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Sensitivity of Freshwater Pulmonate Snails, *Lymnaea luteola* L., to Heavy Metals

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The current alarm of the impacts of metal pollution on living organisms has received much attention with the tragedy of Minimata and later Niigata, in Japan. Although there has been a great deal of the concern about the acute and chronic toxicities of heavy metals to freshwater fishes (Doudoroff and Katz 1953; Pickering and Henderson 1966) and crustaceans (Biesinger and Christensen 1972; Maki 1979; Khangarot and Ray 1987b) but little information is available on the effects of heavy metals to freshwater snails (Wurtz 1962; Gupta et al. 1981a,b; Khangarot and Ray 1987a), which are widely distributed in the aquatic environment. The present study was undertaken to determine the acute toxicities of selected heavy metals to a freshwater pond snail **Lymnaea luteola** Lamarck; a locally abudant species and play an important role in the aquatic food chain(s). Static bioassays were conducted with the salts of cadmium, copper, chromium, mercury, nickel, silver and zinc in hardwater (hardness = 195 mg/L as $CaCO_3$ under ambient summer conditions (32°C).

MATERIALS AND METHODS

Adult **L. luteola** were collected from a local freshwater pond and acclimatized to laboratory conditions in tubewell water for 7-10 days prior to experiments. The specimens used for study averaged 2.1 cm (range 1.9-2.3 cm) in mean height and 0.5 g (range 0.45-0.75 g) in wet weight with shell. The toxicants were reagent grades of metal salts (Table 2). Stock solutions were prepared in distilled water. A series of test concentrations were prepared from the respective stock solution by dilution technique as described by APHA et al. (1977). The snails were fed daily with algae and aquatic plants but feeding was discontinued 48 h before static bioassays. Toxicity studies were carried out in the month of June-July and test water was not aerated during the experimental period. Ten snails were exposed at a time to each concentration and for every concentration there were two replicates. The toxicant solution and the control water were replaced after every 24 h. Seven to ten concentrations were tested for each metal. The criterion for death was the failure of snails to respond to prodding of their 'foot' with a needle. The death was further confirmed by putting the snail in freshwater. If it did not show any movement, it was considered dead. The

*Send reprint requests to P.K. Ray at the above address dead specimens were removed and recorded at 1, 2, 4, 8, 14±2, 24, 33±3, 48, 72 and 96-h. The LC50 values and their 95% confidence limits were calculated by moving-average-angle procedure (Harris 1959). The alkalinity, total hardness, dissolved oxygen, and total solids were analysed at every 24-h for physico-chemical properties (APHA et al. 1977). The measurements of sodium, potassium and calcium were made by flame photometric method.

RESULTS AND DISCUSSION

The physico-chemical properties of test solutions are shown in Table 1. Decreased pH values were recorded at some of the higher test concentrations of Cd, Cr and Zn metals, but these values were never greater than 0.5 pH unit. The test solutions of Zn and Cd showed white precipitation after 4-6 h after the addition of toxicants. In chromium test solutions, dissolved oxygen, alkalinity and total hardness were not determined, but these parameters were measured in control test containers.

Table 1. Physico-chemical properties of test water

Characteristics	Unit	Mean	Range
Temperature (Air)	°C	34	31-36
Water temperature	°C	32	30-33.5
pH		7.4	7.2-7.6
Alkalinity	mg/L as $CaCO_3$	160	120-177
Total hardness	mg/L as $CaCO_3$	195	165-230
Dissolved oxygen	mg/L	6.1	5.2-6.5
Sodium	mg/L	30	25-40
Potassium	mg/L	40	35-46
Calcium	mg/L	22	11-25
Conductivity	uM/cm	950	750-1050
Total solids	mg/L	980	800-1050
Dissolved solids	mg/L	510	420-650

The LC50 values and their 95% confidence limits are given in Table 2. There is a marked and progressive decrease of LC50 values between 24-h and 96-h. The results suggested that Ag was the most toxic heavy metal and Cr, the least toxic; Hg, Cu, Ni, Cd, Zn were intermediate in toxicity. Higher snail mortality appeared in first 48 h of exposure and thereafter mortality rate decreased considerably. From relative potency ratios, it appeared that the acute toxicities of Ag and Hg were 923.8 and 204.2 times more toxic than that of Cr, respectively. In the higher concentrations of toxicants, **L. luteola** spends most of the time at the bottom. However, in lower test concentrations, most of the snails attached to the wall of glass container and most of them survive during the experimental period, i.e., 96-h. Based on the concentration of metal ions that cause 50% mortality at 96-h of exposure (96-h LC50), the decreasing rank order of toxicity of metals was Ag > Hg > Cu > Ni = Cd = Zn > Cr. The rank order toxicity of these metal ions is correlated with the toxicity studies of heavy metals to some other freshwater animals. For example, the rank order toxicity of some of the selected heavy metals to **Daphnia magna** was Hg> Ag> Cu> Zn> Cr = Cd = Pd> Ni (Khangarot and Ray 1987b) and for amphibian tadpoles **Bufo melanostictus**, it was Ag> Hg> Cu> Cd> Zn> Ni> Cr (Khangarot and Ray 1987a). Eisler and Hennekey (1977) conducted static bioassays using starfish, sandworm, hermit crab, softshell clam, mudsnail and mummichog, and they observed that Hg was the most toxic and Ni the least toxic metal and reported the following the rank order toxicity for some of the heavy metals: Hg> Cd> Zn> Cr> Ni. Similarly, Gupta et al. (1981a) studied the acute toxicity of Cu, Zn, Cr, Cd and Cr to a freshwater Indian pond snail **Viviparus bengalensis** and reported that Cu was the most toxic metal and Ni the least toxic; Zn, Cd and Cr showed intermediate toxicity. There is a variation in rank order of toxicity among aquatic animals for Cd, Zn, Cr and Ni metals (Calabrese and Nelson 1974).

The 96-h LC50 values determined for **L. luteola** were 1.68 mg/L for Zn; 0.027 mg/L for Cu; and 0.019 mg/L for Hg at 32°C; while Mathur et al. (1981) reported the LC50 values of 6.13 mg/L for Zn, 0.172 mg/L for Cu and 0.135 mg/L Hg at 27°C for the same species. Wurtz (1962) and Gupta et al. (1981) reported that rise in temperature increased the zinc and copper toxicity in hardwater to ramshorn snail (**Helisoma campannulatum**) and pond snail (**Viviparus bengalensis**), respectively. Similar results were obtained for **Lymnaea luteola** for the acute toxicity of zinc ions with the seasonal variations in temperature (Khangarot and Ray 1987c). Few other comparative studies on zinc and copper to freshwater snails have also been reported. Wurtz (1962) calculated 96-h LC50 values of 0.05 mg/L of Cu and 3.16 mg/L of Zn in hard water (hardness = 100 mg/L as $CaCO_3$), and 0.034 mg/L of Cu and 1.11 mg/L of Zn in softwater (hardness = 20 mg/L as $CaCO_3$) for **Physa heterostropha.** Arthur and Leonard (1970) observed a 96-h LC50 values to **physa integra** as 0.039 mg/L of Cu. In another study, Cairns and Scheier (1958) determined the effect of temperature and hardness of water upon the acute toxicity of zinc to the pond snail, **Physa heterostropha.** Hardness of water is also an important physico-chemical property of test water which could change the heavy metal toxicity. The salts of Zn, Cd, Cu and Ni are less toxic in hardwater than they were in softwater (Pickring and Henderson 1966).

Freshwater pulmonate snails such as **L. luteola** are commonly available in Indian freshwater reserviors throughout the year. They are inexpensive; can be collected in large numbers; they can be easily handled and cultured in the laboratory and are sensitive indicator of harmful levels of environmental pollutants as observed in this study. For a realistic approach to pollution effects, it is essential that additional research on acute and chronic toxicity of variety of environmental pollutants, under different environmental (pH, temperature, hardness, dissolved oxygen, etc.) and biological conditions (age, size,reproductive state, etc.) are needed. The joint toxicity of chemicals should also be carried out. There is also need to understand the mechanism of pollutants at cellular and molecular levels in these animals. Some of these studies are in progress in our laboratory.

Table 2. The LC50 values and their 95% confidence limits for selected heavy metals to **Lymaea luteola L.**

Metal	Salt used	LC50 values and 95% confidence limits at(mg/L of metal)				Relative potencies at 96-h
		24-h	48-h	72-h	96-h	
Silver	$AgNO_3$	0.0194 (0.0112-0.0271)	0.0092 (0.0071-0.0178)	0.0064 (0.0051-0.0081)	0.0042 ---*	923.81
Mercury	$HgCl_2$	0.05 (0.036-0.072)	0.026 (0.021-0.036)	0.023 (0.018-0.023)	0.019 (0.016-0.023)	204.21
Copper	$CuSO_2.5H_2O$	0.081 (0.074-0.087)	0.052 (0.040-0.047)	0.027 (0.022-0.035)	0.027 (0.022-0.035)	143.70
Nickel	$NiCl_2.6H_2O$	6.09 (4.71-8.19)	1.70 (1.47-2.56)	1.70 (1.47-2.56)	1.43 (1.09-1.83)	2.71
Cadmium	$3CdSO_4.8H_2O$	3.41 (2.51-4.16)	2.10 (0.95-3.71)	1.60 (0.91-2.55)	1.52 (1.02-2.07)	2.55
Zinc	$ZnSO_4.7H_2O$	7 ---*	3.80 (2.87-5.22)	3.80 (2.87-5.22)	1.68 (1.15-2.77)	2.31
Chromium	$K_2Cr_2O_7$	16.55 (12.23-20.73)	5.85 (5.0 - 6.80)	4.14 (3.80-4.62)	3.88 (3.49-4.33)	1.00

*95% confidence limits can not be calculated

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