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Toxicity of Metals to a Freshwater Tubificid Worm, *Tubifex tubifex* (Muller)

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Salts of various metals are being released in ever increasing amounts into the aquatic environment from mining operations, metal processing facilities, chemical industries and other similar sources (Goldberg 1976). Although there has been considerable study of the acute and chronic toxicities of metals to freshwater fishes (Doudoroff and Katz 1953; Leland and Kuwabara, 1985), crustaceans (Hale 1977; Khangarot and Ray 1989) and snails (Khangarot and Ray 1988), little information is available on the effects of metals to tubificid worms (Jones 1938; Brkovic-Popovic and Popovic 1977a,b) which are widely distributed in the aquatic environment. Tubificid worms are useful indicators of varying degrees of aquatic pollution (Auston 1973). It is suggested that tubificid worms are an important element in the aquatic environment and therefore their use as a bioassay organism is logical one. The importance of using aquatic oligocheates as test organisms were described in detail by Chapman et al. (1982). The present study was undertaken to determine the acute toxicities of various metals to a freshwater tubificid worm, Tubifex tubifex (Muller), which form an important linke in aquatic food chain(s).

MATERIALS AND METHODS

Tubificid worms, Tubifex tubifex, were collected from Gheru Campus of ITRC, Lucknow, from natural sources and acclimatized to laboratory conditions for 7 days prior to experiments. All test chemicals were at least reagent grade in quality. The chemical formulae of the 32 metal salts used in the present study are shown in Table 2. Stock solutions were prepared in distilled water. During the preparation of stock solutions from the metal salts it was observed that copper sulphate, zinc sulphate and ferrous sulphate were less soluble, therefore, few drops of dilute HCl were added to the stock solution to dissolve the salts. Sodium arsenite, antimony trioxide, and sodium tungstate were boiled in a small amount of dilute HCl to dissolve them. A concentration gradient series from each respective stock solution was prepared in tubewell water. Final concentrations were based on the results of preliminary

acute static bioassays. Test concentrations were selected on a logarithmic scale as outlined in standard methods (APHA et al. 1981). Tests were conducted in 200 ml beakers containing 100 ml of test water. Ten tubificid worms were exposed to each concentration, and each concentration was tested in replicates of three. The concentrations were given as mg/L of metal ion. Test worms were considered dead when there was complete immobilization and no response to pressing with a blunt glass rod. Death was further confirmed by transferring worms back to fresh control tubewell water. The dead specimens were removed and recorded at intervals of 30 min and 1,2,4,8, 14±2, 24, 33±3, 48 and 96h. Test water was renewed every 24 hrs.

EC50 (effective concentration at which 50% immobilization response was recorded) values and 95% confidence limits were calculated by the moving average angle method (Harris 1959). The physicochemical properties of test water were determined by routine procedure (APHA et al. 1981).

RESULTS AND DISCUSSION

The physico-chemical characteristics of tubewell water used in the present study are shown in Table 1. The test solutions of Ba, Fe, Cd, Zn and Sn showed precipitation after 2-3 hours of the addition of metal salts.

Table 1: Physico-chemical properties of tubewell water used for worm toxicity tests.

Characteristics	Unit	Mean	Range
Water temperature	^{o}C	30	29.5-31
pH		7.6	7.5-7.7
Dissolved oxygen	mg/L	5.8	5.2-6.0
Total hardness	mg/L as $CaCO_3$	245	230-250
Total alkalinity	mg/L as $CaCO_3$	400	390-410
Calcium	mg/L	160	151-167
Magnesium	mg/L	90	80-98
Chloride	mg/L	10	7-12

In control tests, tubificid worms remained active during the test period. They were clustered at the bottom of the test container and showed typical tubificid movement. In the higher concentrations of heavy metals, e.g., Ag, Hg, Cu, Pt, Pd and Zn, test animals remained separated at the beginning of the experiment and showed rapid twisting movement. The later phase of the intoxication was the reduced tactile movements of the worm. Segmentation and degeneration of the body took place and death appeared without other noticable signs. At the lethal concentrations of many metals after 24h of exposure, the haemoglobin content disappeared and the

Table 2: Acute toxicity of various metal ions to Tubifex tubifex.

Metal	Salt used	EC50 and 95% confidence limits (mg/L of metal)		
		24h	48h	96h
Os	OsO_4	0.014 (0.009-0.021)	0.009 (0.007-0.0121)	0.0067 (0.0051-0.0072)
Ag	$AgNO_3$	0.041 (0.035-0.048)	0.039 (0.033-0.046)	0.031 (0.021-0.055)
Pb	$Pb(NO_3)_2$	0.237 (0.183-0.316)	0.142 (0.107-0.184)	0.042 (0.033-0.052)
Hg	$HgCl_2$	0.182 (0.218-0.152)	0.121 (0.090-0.061)	0.051 (0.032-0.061)
Pt	$PtCl_2$	0.095 (0.086-0.163)	0.086 (0.073-0.092)	0.061 (0.050-0.079)
Pd	$PdCl_2$	0.237 (0.183-0.316)	0.142 (0.107-0.184)	0.092 (0.033-0.052)
Cu	$CuSO_4.5H_2O$	0.506 (0.437-0.597)	0.282 (0.249-0.323)	0.158 (0.139-0.182)
Cr	$K_2Cr_2O_7$	0.348 (0.289-0.405)	0.196 (0.161-0.223)	0.190 (0.149-0.225)
Bi	$Bi(NO_3)_3.5H_2O$	14.79 (13.11-17.20)	14.79 (13.11-17.20)	0.662 (0.535-0.793)
U	$UO_2(CH_3COO)_2.2H_2O$	8.61 (7.46-10.18)	7.89 (6.36-8.67)	2.05 (1.72-2.26)
Se	$NaHSeO_3$	14.83 (12.41-18.35)	7.94 (6.43-9.73)	7.71 (6.50-9.16)
Li	$LiSO_4.H_2O$	44.77 (35.90-56.12)	11.22 (9.80-12.94)	9.34 (8.91-11.26)
As	Na_3AsO_3	13.01 (11.17-14.62)	8.87 (8.01-9.93)	8.87 (8.01-9.93)
Be	$BeSO_4$	25.97 (21.80-29.33)	18.22 (15.50-20.88)	10.25 (8.40-14.40)
Zn	$ZnSO_4.7H_2O$	22.18 (18.95-25.54)	21.13 (17.58-24.49)	17.78 (13.82-25.66)

(contd.....)

Sn	$SnCl_2.2H_2O$	157.76 (132.37-197.53)	140.28 (113.07-166.47)	21.23 (18.23-23.87)
Mo	$Na_2MoO_4.2H_2O$	56.11 (44.42-75.44)	52.12 (42.48-65.64)	28.91 (26.30-34.72)
La	$La(OH)_3$	33.50 (27.86-38.87)	33.50 (27.86-38.87)	29.38 (24.45-36.39)
Ba	$BaSO_4$	44.98 (34.20-57.71)	33.65 (28.61-38.03)	33.65 (28.61-38.03)
Cd	$CdCl_2.6H_2O$	75.86 (69.50-85.78)	59.43 (51.44-71.18)	47.53 (40.15-56.70)
Ni	$NiCl_2.6H_2O$	120.5 (106.7-134.97)	96.38 (82.95-112.10)	66.75 (40.15-56.70)
Al	$Al(NH_4SO_4)_2.12H_2O$	69.82 (61.82-80.54)	55.85 (48.45-66.89)	50.23 (40.96-64.32)
Fe	$FeCl_3.6H_2O$	112.98 (102.50-123.68)	101.84 (91.83-111.57)	101.84 (91.83-111.57)
Co	$CoCl_2.6H_2O$	447.71 (358.99-561.20)	447.71 (358.99-561.20)	139.32 (113.14-148.79)
Te	K_2TeO_3	510 (480.20-590.82)	320 *	125.60 (102.19-158.73)
Mg	$MgSO_4.7H_2O$	302.79 (265.26-350.81)	164.82 (149.60-191.36)	158.13 (139.48-184.91)
Mn	$MnSO_4,2H_2O$	301.3 (264.93-350.81)	208.06 (248.16-335.71)	170.61 (147.17-197.76)
Zr	$ZrOCl_2$	403.30 (380.41-440.41)	331.81 (320.06-366.24)	221.18 (190.61-231.27)
Sr	$SrCl_2.6H_2O$	540 (511.5-620.4)	320 (308.4-384.1)	240.8 (211.4-290.9)
Ca	$CaCl_2.2H_2O$	814.40 (750.66-910.16)	389.05 (326.66-446.52)	281.19 (249.02-326.60)
Sb	Sb_2O_3	108 (926-1330)	920 (840-1181)	678 (610-840)
Na	NaCl	1250 *	1016 (920-1108)	781 (660-884)
K	KCl	2000 *	1320 (1026-1671)	812.8 (738-937)

*95% confidence limits cannot be calculated.

rear part of the body became white, with disintegration of the body observed. In general, disintegration starts from the rear part of the body and advances towards the front part.

The EC50 values and their 95% confidence limits, chemical formulae of the metal salts used are given in Table 2. The results of the present study suggested that Os and Ag were the most toxic and Na and K the least toxic ions. The rank order toxicity of these metal ions are in good correlation with the toxicity of heavy metals to some other freshwater. For example, the rank order toxicity of selected metals to Daphnia magna was Hg Ag Cu Zn Cd Pb Co Cr^{6+} As Ni Fe Mn Sn Ba W Sr Al Sb K Ca Na (Khangarot and Ray 1989) and for amphibian tadpoles Bufo melanostictus, it was Ag Hg Cu Cd Zn Ni Cr (Khangarot and Ray 1987). In general, Ag, Hg, Cu and Cd are more toxic than Na, K and Mg. The position of metal ion in toxicity sequence may depend largely on several factors, such as the salt used and expression of lethal values (Venugopal and Luckey 1978). The variation in rank order toxicity in aquatic organism is also related to physico-chemical properties of test water of toxicity among aquatic animals for Cd, Zn, Cr and Ni metals (Calabrese and Nelson 1974).

The acute toxicity of metals to tubificid worms under laboratory conditions have been reported by few investigators. Jones (1938) observed that T.tubifex were killed within hours of exposure to Cu and Pb at 1000 mg/L. Brokovic-Popovic and Popovic (1977a) determined the 24h and 48h LC50 values of Cu, Cd, Cr, Hg, Ni and Zn ions in hard and soft water. The 48h LC50 values in mg/L were: Cu, 0.006-0.89; Zn, 0.11-60.2; Cr, 0.06-4.57; Ni, 0.08-61.4; Cd, 0.03-0.72; and Hg, 0.06-0.1. The toxicity of tested metals depends on hardness and alkalinity of test water, except for Hg. The results of the present study also suggested that Hg, Cu and Cd are more toxic than Cr, Zn and Ni. Whitley (1967) found the LC50 values of 49.0 mg/L for Pb and 46 mg/L for Zn and he has also suggested that toxic action is due to formation of mucus metal coomplex which precipitates on the body wall of worms and blocks the exchange of oxygen and carbon dioxide.

The results of the present study suggest that there is a common, non-specific, toxic action for most metal cations, which can be related to their strength of covalent binding to the ionogenic group, for example, with imidazole, carboxyl and sulphydral groups. Thus, it seems reasonable that most of the heavy metal ions are toxic to living organisms because they combine with some ligends of enzymes which are necessary for life. However, for non-transitional metal cations, enzyme inhibition is not likely to be a primary factor in toxicity. But osmatic or other colligative factors operating through physical reactions cause physical damage to the cellular system.

There is a need to understand the mechanisms of metal ion interactions at the cellular and molecular levels in aquatic organisms.

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