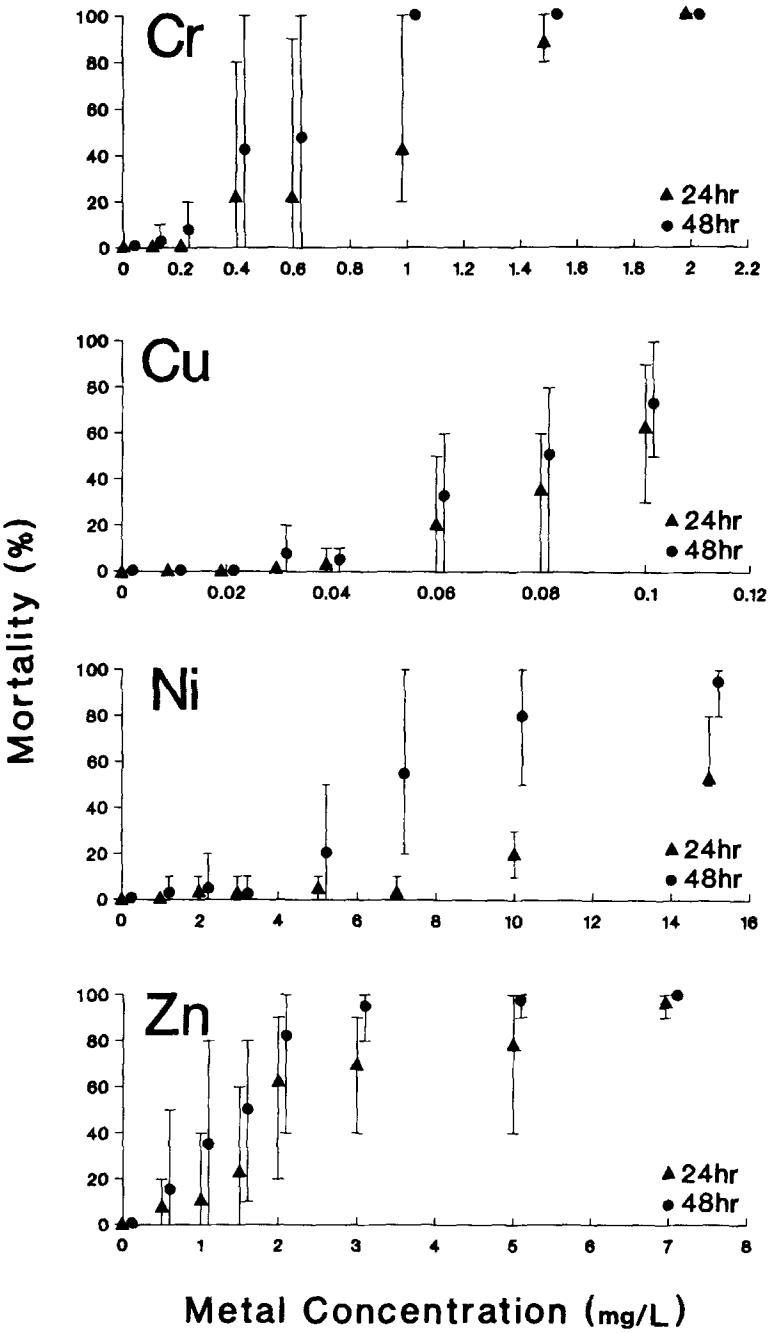

Figure 1. Mortality (%) of M. marocopa exposed to different concentrations of chromium, copper, nickel and zinc for 24 hr and 48 hr. Each point shows the mean and range of 4 replicates.

an index of feeding intensity for M. macrocopa. Frozen animals were transferred to 90% analytical acetone for pigment extraction. Fluorescence of the extracts before and after acidification with 5% HCl was measured with a Turner 112 fluorometer. Gut fullness was calculated using the equations of Dagg (1983).

RESULTS AND DISCUSSION

Mortality of Moina macrocopa in different concentrations of chromium, copper, nickel and zinc is presented in Figure 1. Variability in percentage mortality among replicates was high. In general, mortality increased with metal concentrations. Of the freshwater cladocerans tested, Daphnia magna showed a similar pattern (Biesinger and Christensen 1972, Khangarot and Ray 1987), while a slightly different pattern with copper > chromium > zinc > nickel has been reported for Daphnia hyalina (Baudouin and Scoppa 1974).

Probit analysis (Finney 1971) indicated that copper was clearly more toxic to M. macrocopa than the other metals (Table 1). The 24 and 48 hr LC50 values for copper were one to several orders of magnitude lower than those for the other metals. For copper, there was only a slight difference between the 24 hr LC50 and 48 hr LC50. For the other metals, the 48 hr LC50 was lower than the 24 hr LC50. The difference was about twofold for chromium and zinc, and close to fourfold for nickel. These results demonstrated that there was an increase in toxicity following prolonged exposure. The order of both LC50 values was nickel > zinc > chromium > copper.

Table 1. LC50 values (mg/L) of chromium, copper, nickel and zinc for M. macrocopa. 95% confidence limits are indicated in parentheses.

	24 hr	48 hr
Cr	0.76 (0.69-0.83)	0.36 (0.29-0.40)
Cu	0.09 (0.08-0.10)	0.08 (0.07-0.09)
Ni	23.59 (12.99-34.19)	6.48 (5.55-7.41)
Zn	2.04 (1.75-2.33)	1.17 (1.02-1.32)

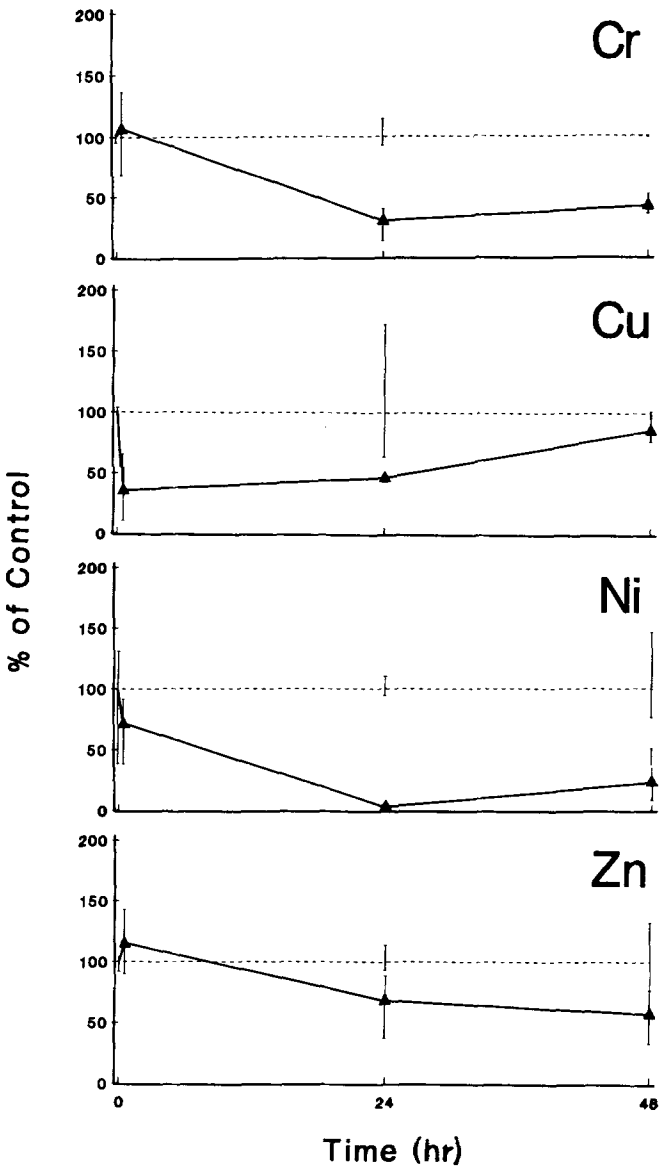

Figure 2. Gut fullness of M. macrocopa after 0.5, 24 and 48 hr of exposure to chromium, copper, nickel and zinc at a concentration equivalent to the 48 hr LC50 of each metal. Gut fullness of control animals is expressed as 100%. Each point represents the mean and range of 6 to 10 fluorometer counts using a total of at least 60 animals.

Comparison among species is approximate because many experimental variables may affect toxicity. In terms of LC50 values, M. macrocopa was less sensitive to heavy metals than some of the Daphnia species. The 48 hr LC50 of 0.08 mg Cu/L for M. macrocopa was similar to those reported for D. magna (Biesinger and Christensen 1972, Khangarot and Ray 1987) and higher than those reported for D. pulex (Ingersoll and Winner 1982) and D. hyalina (Baudouin and Scoppa 1974). For chromium, nickel and zinc, 48 hr LC50 values for M. macrocopa were much higher than those for D. hyalina (Baudouin and Scoppa 1974).

The feeding of M. macrocopa was measured after 0.5, 24 and 48 hr of exposure to metals at concentrations equivalent to the 48 hr LC50 value of each metal (Figure 2). Gut fullness of animals exposed to metals was expressed as a percentage of the control. Results were tested with 2-way ANOVA with exposure time and presence or absence of metal as the main factors. Variability in gut fullness was high among replicates. A significant effect ($P < 0.05$) of metals was noted for copper, chromium and nickel, whereas significant effects ($P < 0.05$) of exposure time was noted for copper, chromium and zinc. For all four metals, there was no evidence of interaction between the main factors. Animals exposed to copper for just 0.5 hr showed a 65 % reduction in gut fullness. At 24 hr, gut fullness was depressed by more than 50 % in animals exposed to chromium, copper and nickel, and by about 30 % in animals exposed to zinc. A continued, but less pronounced, inhibitory effect was observed at 48 hr. Gut fullness of surviving animals was reduced by about 58% in chromium, 77% in nickel, 42 % in zinc and only 14% in copper. Decreased food intake leads to lower reproductive capacity which, in turn, may reduce population size. These results suggested that feeding is a useful indicator of metal exposure.

Acknowledgments. The author thanks Ms. S.W. Hui for her help in performing the experiments. This work was supported by a grant from the Croucher Foundation, Hong Kong.

REFERENCES

Biesinger KE, Christensen GM (1972) Effects of various metals on survival, growth, reproduction, and metabolism of Daphnia magna. J Fish Res Bd Can 29: 1691-1700

Baudouin MF, Scoppa P (1974) Acute toxicity of various metals to freshwater zooplankton. Bull Environ Contam Toxicol 12: 745-751

Chan JP, Cheung MT, Li FP (1974) Trace metals in Hong Kong waters. Mar Pollut Bull 5: 171-174
Chu KH, Cheung WM, Lau SK (1990) Trace metals in bivalves and sediments from Tolo Harbour, Hong Kong. Environ Internat 16: 31-36
Dagg MJ (1983) A method for the determination of copepod feeding rates during short-time intervals. Mar Biol 75: 63-67
Finney DJ (1971) Probit analysis. Cambridge University Press, London
Ingersoll CG, Winner RW (1982) Effect on Daphnia pulex (De Geer) of daily pulse exposures to copper or cadmium. Environ Toxicol Chem 1: 321-327
Khangarot BS, Ray PK (1987) Correlation between heavy metal acute toxicity values in Daphnia magna and fish. Bull Environ Contam Toxicol 38: 722-726
Mance G (1987) Pollution threats of heavy metals in aquatic environments. Elsevier Applied Science, New York
Phillips DJH (1989) Trace metals and organochlorines in the coastal waters of Hong Kong. Mar Pollut Bull 20: 319-327
Winner RW, Farrell MP (1976) Acute and chronic toxicity of copper to four species of Daphnia. J Fish Res Board Can 33: 1685-1691
Wong CK (1989) Effects of cadmium on the feeding behaviour of the freshwater cladoceran Moina macrocopa. Chemosphere 18: 1681-1687

Received November 20, 1991; accepted April 9, 1992.