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Acute Toxicity of Copper, Cadmium, and Zinc to the Water Flea, *Moina irrasa* (Cladocera)

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As a result of industrial activities, the aquatic ecosystem has been increasingly contaminated by heavy metals. This poses great stresses to aquatic organisms in particular and to the whole ecosystem in general. Because of the cladocerans' role as an important link in the food webs of freshwater ecosystems, as well as their high vulnerability to toxicants, many species have been used as bio-indicators to assess heavy metal toxicity. These include *Daphnia magna*, *D. pulex* (Hall *et al.* 1986), *D. carinata*, *Echinisca triserialis* (Chandini 1988), *Ceriodaphnia dubia* (Belanger and Cherry 1990), and *C. reticulata* (Hall *et al.* 1986). However, information on the toxic effects of heavy metals on the *Moina* species is rare. The present study investigates the acute toxicity of copper, cadmium, and zinc to *Moina irrasa*, a water flea commonly found in the freshwaters of the Yangtze delta of China. An attempt has also been made to examine the modification of heavy metal toxicity by environmental variables such as pH and temperature.

MATERIALS AND METHODS

Moina irrasa were obtained from a stock maintained by the aquatic invertebrate laboratory of the East China Normal University of Shanghai. For bioassay testing, a *M. irrasa* stock culture was recloned by cultivating a single parthenogenic female in distilled water at a temperature of 25 ± 0.5 °C, pH 7.0 ± 0.2, and fed at $10^6 \sim 10^7$ yeast cells/ml medium. The experiments were not started until sufficient numbers of egg-bearing *M. irrasa* were obtained. All toxicity tests were carried out on neonates (age < 24 hr) obtained by isolating adults from the stock culture a day earlier.

The metals tested included copper, cadmium, and zinc. Stock

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solutions were prepared by dissolving analytical grade salts in distilled water. To examine the effects of pH and temperature on heavy metal toxicity, a series of experiments were designed in which the toxicity of an individual metal was examined under various combinations of pH and thermal conditions. The experimental pH levels and temperatures were all within the physiological range for *M. irrasa* (Chen 1989). There was a total of nine sets of experiments for each metal. Six concentration gradients and one control were performed. The ranges of metal-salt concentration administered under various test conditions were determined by progressively narrowing the concentration range (Table 1).

At each concentration level in each experiment, 10 neonates (age < 24 hr) were introduced into a 50-ml beaker containing 20 ml of test solution. The solution was prepared by adding aliquots of the corresponding stock solution to distilled water at the experimental pH in the 50-ml beaker. The selected water quality variables at 25°C were as follows: conductivity 0.5~2.0 uS/cm, hardness less than 5 mg/l as $CaCO_3$ and dissolved oxygen 6.2~6.5 mg/l. Each pH (5.0, 6.5, 8.0) was adjusted with either dilute HCL or NaOH. Because the use of a buffer system was avoided, the pH of the test solution was readjusted daily. Mortalities were observed and recorded every 24 hr, and experiments were terminated after 96 hr, except where high mortalities occurred at all concentration levels, leading to earlier termination. The LC50 values were expressed in ug metal salt per liter.

Probit analysis was used to calculate the daily LC50 as described by Gad and Weil (1984). Three-way ANOVA as described by Tu (1985) was used to examine the effects of pH, temperature and metal types on metal toxicity to *M. irrasa*.

RESULTS AND DISCUSSION

Daily LC50 values for copper, cadmium, and zinc under various test conditions are shown in Table 2. It was clearly demonstrated that metal toxicity to *M. irrasa* increased with increasing exposure time. At higher temperatures, high mortalities occurred at almost all concentrations. Therefore, only 24- and 48-hr LC50 values at 25°C, and 24-hr LC50 values at 30°C were obtained (Table 2).

Table 1. Concentration gradients used under various test conditions. Concentrations in ug metal salt/liter.

	20 °C							25 °C							30 °C						
$CuCl_2$	0	5	7.5	10	15	20	50	0	1	5	7.5	10	15	20	0	0.5	1	5	7.5	10	15
$CdCl_2$	0	10	20	40	60	80	100	0	10	20	40	60	80	100	0	5	10	20	40	60	80
$ZnCl_2$	0	50	200	400	600	800	1000	0	50	200	400	600	800	1000	0	50	100	200	400	600	800

Table 2. Daily LC50 values for *Moina irrasa* exposed to copper, cadmium, and zinc under various test conditions. LC50 values in ug metal salt/liter.

		20 °C				25 °C				30 °C			
	pH	24-hr	48-hr	72-hr	96-hr	24-hr	48-hr	72-hr	96-hr	24-hr	48-hr	72-hr	96-hr
	5.0	6.49	6.03	4.92	3.30	10.06	5.51	-	-	1.42	-	-	-
$CuCl_2$	6.5	8.09	8.09	8.09	6.06	6.24	3.73	-	-	2.87	-	-	-
	8.0	19.67	12.61	12.61	7.48	12.84	11.84	-	-	12.74	-	-	-
	5.0	38.25	29.13	22.21	10.45	32.58	17.43	-	-	29.04	-	-	-
$CdCl_2$	6.5	42.32	25.04	13.46	9.57	27.55	5.26	-	-	20.98	-	-	-
	8.0	84.02	55.16	7.48	2.52	*	33.10	11.68	-	68.88	-	-	-
	5.0	147.30	77.46	70.05	-	119.4	49.99	-	-	*	-	-	-
$ZnCl_2$	6.5	306.49	152.51	124.59	102.87	182.31	92.88	-	-	81.28	-	-	-
	8.0	327.27	205.31	33.76	-	*	123.42	-	-	254.86	-	-	-

* LC50 values are incalculable because of low mortalities and are compensated for as described by Tu (1985) for ANOVA.

Table 3. Comparison of acute toxicity of copper, cadmium and zinc for some cladocerans.

	Species	pH	Temperature (^{0} C)	Water Hardness (mg/l as $CaCO_3$)	48-hr LC50 (ug/l)	References
	Ceriodaphnia reticulata	7.8±0.3	22	120±10	110	Hall *et al.* (1986)*
	Echinisca triserialis	7.0±1.2	24±1	dw	345	Chandini (1988)*
Cd	*Daphnia pulex*	7.8±0.3	22	120±10	80	Hall *et al.* (1986)*
	D. carinata	7.0±0.2	24±1	dw	265	Chandini (1988)*
	D. magna	7.8±0.3	22	120±10	50	Hall *et al.* (1986)*
	Moina irrrasa	8.0	20	<5	15.27	present study**
	Ceriodaphnia dubia	8	25	97.6±3.9	28	Belanger and Cherry (1990)
Cu	*Daphnia magna*	7.6	12~15	240	97	Khangarot *et al.* (1987)
	Moina irrasa	8	20	<5	5.93	present study**
Zn	*Ceriodaphnia dubia*	8	25	97.6±3.9	101	Belanger and Cherry (1990)
	Moina irrasa	8	25	<5	59.24	present study**

* Data for organisms fed during test.
** For the sake of comparison, LC50 data for *M. irrasa* are calculated in ug metal/l.

Table 3 shows the comparison of LC50 values for copper, cadmium, and zinc obtained for *M. irrasa* with those for other cladocerans. It appears that the LC50 values for either copper or zinc for *M. irrasa* were much lower than those for other cladocerans. However, an animal's response to heavy metals can be changed by extraneous factors such as water hardness and food (Chandini 1988; Belanger and Cherry 1990). In this investigation, the use of distilled water as the test medium and food deprivation during experiments might have contributed to the high sensitivity of *M. irrasa* to the toxicants tested. The remarkably large margin between LC50 values for *M. irrasa* and for other species may still indicate that *M. irrasa* is more sensitive to metal toxicants. Accordingly, they may be a better biological indicator of metal pollution in the aquatic environment.

The results of three-way ANOVA show that the metal type has a significant effect on both 24- and 48-hr LC50 values (Table 4). The

Table 4. Data for three-way ANOVA. A=Metal, B=pH. C=Temperature.

Variable	F-value	df1/df2	Significant Level
A	24-hr LC50 98.48	2/5	***
	48-hr LC50 195.12	2/4	***
B	24-hr LC50 18.40	2/5	***
	48-hr LC50 26.04	2/4	**
C	24-hr LC50 7.58	2/5	*
	48-hr LC50 27.86	1/4	**
AB	24-hr LC50 8.94	4/5	*
	48-hr LC50 13.60	4/4	*
AC	24-hr LC50 4.98	4/5	NS
	48-hr LC50 11.32	2/4	*
BC	24-hr LC50 0.670	4/5	NS
	48-hr LC50 1.77	2/4	NS

***: P < 0.005, **: P < 0.01, *: P < 0.05, NS: Not Significant.

24-hr LC50 values for copper under all test conditions (ranging from 1.42 to 19.67 ug metal salt/l) were lower than those for cadmium (ranging from 20.98 to 84.02 ug metal salt/l), and much lower than those for zinc (ranging from 81.28 to 412.86 ug metal salt/l), suggesting that the toxicity sequence for these metals is Cu > Cd > Zn.

Three-way ANOVA also demonstrated that pH had a significant effect (Table 4), with metal toxicity at lower pH being generally higher than at basic pH (Table 2). Similar results were also reported by Belanger and Cherry (1990) in *Ceriodaphnia dubia*. Temperature can also significantly influence metal toxicity (Table 4), with higher temperature generally enhancing a metal's toxicity (Table 2).

No interactions were found between pH and temperature (Table 4), while significant interactions were observed between pH and metal type and between temperature and metal type (Table 4). The existence of the interactions observed indicated that the order of toxicity among metals to *M. irrasa* was determined not only by metal factor, but by pH and termperature as well. Therefore, caution must be given when comparing metal toxicity among metals in view of the possible interacting effects of test conditions. In the present investigation, however, owing to the overwhelming effects of metal factor as shown in Tables 2 and 4, it is still safe to conclude that toxicity sequence for the three metals used was Cu > Cd > Zn. The existence of interactions between test factors stresses the importance of multi-way ANOVA in comparative studies of heavy metal toxicity and the modification of metal toxicity by test conditions.

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