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Sensitivity of Midge Larvae of *Chironomus tentans* Fabricius (Diptera Chironomidae) to Heavy Metals

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The discharge of heavy metals into the natural waters has numerous obvious impacts on physical, chemical and biological parameters of aquatic ecosystem. Bioassay tests are important steps in establishing appropriate water quality criteria and standards for diverse use of ponds, lakes, streams and river waters; if ecologically relevant and the most sensitive life stages of animals are used for such purposes. Therefore, the acute toxicities of various heavy metals to water flea Daphnia magna (Khangarot and Ray 1987a; Khangarot et al 1987), and snail Lymnaea acuminata (Khangarot et al 1982), and toad tadpoles Bufo mentanostictus (Khangarot and Ray 1987b), have been reported from our laboratory. Gauss et al (1985) suggested that chironomid larvae might be particularly useful as indicators of water quality because they are widely distributed in freshwater systems and often from diverse communities within particular habitat. Some studies have been reported on the toxic effects of metal ions to chironomid larvae (Rehwoldt et al 1973; Anderson et al 1980; Hepakeyama and Yasono 1981; Rao and Saxena 1981; Powlesland and George 1986). The aim of this study was to determine the acute toxicity of ten heavy metals to the midge larvae Chironomus tentans Fabricius, which forms an important link in aquatic food chain(s).

MATERIALS AND METHODS

Chironomid larvae were collected from a natural pond and acclimatized to laboratory conditions for 48 h prior to heavy metals exposure. Acute static bioassays were carried out in natural water at 13 ± 2°C according to method described in the Standard Methods (APHA et al 1981). This method recommends the 48 h of test duration in view of the effects of starvation to larvae and their possible development into succeeding instar during the course of the development. Tests were carried out in 250-mL glass beakers

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Table 1: Physico-chemical properties of test water

Characteristics	Unit	Mean	Range
Temperature	°C	14	13-17
pH		6.3	6.1-6.6
Acidity	mg/L as $CaCO_3$	20	16-28
Alkalinity	mg/L as $CaCO_3$	25	20-33
Total hardness	mg/L as $CaCO_3$	25	18-35
Dissolved oxygen	mg/L	6.5	5.5-8.0
Calcium	mg/L	7.0	6.4-8.5
Magnesium	mg/L	0.65	0.60-0.85

containing 200 mL of test water. Ten chironomid larvae (third instar stage) were placed in beaker and each test concentration was repeated twice. A series of test concentrations (7 to 10) of toxicant and controls were run during the study. Metal salts used in this study are listed in Table 2. Stock solutions were prepared in glass distilled water. As_2O_3 was insoluble in water, therefore, it was first dissolved in small amount of boiled water and dilute HCl and then the stock solution was prepared. The test concentrations are given as mg/L of metal. Immobilized chironomid larvae were inspected at 30 min during the first 4 to 6 h and hourly up to 6 to 8 h. Additional observations were made at 4 to 6-h intervals for remainder period of each test. Observations were continued until test animals immobilized or until the end of experiment, i.e., 48 h. The criteria for determining death was the complete immobilization, the lack of body movement, and papillae or mouth parts when subjected to gentle mechanical stimulation with a blunt glass rod. Immobilized chironomids were removed from the test container and counted. Effective concentrations (EC50), defined as the metal concentration required to immobilize 50% of the chironomid in a given time were calculated using moving-average-angle procedure (Harris 1959). No immobilization of test animal was observed in control test during 48 h of exposure. Alkalinity, dissolved oxygen, total hardness, calcium and magnesium were analyzed initially and after 48 h of test period. Physico-chemical analysis of water were carried out using the Standard Methods (APHA et al 1981).

RESULTS AND DISCUSSION

The mean and ranges of physico-chemical properties of test solutions are given in Table 1. Dissolved oxygen concentrations ranged from 5.5 to 8 mg/L. Temperature was the same as found in the pond water from which these chironomid larvae were collected. Decreased pH values were recorded at some of the higher test concentrations of Zn, Ni and Cr test solutions, but these values were never greater than 0.5 pH unit. In chromium test solutions,

Table 2: Acute toxicity of various heavy metals to *Chironomus tentans* larvae

Metal Ox[a]	Salt used	EC50 and 95% confidence limits (mg/L of metal)		Relative potancies at 48 h
		24 h	48 h	
Silver (I)	$AgNO_3$	0.0102 (0.0103-02012)[b]	0.0104 (0.0092-0.114)	6682.69
Mercury (II)	$HgCl_2$	0.119 (0.088-0.340)	0.029 (0.014-0.047)	2396.55
Copper (II)	$CuSO_4$ $5H_2O$	0.701 (0.50-0.946)	0.327 (0.134-0.527)	212.54
Arsenic (III)	AS_2O_3	1.309 (0.890-14.28)	0.68 (0.47-0.30)	102.21
Cadmium (II)	$CdCl_2$ $2H_2O$	23.25 (19.45-27.60)	8.05 (7.03-9.02)	8.63
Zinc (II)	$ZnSO_4$ $7H_2O$	10.83 (9.02-14.28)	8.20 (6.65-9.69)	8.43
Chromium (VI)	$K_2Cr_2O_7$	21.95 (19.45-24.15)	11.80 (8.27-15.23)	5.89
Lead (II)	$Pb(NO_3)_2$	52.87 (43.05-61.38)	34.67 - c	2.05
Cobalt (II)	$COCl_2$ $6H_2O$	67.92 (41.21-76.74)	56.87 (49.20-64.57)	1.22
Nickel (II)	$NiCl_2$ $6H_2O$	78.05 (65.16-88.51)	69.50 (40.60-81.10)	1.00

a Oxidation number

b Values given in parenthesis represent 95% confidence of EC50

c 95% confidence limits can not be calculated.

Table 3: Toxicity sequences for various metal ions to aquatic organisms

Organisms	Decreasing order of toxicity	Reference
Invertebrates		
Daphnia magna	Hg > Ag > Cu > Zn > Cr > Cd > Pb > Ni	Khangarot & Ray (1987a)
Daphnia hylalina	Hg > Cu > Cr > Zn > Cd > Pb > Co > Mg > Sr > Cs > Ca	Bandouin and Scoppa (1974)
Cyclops abysscrum prealpinus	Hg > Cu > Cd > Zn > Pb > Cr > Ni > Co > Mg > Sr > Cs > Ca >	Baudouin and Scoppa (1974)
Tubifex tubifex	Hg > Cd > Cu > Cr > Zn > Ni	Brkovic-Popovic and Popovic (1977)
Chironomus Tendipes	Hg > Cd > Pb > Zn > Mn	Rao and Saxena (1981)
Viviparus bengalensis	Cu > Zn > Cr > Cd > Ni	Gupta et al (1981)
Lymnaea acuminata	Hg > Cu > Cd > Ni > Cr > Zn	Khangarot et al (1982)
Mya arenaria	Hg > Cd > Zn > Cr > Ni	Eisler and Hennkey (1977)
Vertebrates		
Salmo gairdneri	Ag=Hg > Cu > Zn > Cd > Pb > Cr > Ni	See-Khangarot and Ray (1987a)
Lepomis macrochirus	Cu > Zn > Ni > Cd > Cr > Pb	Pickering and Henderson (1966)
Bufo melanostictus	Ag > Hg > Cu > Cd > Zn > Ni > Cr	Khangarot and Ray (1987b)
Rana hexadacytla	Ag > Cu > Hg > As > Zn > Co > Fe > Pb > Cr	Khangarot et al (1985)

[a] The atomic symbols represent hexavalent ion for Cr; trivalent ion for As; divalent ions for Cd, Co, Cu, Ca, Hg, Pb, Ni, Mg, Sr. Fe and Zn; and monovalent ions for Ag and Cs.

[b] In these sequences the metal concentrations resulting from the toxicity data (96 h LC50 or 48 h EC50 values) were expressed on a mg/L of metal ion basis.

The dissolved oxygen, alkalinity and hardness parameters were not determined but these water characteristics were analyzed in control test containers. EC50 values and their 95% confidence limits at 24 h and 48 h are shown in Table 2. The results suggest that metal toxicity increased with the increase of exposure time. Control animals appeared normal and healthy at the end of the experiment (48 h). The EC50 values indicates that Ag and Hg were the most toxic and Ni and Co the least toxic among the heavy metals tested. From relative potency ratio it appears that at 48 h, the toxicity of Ag and Hg ions were 6683 and 2397 times more toxic than that of Ni ions. The 48 h EC50 values of tested heavy metallic ions showed the decreasing rank order of toxicity as follows: Ag > Hg > Cu > As > Cd > Zn > Cr > Pb > Co > Ni. A selection of toxicity sequences of metal ions is shown in Table 3 for various aquatic animals. There are similarities between sequences for different organisms for various heavy metals. In general, Ag, Hg, and Cu ions are more toxic than Fe, Co and Ni. the position of a metal in a toxicity sequence may largely depend on several factors such as nature of salt used (e.g., chloride, sulphate, nitrate) and concentration scale (e.g. mg/L or molarity) to expressed lethal values (Venugopal and Luckey 1978). The variation in rank order of toxicity of metals to aquatic organisms is also related to the physico-chemical properties of test water (Khangarot and Ray 19 87a). Thus a toxicity ranking is approximately, because so many physical, chemical and biological factors may influence a pollutant's toxicity (Eisler and Honnekey 1977; Khangaroot and Ray 1987b).

In the present study, the 48-h EC50 values observed for C. tentans in mg/L were 9.2 for Zn, 0.029 for Hg and 34.87 for Pb; while Rao and Saxena (1981) reported the 48-h EC50 values of 62 mg/L for Zn, 0.664 mg/L for Hg and 50 mg/L for pB to C. tendipes. Our EC50 values are comparatively lower than those reported for Chironomus sp. by Qurshi et al (1980). They observed the 40hr EC50 of 1.8 mg/L for Hg, 80 mg/L for Zn, and 200 mg/L for Pb using static bioasay techniques. Water characteristics, especially hardness, are known to influence the acute toxicity of heavy metals to aquatic organisms. The present studies were carried out in soft water (see Table 1). The salts of Pb, Cu, Zn and Ni are less toxic in hard water than they need to agree are in soft water (Pickering and Henderson 1966).

Fresh water invertebrates have an important role to play in nutrient turnover and energy flow in the aquatic ecosystem. Therefore, studies on many species to taxonomically and ecologically divergent groups are required in order to evaluate in a satisfactory manner, The potentially hazardous compounds in polluted water. The results of such studies can be useful for developing the water quality criteria and standards for diverse uses of natural waters for recreation, food, energy, aquaculture, irrigation and industry.

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