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pH-Dependent Toxicity of Heavy Metals to a Freshwater Sludgeworm *Tubifex tubifex* Müller

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The effect of a metal on aquatic organisms is influenced by a number of environmental factors, which may alter the rate of metabolism, state and availability of the pollutant (Rattner and Heath 1995). Sprague (1985) presents an excellent review on the effects of environmental factors on the toxicity of heavy metals and other toxicants upon the fish and fish-food organisms. In determining the toxicity of trace metals to aquatic organisms, pH plays an important role because it affects the speciation and bioavailability of metals (Guy and Chakrabarti 1976). Attempts have been made to determine the effects of pH on the toxicity of metals to fishes (Howarth and Sprague 1978; Miller and Mackay 1980); however, little information is available on the effect on sludgeworms, which are an important link in aquatic food chains (Chapman et al. 1982a, b; Schübauer-Berigan et al. 1993). Sludgeworms are important members of benthic fauna in the aquatic environment, and harmful effects of heavy metals on these organisms are likely to be reflected in the whole ecosystem (Auston 1973; Reynoldson et al. 1991; Khangarot 1991; Fargasovä 1994; Boüché et al. 2000). Chapman et al. (1982a) studied the tolerance of 12 species of oligochaetes to a variety of pollutants including Hg and Cd under different conditions of pH (6, 7, 8). Toxicity within a pH range of 6 to 8 is of great interest because several industrial effluents and sediment have these pH values.

The purpose of the present study was to examine the effect of altered pH on heavy metal toxicity to a commonly available freshwater sludgeworm *Tubifex tubifex*. Short-term acute toxicity tests for 96 hr were conducted in hard water (305 mg/L as $CaCO_3$) at three pHs (6, 7,and 8). The tested heavy metals (Cu, Cd, Co, Cr, Hg, Pb, Ni, Fe, Mn and Zn) are frequently observed pollutants of industrial and sewage effluents (Heath 1987).

MATERIALS AND METHODS

The ten heavy metals studied in this research were cadmium (as $CdCl_2 . 2.5H_2O$), chromium (as $K_2Cr_2O_7$), cobalt (as $CoCl_2 . 6H_2O$), copper (as $CuSO_4 . 5H_2O$), iron (as $FeCl_3 . 6H_2O$), lead (as $Pb(NO_3)_2$, manganese (as $MnSO_4 . H_2O$), mercury (as $HgCl_2$) nickel (as $NiCl_2 . 6H_2O$) and zinc (as $ZnSO_4 . 7H_2O$). Reagent grade metallic salts were used in the study. Stock solutions of heavy metallic salts were

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prepared in double-glass distilled water. Static bioassay tests were conducted in hard reconstituted water (HRW; hardness = 305 mg/L as $CaCO_3$; alkalinity 225 mg/L as $CaCO_3$). Stock and dilution water solutions were adjusted to the desired pH (6, 7, and 8) using analytical-grade 1N HCl or 1N NaOH (E. Merck, India). Test concentrations were prepared using a 50% dilution series with hard reconstituted water (USEPA 1989) and adjusted to the target pH. Tests were conducted in 50 mL polystyrene petri dishes and sealed with polystyrene lids to prevent headspace gas exchange and to provide pH control for tests conducted at pH values 6, 7 and 8. The pH values generally drifted from the target values by <0.3 unit. Therefore, the test pH ranges were 6.0 to 6.3, 7.0 to 7.3 and 8.0 to 8.3. For simplification purposes, they will henceforth be referred to as pH values 6.0, 7.0 and 8.0 respectively. Toxicity tests were conducted in an environmental growth chamber at $20 \pm 1^{0}C$.

Test organism sludgeworm *Tubifex tubifex* Müller were collected from local sources and cultured in the laboratory. All tests were carried out in duplicate or triplicate for 96 hr using ten organisms per replicate in an environmental growth chamber at $20 \pm 1^{0}C$ and photoperiod (12:12 light: dark). Trials were run without aeration and renewal of test water. Dissolved oxygen was measured at test termination and was never below 5 mg/L (60 % saturation). The details static bioassay test procedures as outlined in standard methods were followed (APHA et al. 1993). Test pH values were measured at the beginning and at termination of test using a Systronic Instrument Pvt. Ltd. (India) pH meter equipped with a Systronic KCl-filled probe. The criterion for determining death of sludgeworms was lack of movement. Control trials with mortality greater than 10 percent were excluded from the results and repeated. Median effective concentrations (EC_{50}`s) and corresponding 95 percent confidence limits were calculated using the moving-average-angle method (Harris, 1959). The EC_{50} values were calculated using nominal metal concentrations.

RESULTS AND DISCUSSION

All control test animals survived in pH 6, 7, and 8 in the present study. EC_{50} values and 95 percent confidence limits calculated from mortality data by moving-average-angle methods are shown in Table 1. There was pH dependence in toxicity for Zn, Cu, Cd and Pb. Sludgeworms survived better at pH 7 than at pH 6 and 8. Percentage mortality of *T. tubifex* at different pH values in 48 and 96 hr of exposure of heavy metals concentrations suggested that most mortality appeared in the first 24-48 hours of metal exposure. At pH 6, with concentrations of 0.32 and 0.1 mg/L of Cu, the percent mortalities at 96 hr were 100 and 80, respectively. Similar trends in sludgeworm mortality were observed in Zn, Pb and Cd. At pH 7 and 8 lesser rate of mortality were noticed as compared to pH 6. These results indicate that acute toxicity of Zn, Cu, Pb and Cd to sludgeworm differ with changes in pH. Copper toxicity was much greater than Zn, Cd, Pb Fe, Mn, Ni and Co but lesser toxic than Hg. The toxicity of Mn and Ni were greater at pH 6 and pH 8. The acute toxicity of Hg was less pH-dependent for *T. tubifex*. Lower EC_{50} values were obtained for Cu, Cd, Zn and Pb when tested at pH 6 values compared to pH 7 and 8 values. The 96 hr EC_{50} values and their 95 percent confidence limits at pH 6 were 156.6 (109.4-212.1) while at pH 7 values were 203.1 (169.3-237.1) mg/L of Co. These values indicate that the toxicity of Co is less pH dependant. The results suggested that Zn, Cu, Pb and Cd were less toxic at pH 8 while at pH 6 these metals were more toxic. Thus, acute toxicity of heavy metals at different pH values varied considerably.

Characteristic morphological and behavioral changes of sludgeworm were noted when exposed to various heavy metals. In higher concentrations of Cu, Hg, Cd and Zn in initial hours of exposure, worms loss their movement, were less active and in later hours showed slow movement and remained separated in the test solutions. The morphological changes were uncoiling of the body followed by degeneration from the posterior body segments and loss of hemoglobin content, pale yellow body, breakage in body segment, and complete loss of rear body segments. At lower concentration no loss of body segments were noticed during 96 hr of exposure. In Cr and Mn, bioassay tests, there was no loss of body segments, but bodies became uncoiled and loss the characteristic tubificid movement. In addition, beading of the body and the massive blood clotting were observed in worms exposed to both higher and lower pH values and at various concentrations of Cr, and Hg. In Mn tests, the worms were typically uncoiled, lost color and became quiescent. All the tubificid worms in control tests at pH 6, 7, and 8 survived and remained healthy and appeared normal at the end of the experiment.

Although there have been some laboratory studies on the effects of heavy metal ions on sludgeworms (Whitely and Sikora 1970; Khangarot 1991; Reynoldson et al. 1991; Boüché et al. 2000; Rathore and Khangarot 2002). Studies on effects of pH on metals acute toxicity to freshwater invertebrates are few. Whitely (1967) observed that tubificid worms had high tolerance of Pb and Zn in test solutions. The 24 hr LC_{50} value was 49 mg/L at pH of 6.5 and 27.5 mg/L at a pH 8.5. In a review article, Auston (1973) suggested that tubificids could be used as biological indicators of water quality especially for water receiving sewage or other organic effluents. Few studies have also examined the resistance of oligochaetes to high and low pH values. Rice (1939) found that *T. tubifex* could survive in a range of pH between 5 and 9.5 for 18 hours. The present investigations were carried out in the pH range of 6 to 8. No similar comparable data are available in the literature. There have been few studies of oligochaete tolerance to heavy metals under variable environmental conditions. Chapman et al. (1982a, b) studied the relative tolerance of selected freshwater and marine oligochaetes to mercury and cadmium toxicity at pH 6, 7, and 8. They reported that the effect of pH changes on oligochaetes tolerance to Cd was species-specific. A study of more relevance to the present investigation was carried out by Schübauer-Berigan et al. (1993) and measured the pH dependant acute toxicity of Cd. Cu, Pb, Ni, and Zn to the freshwater cladoceran *Ceriodaphnia dubia*, fathead minnow, *Pimephales promelas, Hyalella azetca* and an oligochaete *Lumbriculus variegates* at three pH values (6, 7.3 and 8.3) in vary hard reconstituted water (hardness= 300-320 mg/L as $CaCO_3$). They reported that toxicity of Cd, Ni and Zn was greatest at pH 8.3 and least at pH 6.3 to most of these species. However, the toxicity of Cu and Pb

Table 1. Effect of heavy metals in hard water at different pH 6, 7, and 8 on survival of freshwater sludgeworm *Tubifex tubifex*

Water pH	EC_{50} values and their 95 percent confidence limits (mg/L) 24 hr	48 hr	72 hr	96 hr
		Cadmium		
6	62.68	14.39	11.53	8.279
	(49.76-74.87)	(12.03-17.77)	(9.53-13.21)	(7.11-9.9)
7	53.45	34.41	30.06	30.06
	(46.33-65.03)	(27.56-40.73)	(24.46-40.49)	(24.46-40.49)
8	4.50	2.45	1.486	1.131
	(3.50-5.92)	(1.98-3.34)	(1.27-1.78)	(1.11-1.33)
		Chromium		
6	29.77	6.40	4.48	3.49
	(24.47-38.58)	(4.95-8.02)	(3.83-5.26)	(2.83-4.16)
7	20.92	7.57	20.92	2.019
	(15.39-26.83)	(6.31-10.80)	(15.40-26.83)	(1.12-3.02)
8	26.78	13.78	8.39	6.24
	(23.64-30.85)	(12.10-15.82)	(6.99-10.32)	(4.83-7.59)
		Cobalt		
6	694.8	357.7	313.3	156.6
	(563.8-830.6)	(300.5-417.1)	(153.1-541.9)	(109.4-212.1)
7	395.6	216.7	203.1	203.1
	(365.5-437.2)	(181.0-252.3)	(169.3-237.1)	(169.3-237.1)
8	397.1	282.7	806.4	585.7
	(334.1-459.4)	(242.6-339.3)	(691.7-962.9)	(479.9-653.5)
		Copper		
6	0.579	0.422	0.148	0.148
	(0.458-0.693)	(0.361-0.496)	(0.110-0.188)	(0.110-0.188)
7	48.44	17.97	13.397	4.222
	(41.64-57.61)	(15.28-22.82)	(11.051-16.286	(3.519-4.956)
8	2.428	1.079	0.681	0.275
	(1.461-5.110)	(0.882-1.252)	(0.578-0.841)	(0.236-0.329)
		Iron		
6	29.30	29.30	29.30	27.56
	(24.36-34.13)	(24.36-34.13)	(24.36-34.13)	(23.60-32.95)
7	74.67	58.57	53.46	49.71
	(63.55-88.16)	(47.99-68.27)	(46.25-64.97)	(42.83-59.41)
8	26.17	20.05	17.15	17.15
	(21.16-30.97)	(15.77-24.84)	(14.72-21.04)	(14.72-21.04)
		Lead		
6	166.48	134.05	111.69	102.13
	(143.36-204.33)	(113.67-158.33)	(88.86-132.82)	(79.82-122.04)
7	> 320	> 320	> 320	> 320
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8	185.15 (158.17-223.27)	74.79 (63.55-88.16)	74.79 (63.55-88.16)	74.79 (63.55-88.16)
		Manganese		
6	124.0 (103.9-145.3)	97.5 (82.7-117.9)	90.8 (77.7-109.6)	78.8 (67.1-107.5)
7	> 560 ------*	> 560 ------*	> 560 ------*	252.5 (195.0-335.2)
8	212.9 (180.0-249.5)	179.7 (152.8-220.1)	158.8 (135.4-190.9)	148.6 (127.3-181.6)
		Mercury		
6	0.075 (0.064-0.088)	0.075 (0.064-0.088)	0.07 (0.059-0.082)	0.07 (0.059-0.082)
7	0.056 (0.046-0.065)	0.095 (0.082-0.117)	0.075 (0.064-0.088)	0.075 (0.064-0.088)
8	0.075 (0.064-0.088)	0.075 (0.064-0.088)	0.075 (0.064-0.088)	0.075 (0.064-0.088)
		Nickel		
6	< 560 ------*	97.1 (82.7-118.0)	84.9 (73.2-102.3)	74.8 (63.54-88.1)
7	126.9 (106.7-148.9)	51.4 (42.9-61.5)	60.8 (48.1-72.1)	60.8 (48.1-72.1)
8	95.4 (82.5-116.7)	86.1 (73.6-103.1)	86.1 (73.6-103.1)	27.5 (22.4-35.1)
		Zinc		
6	36.11 (33.86-41.95)	31.89 (27.28-38.42)	20.30 (16.94-23.68)	17.97 (15.28-22.02)
7	55.91 (47.90-67.62)	44.67 (40.31-56.37)	38.78 (32.97-46.55)	36.23 (34.95-42.95)
8	20.17 (11.21-30.20)	8.79 (7.34-10.91)	7.82 (6.54-10.42)	6.49 (5.79-7.59)

* 95 percent confidence limits cannot be calculated.

was greatest at pH 6.3 and least at 8.3. Chapman *et al* (1982a) measured 96 hr LC_{50} values of Hg and Cd for tubificid worm *T. tubifex* and eight other freshwater oligochaetes. The 96 hr LC_{50} values for *T. tubifex* were 0.14 and 0.32 mg/L for Hg and Cd, respectively at pH 7.0. Broković-Popović and Popović (1977) observed 24 and 48 hr LC_{50} values exposed to Cu, Cd, Hg, Cr, Ni Zn in vary soft and hard waters. They found that acute toxicity of these heavy metals is hardness and alkalinity dependent. Their 48 hr LC_{50} values in mg/L were: Cu, 0.06-0.89; Cr, 0.06-4.5; Ni, 0.8-61.4; Cd, 0.03-0.72; Hg, 0.06-0.1, and Zn, 0.11-60.2. The LC_{50} values suggested that *T. tubifex* is more sensitive to Cu, Cd and Hg than Zn, Cr and Pb. A similar rank order of heavy metal toxicity was observed is our study. Pervious published Cu, Hg and Zn LC_{50} or EC_{50} values for *T. tubifex* exposed in hard water closely agree with the range of EC_{50}\`s observed for *T. tubifex* in the present study. In an experiment of hard water (hardness = 245 mg/L as $CaCO_3$) exposure, Khangarot (1991) reported 96 hr EC_{50} values 17.78

mg/L for Zn, 47.53 mg/L for Cd and 0.05 mg/L for Hg for sludgeworm. Difference in acute toxicity of heavy metals to *T. tubifex* between the present study and those found by other investigations is mainly due to differences in, temperature, total hardness and pH between exposures. Because of the effect of pH and other environmental factors on EC_{50} values for heavy metals as observed in the present study and by other investigators, considerable caution should be exercised when comparing the sensitivities of species tested in different waters. Slight differences in experimental conditions, especially pH, result in changes in EC_{50} values that make direct comparisons of relative acute sensitivities impractical. For example, Holcombe and Andrew (1978) reported that at constant hardness and alkalinity, the LC_{50} for trout varies by a factor of approximately two fold for each unit change in pH. Thus for determining the safe concentrations, developing water quality criteria and standards for diverse uses and protecting aquatic fauna and flora and human health, the water quality must be same for the acute and chronic toxicity tests.

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