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Sensitivity of Toad Tadpoles, *Bufo melanostictus* (Schneider), to Heavy Metals

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Amphibian larval stages have several qualities which make them as a useful indicator of harmful levels of pollutants in bioassay tests (Cooke 1981). They are inexpensive and can be collected in large numbers during their breeding season; they can be easily handled in the laboratory and are good representatives of freshwater life. Amphibian tadpoles show a variety of sublethal responses such as changes in growth, development rates, pigmentation and expression of morphological deformities in a lesser time of exposure to the environmental pollutants.

Pollution of the aquatic environment by heavy metals is a subject of great attention. These pollutants are generally discharged into the environment as a result of industrial growth and mining process, and are a major problem because they are toxic and tend to accumulate in living system. In recent years, tadpoles have been used widely, as test organisms in bioassay tests. Studies have been conducted with heavy metals (Chang et al. 1973; Dial 1976; Khangarot et al. 1981) and pesticides (Cooke 1977; Dutta and Mohanty-hejmadi 1978; Hall and Swineford 1980). The objective of the work reported in this paper was to determine the acute toxicity of cadmium, copper, chromium, mercury, nickel, silver and zinc to the tadpoles of toad Bufo melanostictus (Schneider), which is commonly available and breed in aquatic habitats exhibiting a wide range of temperature and varying water quality (Daniel 1963).

MATERIAL AND METHODS

Tadpoles of B. melanostictus were collected from a pond of Lucknow (India) and acclimatized to laboratory conditions for seven days prior to exposure of metallic compounds. The animals used for study were 1.95 cm (range 1.8-2.2 cm) in length and 100 mg (range 90-120 mg) in wet weight. Tadpoles were fed freshwater algae during acclimatization period but were starved for 24 h before static bioassay and during the test period. The ground well water was used as a diluent water in all the tests. The toxicants selected in the present study were: Ag as $AgNO_3$, Cd as $3CdSO_4.8H_2O$, Cu as $CuSO_4.5H_2O$, Zn as $ZnSO_4.7H_2O$, Ni as $NiSO_4.6H_2O$, Cr as $K_2Cr_2O_7$ and Hg as $HgCl_2$. Stock solutions were prepared in distilled water. Acute bioassay procedure was followed

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during this study (APHA et al 1976). At the beginning of test 10 tadpoles were placed in 1000 ml test water. A logarithmic series of test concentrations (7-10) of toxicant and control were used in this study. All tests were performed in three replicate sets.

The physico-chemical properties of water were determined according to methods described in Standard Methods (APHA et al 1976). The LC50 values and 95 per cent confidence limits were calculated by moving-average-angle procedure (Harris 1959).

RESULTS AND DISCUSSION

Physico-chemical properties of water are given in Table 1. The higher Zn and Cd test concentrations dramatically affected the water quality; the alkalinity and pH decreased with increase of Zn and Cd concentrations, because of the precipitation of metal in the form of carbonates. The loss of carbonate from water caused a decrease in pH because of the release of hydrogen ions tied up in bicarbonate buffering system as HCO_3. However, the pH values never decrease less than 0.5 unit. Conductivity, total solids and dissolved solids increased with the increase of test concentrations.

Table 1. Physico-chemical properties of test water

Characteristics	Unit	Mean	Range
Temperature (Air)	°C	33.5	31-36
Water temperature	°C	31.0	29-34
pH		7.4	7.1-7.6
Alkalinity	mg/L as $CaCO_3$	135	120-160
Total hardness	mg/L as $CaCO_3$	185	165-215
Dissolved oxygen	mg/L	6.5	5.8-7.8
Conductivity	µM/cm	950	750-1100
Total solids	mg/L	920	650-1250
Dissolved solids	mg/L	520	390-630

The behavioral responses of the tadpoles to toxicants varied with metals and test concentrations. In higher concentrations (Ag, Cu and Hg), the behavioral changes were surfacing, increased irratic body movement and loss of equilibrium in 1-4 h of exposure. At lower concentrations, behavioral changes were noted only before death such as surfacing, loss of equilibrium and gulping of air from the surface. The control animals appeared normal and healthy at the end of experiment (96 h).

A summary of the LC50 values and 95 per cent confidence limits and relative potencies calculated from the mortality data are shown in Table 2. The results indicate that Ag was the most toxic metal, while Cr was the least toxic among the seven tested metals. From relative potency ratio it is clear that at 96 h, the toxicity of Ag was 12021 times more than that of Cr. The 96 h LC50 values suggested the decreasing rank order of toxicity of metals as follows: Ag > Hg > Cu > Cd > Zn > Ni > Cr.

Table 2. The LC50 values and 95% confidence limits for heavy metals to tadpoles of _Bufo metanostictus_ at different time intervals

Metal	LC50 values and 95% confidence limits at (mg/L)				Relative potencies at 96 h
	12 h	24 h	48 h	96 h	
Silver	0.0127 (0.0104-0.1505)	0.0073 (0.00637-0.00809)	0.0062 (0.00504-0.00708)	0.0041 (0.00364-0.00461)	12021.95
Mercury	0.0698 *---	0.0528 (0.0436-0.0615)	0.0456 (0.0409-0.0567)	0.0436 (0.0368-0.0585)	1130.5
Copper	1.97 (1.46-2.53)	0.843 (0.731-0.936)	0.446 (0.36-0.55)	0.32 *---	154.03
Cadmium	22.42 (20.19-24.3)	19.81 (17.39-29.15)	11.91 (9.99-13.95)	8.18 (6.96-9.53)	6.02
Zinc	50.03 (45.01-55.76)	47.26 (35.48-58.19)	25.65 (23.73-27.74)	19.86 (17.68-23.90)	2.48
Nickel	61.41 (56.18-66.25)	53.21 (49.56-58.37)	34.3 (32.9-37.21)	25.32 (22.8-28.62)	1.9
Chromium	74.25 (61.52-105.6)	57.97 (49.14-65.48)	53.43 (49.21-61.67)	49.29 (43.63-56.39)	1.00

*95% confidence limits cannot be calculated.

The rank order toxicity of tested metals is in good agreement with the comparative studies of heavy metals toxicity to aquatic organisms. For example, the rank order toxicity of some of the heavy metals to tadpoles Rana hexadactyta was Ag > Cu > Hg > As > Zn > Co > Fe > Pb > Cr (Khangarot et al 1985) and for a freshwater pond snail Viviparus bengalensis it was Cu > Zn > Cr > Cd > Ni (Gupta et al 1981). There is a variation in rank order of toxicity among aquatic animals for Cd, Zn, Cr and Ni metals (Calabrese and Nelson 1974). The changes in rank order toxicity is largely related to the physico-chemical properties of test water, species and life stages of animal (Vernberg et al 1974). In general, the acute toxicity of heavy metals to marine life was Hg > Cd > Ag > Ni > Se > Pb > Cu > Cr > As > Zn. They are listed here in the order of decreasing toxicity given by Ketchun et al (1975). Though this particular ranking order could be a matter of some debate (Waldichuk 1974).

Water characteristics, especially hardness, are known to influence the acute toxicity of heavy metals for aquatic organisms. The 96 h LC50 for Zn is 19.86 mg/L for tadpoles of Bufo melanosticutus and for frog tadpoles (Rana hexadactyla); the LC50 values are as low as 2.10 mg/L. The total hardness of water express as 20 mg/L of $CaCO_3$ (Khangarot et al 1984). The salts of Zn, Cu and Cd are less toxic in hardwater than they were in softwater (Pickering and Henderson 1966).

Tadpoles of frog and toad are commonly available in freshwater reservoirs during breeding season. There is a paucity of information on the acute and chronic toxicity of pollutants. Additional research on acute and chronic toxicity of aquatic pollutants to tadpoles under different environmental conditions are needed. The results of such studies can be useful for developing the water quality criteria for diverse uses.

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