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Effects of Chromium, Copper, Nickel, and Zinc on Longevity and Reproduction of the Cladoceran *Moina macrocopa*

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Most rivers and ponds in Hong Kong are polluted to various extents by domestic sewage, livestock wastes and industrial effluents. Spillage of wastewater from electroplating factories and unauthorized connections of industrial discharges to stormwater drains in multi-story industrial buildings are the most important sources of heavy metal pollution. The worst situation, in terms of heavy metal pollution, is the Shing Mun River which flows through the industrial areas of Fo Tan. A recent survey of water quality in the Fo Tan nullah (Environmental Protection Department, 1989) showed that the concentration of chromium averaged 0.02 mg/L, copper 0.85 mg/L and zinc 0.16 mg/L.

The toxicity of heavy metals to aquatic organisms has been studied by many investigators (Mance 1987). Of the many organisms in freshwater environments, crustacean zooplankton are the most sensitive to heavy metal pollution (Mance 1987). The freshwater cladoceran Moina macrocopa is widely distributed (Edmondson 1959). In Hong Kong it is common in ponds, muddy pools and rice paddies and is mass cultured by some local fish farmers as a high quality fish food. Electroplating wastewater in Hong Kong contains high levels of chromium, copper, nickel and zinc. Toxicity of these metals to M. macrocopa has been considered on an acute basis (Wong, in press). However, reduced reproduction has been found to be a more sensitive measure of heavy metal toxicity than survival (Bertram and Hart 1979; Biesinger and Christensen 1972; Wong and Wong 1990). The present study was initiated to determine the chronic effects of metals on longevity and reproduction of M. macrocopa.

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MATERIALS AND METHODS

The test organisms in this study, Moina macrocopa, came from a laboratory population raised from a single parthenogenetic female. Animals were grown in water (pH 6.5-7.0) from a large (300 L) aquarium and fed Chlorella pyrenoidosa cells from an axenic culture. Analysis by atomic absorption spectrophotometry indicated that the concentration of chromium, copper, nickel and zinc in the aquarium water was < 1.0 µg/L. Egg-bearing adult females were isolated 16-18 hr prior to an experiment so that a cohort of newborn animals of nearly the same age could be collected the next morning for toxicity experiments.

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. Aliquots of stock solution were diluted with filtered (Whatman GF/C) aquarium water to obtain the appropriate test concentrations. Since the test solutions were not analyzed for the metals, the toxicity experiments recorded the nominal concentrations.

Toxicity experiments were carried out in 50 mL beakers containing 40 mL of test solution. Each metal ion was tested at 6 concentrations. Twenty to 40 animals, 10 in each beaker, were tested for each metal concentration. Animals were transferred daily to clean beakers with fresh test solutions. During each transfer, the number of surviving animals was recorded. Moribund animals were examined under a dissecting microscope. Individuals without heartbeats were considered dead. Newborn young were counted and discarded. Chlorella pyrenoidosa cells were centrifuged, resuspended in aquarium water and added to the beakers at a cell concentration of 200,000 cells/mL to feed the animals after each transfer. Surplus algal cells were always observed still in suspension after 24 hr. To minimize algal uptake of heavy metals, the beakers were only weakly illuminated during day time by natural light. Temperature during experiments ranged from 24 to 27^{o} C.

Life table parameters for M. macrocopa were calculated according to Hedrick (1984). Survivorship at age x (l_x) was measured as the proportion of individuals in the population that survived to day x. The median age of death (LT_{50}) was the time at which 50% of the animals had died. The average longevity represented the arithmetic average of time to death. Reproductive performance at age x (m_x) represented the average number of offspring produced by a female at age x.

Net reproductive rate (R_0), defined as the total number of offspring produced by a female during her entire lifetime, was calculated from the equation:

$$R_0 = \sum l_x m_x$$

Mean generation time (T) represented the number of days required for a population to increase by the R_0. It was calculated as:

$$T = \ln R_0 / r$$

where r was the intrinsic rate of population increase computed by solving the equation :

$$\sum l_x m_x e^{-rx} = 1$$

RESULTS AND DISCUSSION

Survivorship curves of Moina macrocopa at different concentrations of chromium, copper, nickel and zinc are presented in Figure 1. The difference between control and treatment groups was compared using the Wilcoxon paired-sample test, with day of death as the ranked observation. Copper was clearly more toxic to M. macrocopa than the other metals. Survivorship at 0.02 mg Cu/L and all higher concentrations was significantly different from survivorship of control. In the case of chromium, a significant effect on survivorship was observed at concentrations > 0.04 mg/L. Nickel and zinc were less toxic than copper and chromium. Significant reduction in survivorship was first observed at 0.25 mg/L for nickel and 0.50 mg/L for zinc.

LT50 was reduced by more than 2 days at concentrations of Cr > 0.04 mg/L, Cu > 0.02 mg/L, Ni > 0.25 mg/L and Zn > 0.45 mg/L (Table 1). LT50 was < 1 day at 0.25 mg Cu/L. The average lifespan for control animals was 9.70 days. In all treatment groups except 0.02 mg Cr/L and 0.01 mg Zn/L, the average lifespan was shorter than that for control animals. Average lifespan was reduced by more than 50% at concentrations of Cr > 0.35 mg/L, Cu > 0.15 mg/L, Ni > 0.50 mg/L and Zn > 0.70 mg/L.

Most control animals produced 3 broods. Exposure to heavy metals caused a reduction in average lifespan and number of broods per female. No clear differences in initial age of reproduction were observed among control and treatment groups. In all groups that

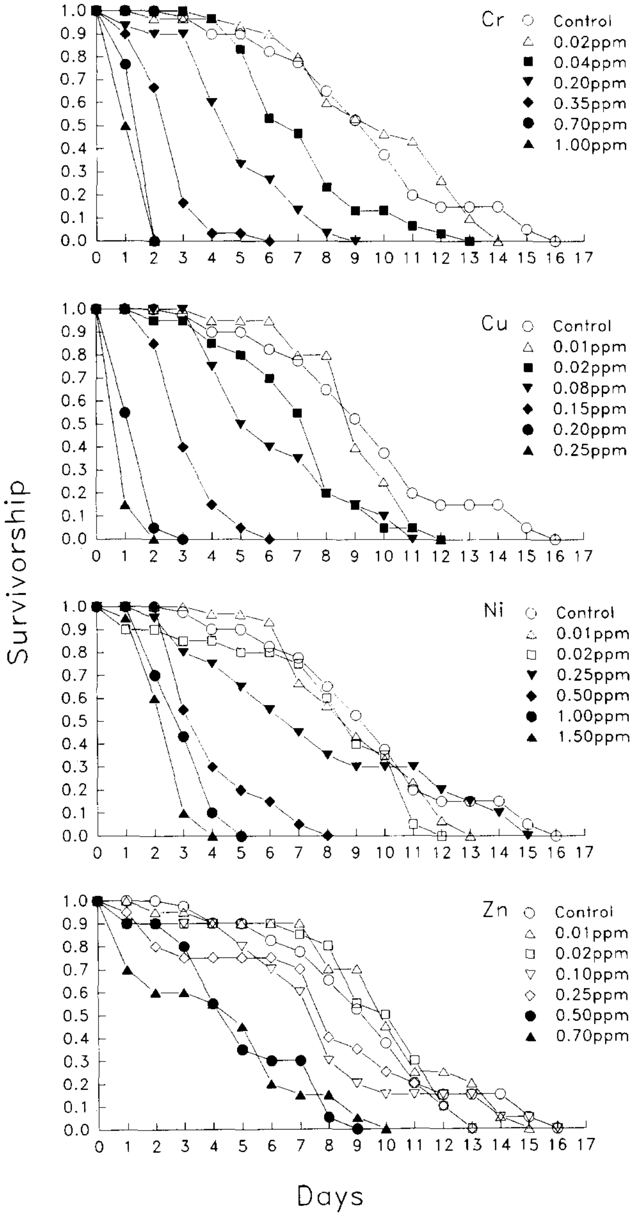

Figure 1. Survivorship of <u>M. marocopa</u> at different concentrations of chromium, copper, nickel and zinc. Significant ($P < 0.05$) difference in survivorship relative to control is indicated by closed symbols.

Table 1. Life table parameters for <u>M. macrocopa</u> exposed to various concentrations of chromium, copper, nickel and zinc.

Conc. (mg/L)	LT50	Average longevity (days)	Initial age of reproduction (days)	R_0	T	r
Control	9.16	9.70	3	13.43	5.72	0.62
Cr						
0.02	9.43	9.83	3	13.46	6.51	0.55
0.04	6.43	7.40	3	11.17	5.29	0.57
0.20	4.37	5.10	3	7.45	3.36	0.62
0.35	2.32	2.93	4	0.33	4.00	-0.27
0.70	1.34	1.77	-	0	-	-
1.00	1.00	1.00	-	0	-	-
Cu						
0.01	8.75	9.15	4	12.90	6.32	0.45
0.02	7.14	7.25	4	6.05	7.46	0.26
0.08	5.00	6.45	4	0.75	5.47	-0.05
0.15	2.78	3.45	-	0	-	-
0.20	1.10	1.60	-	0	-	-
0.25	0.59	1.15	-	0	-	-
Ni						
0.01	8.50	9.16	2	7.47	5.86	0.47
0.02	8.50	8.25	2	13.25	5.44	0.59
0.25	6.50	7.85	3	8.5	6.26	0.45
0.50	3.20	4.25	6	0.3	6.00	-0.20
1.00	2.74	3.23	3	0.03	3.00	-1.14
1.50	2.20	2.65	-	0	-	-
Zn						
0.01	9.80	10.10	3	9.85	5.82	0.52
0.02	10.00	9.50	2	11.80	6.14	0.48
0.10	7.33	7.90	3	11.20	6.79	0.48
0.25	7.67	7.75	2	11.55	6.04	0.52
0.50	4.25	5.15	3	9.55	4.44	0.59
0.70	4.50	4.45	2	3.85	4.94	0.31

produced broods, except 0.50 mg Ni/L, newborn young were first collected between days 2 and 4. This result agrees with previous studies which showed that the initial age of reproduction in <u>M. macrocopa</u> (Wong and Wong 1990) and <u>Daphnia pulex</u> (Bertram and Hart

1979) was not affected by exposure to cadmium. No reproduction was recorded at Cr > 0.70 mg/L, Cu > 0.15 mg/L, and Ni at 1.50 mg/L. The average initial age of reproduction among groups that produced broods was 3.11 days. Females at 0.70 mg Cr/L, 1.00 mg Cr/L, 0.20 mg Cu/L and 0.25 mg Cu/L did not survive to onset of reproduction.

Net reproductive rate, denoted by R_0, decreased rather abruptly at 0.35 mg Cr/L, 0.08 mg Cu/L, 0.50 mg Ni/L and 0.70 mg Zn/L (Table 1). In the case of copper, R_0 at 0.02 mg/L was less than 50% that of control. R_0 was < 1 at 0.08 mg/L, indicating that reproduction was reduced to below the replacement level. For chromium and nickel, R_0 was below the replacement level at concentrations $\geq$ 0.35 mg/L and 0.50 mg/L, respectively.

The intrinsic rate of population increase, r, measures the growth performance of the populations under different concentrations of heavy metals (Table 1). The values decreased abruptly at 0.35 mg Cr/L, 0.08 mg Cu/L, 0.50 mg Ni/L and 0.70 mg Zn/L because of the drastic decrease in reproduction. A slight decrease in generation time, T, with increasing metal concentration was recorded. In _D. pulex_ this phenomenon was attributed to the decrease in R_0 with increased exposure to metal and the ability of cladocerans to maintain high reproductive levels for the first one or two broods (Bertram and Hart 1979).

Comparison among species is hampered by the scarcity of chronic toxicity data for planktonic crustaceans. In addition, toxicity data for freshwater planktonic crustaceans are almost exclusively confined to the genus _Daphnia_. However, it is noticeable that _M. macrocopa_ was less sensitive to the four tested metals than _Daphnia magna_ (Biesinger and Christensen 1972; Trabalka and Gehrs 1977; Winner 1981). On the basis of survival and reproduction, the order of toxicity was Cu > Cr > Ni > Zn. This was different from the results of acute toxicity tests which showed a toxicity pattern of Cu > Cr > Zn > Ni (Wong, in press). Studies with _D. magna_ (Biesinger and Christensen 1972) also revealed that the order of toxicity in terms of LC50 values was Zn > Ni in acute tests and Ni > Zn under chronic test conditins. These results suggests that cladocerans might become more sensitive to nickel after prolonged exposures. For all four tested metals, concentrations for 50% reduction in longevity and R_0 were lower than 48 hr LC50's obtained in an earlier study (Wong, in press). Lee and Buikema (1979) noted that molting _D. pulex_ exhibited greater sensitivity to chromium. Since newborn M. macrocopa did not molt during 48 hr acute tests, the increased sensitivity to metals under chronic test conditions was likely to be molt-related.

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