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Sensitivity of the freshwater tropical oligochaete, *Branchiura sowerbyi* (Beddard, 1892) to the grey list metal, Zinc

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ABSTRACT

Although the freshwater oligochaete, *Branchiura sowerbyi* Beddard, 1892 (Oligochaeta: Naididae: Rhyacodrilinae) has been used as an indicator organism to monitor aquatic pollution, the data on the toxic effects of the grey list metal, zinc on this worm is remained scanty. The present study was undertaken to determine the sensitivity of the worm, *B. sowerbyi* to zinc on the basis of 24, 48, 72 and 96h lethal concentrations ($LC_{1, 5, 10, 15, 50, 85, 90, 95, 99}$) and their behavioural responses. The 96h LC_{50} value of zinc with 95% confidence limit to the worm was recorded as 45.48 (41.32-49.33) mg/l. The rate of mortality of the worm was significantly increased ($p<0.05$) with increasing concentrations and time of exposure (24, 48, 72 and 96h). The study further focussed on estimation of the toxicity factor at different time scale and possible safe level of zinc to the worm to strengthen the base line data that could be used to set up local water quality criteria (WQC) for the toxicant. The worms showed behavioural changes like clumping tendency, movement and mucous secretion with increasing concentration of the toxicant and the progress of time. The findings can be used in determination of ecological risk assessment for the worm to zinc toxicity as well as to understand its mode of action in the light of their ethological changes.

Key words: Zinc, *Branchiura sowerbyi*, acute toxicity, toxicity factor, safe level, behavioural responses

INTRODUCTION

The bottom dwelling tropical oligochaete, *Branchiura sowerbyi* Beddard, 1892 (Oligochaeta: Naididae: Rhyacodrilinae) is broadly distributed in the

sediments of freshwater bodies like lakes, rivers and occasionally in sewer lines (Tyler, 2009). They feed on sediment which involves in the intake of large amounts of substrate (Wang and Matisoff, 1997). Again they are in turn fed on by the higher tropic level organisms and thus they form important links in detritus food chain. Generally, the heavy metals bind to sediments initially and then they gradually become available to bottom dwelling organisms (Claesson, 2000; Öhlander, 2003). This phenomenon accelerates the biomagnification of heavy metals from the bottom sediments to the successive level of food chain due to their strong bioaccumulative potentiality. *B. sowerbyi*is used as an indicator organism to monitor aquatic pollution but the data on the toxic effects of zinc on this worm are still scanty (Kaviraj and Konar, 1982; Casellato *et al*., 2013; Del Piero *et al*., 2014; Dhara *et al*., 2014, 2018, 2019;Ducrot *et al*., 2007; Lobo and Espindola, 2014; Lobo *et al*., 2016). Zinc (Zn) is the second most abundant trace element after Fe (Authman *et al*., 2015), but it is one of the most toxic heavy metals included in the grey list of the international convention (Taylor *et al*., 1985). Owing to its non-biodegradability and tendency to accumulate in the animal tissues as a heavy metal, zinc is regarded as the serious environmental threat (Soegianto *et al*., 2008). It is ubiquitous aquatic pollutant, which has also been detected in higher concentration in the aquatic environment (Lobo *et al*., 2016). Like many other heavy metals, the source of zinc in natural waterbodies is from geological rock weathering or from anthropogenic activities such as industrial and domestic wastes water discharge (Wheatherley *et al*., 1980). It is a major effluent from the industries such as soft drink flavouring, fur dressing and dyeing, fish processing, laundry (DWAF, 1996). Zinc in the form of sulphate is used in rayon manufacture, agriculture, zinc plating, and as a chemical intermediate and mordant (Lloyd, 1984; ATSDR, 1989). While in higher concentration, it can be a potential toxicant to the aquatic organisms by interfering with the internal dynamics of the aquatic ecosystem into irreversible and inflexible condition leading to severe damage and even death of aqutic fauna (Lucky and Venugopal, 1977; Zhang and wang, 2005). In polluted environments, aquatic organisms are continuously exposed to ambient zinc and enter through body surface, gills and nutrients (Srivastava and Tyagi, 2006). Excess zinc interacts with free thiol groups on macromolecules, so blocks the active sites of enzymes, co-enzymes and membrane receptors like other heavy metals (Chandra, 1984) and thuscauses physiological toxic effects. Very high levels of zinc can disturb the protein metabolism and lipid profile (Hopper *et al*., 1980; ATSDR, 1994), impair immune and inflammatory responses (Chandra, 1984). Zinc is also known for its inhibition of glycolysis, tricarboxylic acid cycle, and electron transport chain and glutamate release. It also results in a lower ATP production (Strydom *et al*., 2006). Dineley *et al*. (2000) reported that increased intracellular free zinc (Zn^{2+}) is toxic to neurons. It can induce cell death either through apoptosis or by necrosis, depending upon the intensity of the Zn^{2+}exposure (Lobner *et al*., 2000). Zinc induced neuronal death was also reported by some workers (Shelin *et al*., 2000; Pong *et al*., 2002).

In the light of the above, the present investigation was undertaken to determine the sensitivity of *B.sowerbyion* the basis of 24, 48, 72 and 96h lethal toxicity of zinc.Their behavioural changes due to toxic stress were also analyzed. The study further focussed on the toxicity factors at different time scale and on the estimation of the possible safe level of zinc. The findings may help to design environmental monitoring strategies and ecosystem conservation measures.

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MATERIALS AND METHODS

Healthy, mature and acclimatized specimens of *Branchiura sowerbyi* (mean length 2.02 ± 0.72 cm; mean weight 2.05 ± 0.75 mg) collected from single population were undertaken for 96h bioassay.

Analytical grade zinc sulphate, $ZnSO_4,7H_2O$ (purity 98%, molecular weight 161.47 g/mol; E. Merck (India) Ltd., Mumbai) was used as the test chemical.

Static replacement bioassays were conducted in 500 ml Borosil glass beakers each containing 300 ml water under the laboratory condition to determine the sensitivity and behavioural changes of the worms.

Water chemical analysis and the bioassays were done following the methods outlined in American Public Health Association (APHA, 2012). Tap water stored in the glass aquaria (temperature 27 ± 0.45 °C, pH 7.4 ± 0.21, free CO_2 8.0 ± 0.21 mg/l, DO 5.54 ± 0.42 mg/l, alkalinity 176 ± 7.01 mg/l as $CaCO_3$, hardness 120 ± 7.0 mg/l as $CaCO_3$) was used as a diluent medium.

During 96h acute toxicity test, *B. sowerbyi* were subjected to different concentrations of the toxicant. Mortality rate of the worms at different concentrations of the toxicant and at different times of exposure (24, 48, 72, 96h) was analyzed using the computer software R version 2.14.0 (US EPA, 1999) and probit analysis by Finney (1971) for determining lethal toxicity ($LC_{1,5,10,15,50,85,\ 90,95,99}$) with 95% confidence limits of zinc to the test organism. The relation between mortality rate with exposure time and doses was determined by analysis of variance (ANOVA) followed by DMRT (Gomez and Gomez, 1984).

On the basis of acute toxicity values, toxicity factors at different exposure period (24, 48, 72 and 96h) were assessed following the formula coined by Ayoola *et al*. (2011):
Toxicity factor (TF): (LC_{50} at 24h/ LC_{50} at any other exposure time)

The safe level estimation was calculated by multiplying the 96h LC_{50} with different application factors (AF) based on Sprague (1971), Committee on Water Quality Criteria (CWQC, 1972), National Academy of Sciences/ National Academy of Engineering (NAS/ NAE, 1973), International Joint Commission (IJC, 1977) and Canadian Council of Resources and Environmental Ministry (CCREM, 1991) and also based on the formula developed by Hart *et al*. (1948).

The behavioural changes of the test organisms exposed to various doses of each toxicant like movement, clumping tendency and mucous secretion were recorded systematically by naked eye observation during the bioassay following the method of Rand (1985).

RESULTS

The acute toxicity of zinc ($LC_{1,5,10,15,50,85,90,95,99}$values) with 95% confidence limit to *Branchiura sowerbyi* during the exposure period of 24, 48, 72 and 96h are given in Table 1, 2, 3 and 4 respectively. No mortality was observed in the control group during the experiment.

Significant relationship ($p<0.05$) between mortality rate of *B. sowerbyi*and exposure times (24, 48, 72 and 96h) was recorded at all concentrations of the toxicant except 38 and 66 mg/l concentrations of the toxicant (Table 5). But the mortality rate of the worms showed significant variation ($p<0.05$) at all concentrations irrespective of exposure times (Table 5).

The toxicity factors as calculated from the medial lethal toxicity values at different time of exposure are tabulated in Table 6.

The estimated possible safe level of zinc for the worm as calculated by multiplying their 96h LC_{50} values with different application factors are recorded in Table 7. In the present study, the safe level estimated for the toxicant is varied from 4.548-0.000455 mg/l.

The behavioural changes observed in the test organisms exposed to various lethal concentrations of zinc are summarized in Table 8. The worms without any treatment were active throughout the test period and showed clumping tendency with their normal movements. The clumping tendency was decreased with the increasing concentration and time of exposure in the treated worms. It was pronounced at 72 and 96h.

Table 1: 24h lethal concentration ($LC_{1,5,10,15,50,85,90,95,99}$) values with 95% confidence limits of zinc to *Branchiurasowerbyi*(Control group theoretical spontaneous response rate = 0.0000)

Lethal Concentration points	Concentration values with 95% confidence limits (mg/l)	Slope ± SE	Intercept ± SE
LC_1	28.659 (8.746-37.010)	7.988±2.704	-8.966±4.640
LC_5	34.879 (15.533-42.034)		
LC_{10}	38.730 (21.004-45.189)		
LC_{15}	41.567 (25.642-47.648)		
LC_{50}	**56.041 (49.251-72.122)**		
LC_{85}	75.554 (63.079-163.713)		
LC_{90}	81.087 (66.115-201.060)		
LC_{95}	90.040 (70.742-273.163)		
LC_{99}	109.583 (80.037-487.008)		

Table 2: 48h lethal concentration ($LC_{1,5,10,15,50,85,90,95,99}$) values with 95% confidence limits of zinc to *Branchiurasowerbyi*(Control group theoretical spontaneous response rate = 0.0000)

Lethal Concentration points	Concentration values with 95% confidence limits (mg/l)	Slope ± SE	Intercept ± SE
LC_1	24.843 (5.526-33.606)	7.428±2.571	-7.689±4.379
LC_5	30.686 (10.580-38.390)		
LC_{10}	34.344 (14.914-41.339)		
LC_{15}	37.056 (18.755-43.569)		
LC_{50}	**51.097 (43.383-61.944)**		
LC_{85}	70.458 (59.323-148.981)		
LC_{90}	76.022 (62.452-187.559)		
LC_{95}	85.085 (67.188-264.624)		
LC_{99}	105.097 (76.696-507.087)		

Table 3: 72h lethal concentration ($LC_{1,5,10,15,50,85,90,95,99}$) values with 95% confidence limits of zinc to *Branchiurasowerbyi*(Control group theoretical spontaneous response rate = 0.0000)

Lethal Concentration points	Concentration values with 95% confidence limits (mg/l)	Slope ± SE	Intercept ± SE
LC_1	22.028 (8.843-29.301)	6.801±1.883	-6.459±3.149
LC_5	27.743 (14.516-34.325)		
LC_{10}	31.375 (18.839-37.482)		
LC_{15}	34.090 (22.398-39.892)		
LC_{50}	**48.420 (41.913-57.639)**		
LC_{85}	68.772 (57.737-113.129)		
LC_{90}	74.725 (61.311-134.800)		
LC_{95}	84.506 (66.823-175.277)		
LC_{99}	106.433 (78.155-288.205)		

Table 4.96h lethal concentration ($LC_{1,5,10,15,50,85,90,95,99}$) values with 95% confidence limits of zinc to *Branchiura sowerbyi* (Control group theoretical spontaneous response rate = 0.0000)

Lethal Concentration points	Concentration values with 95% confidence limits (mg/l)	Slope ± SE	Intercept ± SE
LC_1	22.365 (14.805-27.519)	7.547 ± 1.328	–7.512 ± 2.235
LC_5	27.533 (20.244-32.261)		
LC_{10}	30.761 (23.885-35.166)		
LC_{15}	33.150 (26.677-37.313)		
LC_{50}	**45.479 (41.317-49.334)**		
LC_{85}	62.393 (56.471-74.066)		
LC_{90}	67.240 (60.043-82.553)		
LC_{95}	75.121 (65.568-97.224)		
LC_{99}	92.481 (76.988-132.736)		

Table 5 : Mean values of mortality (%) of *Branchiurasowerbyi*exposed to various lethal concentrations of zinc in water at different times of exposure (24, 48, 72 and 96h).Mean values within columns indicated by different superscript letters (a, b, c, d and e) and mean values within rows indicated by different superscript letters (m, n) are significantly different (DMRT, $p < 0.05$).

Dose (mg/l)	**Mean values of mortality (%) of *Branchiurasowerbyi***			
	24h	**48h**	**72h**	**96h**
0.000	0^{am}	0^{am}	0^{am}	0^{am}
30	0^{am}	0^{am}	10^{abmn}	20^{bn}
38	10^{abm}	20^{abm}	20^{bm}	30^{bm}
46	20^{bm}	30^{bmn}	40^{cmn}	50^{cn}
54	50^{cm}	60^{cmn}	70^{dn}	70^{dn}
66	70^{dm}	80^{cm}	80^{dm}	90^{em}

Table 6 : Toxicity factors for *Branchiurasowerbyi*exposed to zinc at different time scale (24, 48, 72 and 96h).

Exposed time (h)	**Toxicity factor value**
24	1.000
48	1.097
72	1.157
96	1.232

Table 7: Estimate of safe levels of zinc to *Branchiurasowerbyi*at 96h ofexposure time

Name of the test organism	**96h LC_{50} value (mg/l)**	**Method**	**Application factor (AF)**	**Safe level (mg/l)**
Branchiurasowerbyi	45.479	Hart *et al.* (1948)*	-	1.274
		Sprague (1971)	0.1	4.548
		CWQC (1972)	0.01	0.455
		NAS/NAE (1973)	0.1-0.00001	4.548-0.000455
		IJC (1977)	5% of 96h LC_{50}	2.274
		CCREM (1991)	0.05	2.273

(*C= 48h LC_{50} X $0.03/S^2$, where C is the presumable harmless concentration and S = 24h LC_{50}/48h LC_{50})

Table 8. Impact of zinc on the behavioural responses of *Branchiurasowerbyi*(CT: clumping tendency; M: movement; MS: mucous secretion; –: none; +: mild; ++: moderate; +++: strong) at various concentrations during different hours of exposure.

Dose (mg/l)	**24h**			**48h**			**72h**			**96h**		
	CT	**M**	**MS**	**CT**	**M**	**MS**	**CT**	**M**	**MS**	**CT**	**M**	**MS**
0.000	+++	+++	–	+++	+++	–	+++	+++	–	+++	+++	–
30	+++	+++	+	+++	+++	+	++	+++	+	++	++	++
38	+++	+++	+	+++	++	+	++	++	+	++	+	++
46	+++	+++	+	+++	++	+	++	++	+	+	+	++
54	+++	++	+	++	++	+	++	++	++	–	+	++
66	++	++	+	++	++	++	+	+	++	–	+	++
70	++	++	+	+	+	++	+	+	++	–	–	–

The worms were separated from each other and remained coiled after 72h at all the treatments. With the progress of time and increasing concentrations the worms showed comparatively slower movement than that of control. Mucous secretion was observed in all the treated worms. It was pronounced at 96h. The necrosis was observed at both the ends of the body of the treated worms before death at 96h of exposure. With the progress of time the body colour of the treated worms was gradually changed from red to white. Finally the worms died and were brittle.

DISCUSSION

The stress response of the zinc compound tested in the present study expressed as their 24, 48, 72 96h $LC_{1,5,10,15,50,85,90,95,99}$ values to*Branchiurasowerbyi* (Table 1,2,3 and 4) indicates that the metal is highly toxic to the worms probablyit combines with some enzymes which are essential for life (Khangarot, 1991). The 96h median lethal concentration for zinc (45.479 mg/l) recorded in the study were much higher than the findings of the earlier workers, which are also very pervasive. The 96h LC_{50} value for zinc to *Naiselinguis*was recorded as 0.91 mg/l byShuhaimi-Othman et. el.(2012).Wurtz and Bridges (1961) and Bailey and Liu (1985)reported variable96h LC_{50} valuesfor the aquatic oligochaete worm, *Lumbriculus variegates*(10.0 and 6.3 mg/l respectively). Khangarot (1991) found much lower 96h LC_{50} value for zinc to another freshwater oligochaete, *Tubifextubifex*(10.0 mg/l) at higher water hardness (225 mg/l as $CaCO_3$) than the present study. On the contrary, Qureshi *et al*. (1980) recorded much higher 48h LC_{50} value (130 mg/l) for zinc to *T. tubifex* at higher water hardness (224 mg/l as $CaCO_3$) than the present observation (51.097 mg/l). Brkovic-Popovic and Popovic (1977) found higher sensitivity (2.98 mg/l as 48h LC_{50} value) for the same species at lower water hardness(34 mg/l as $CaCO_3$). Toxicity of zinc on *L. variegates* depending on pH was also observed by Schubauer-Berigan *et al*. (1993).Lobo *et al*. (2016) recorded 0.97 mg/l as 96h LC_{50} value for zinc to *B. sowerbyi* at the temperature of 25° C and total hardness of 40 mg/l as $CaCO_3$.Similareffects of zinc have also been observed by Rathore and Khangarot (2002) on the sensitivity of sludge worm, *T. tubifex*at different temperatures (10.99 mg/l at 15° and 3.37 mg/l at 30° C).They concluded that the acute toxicity of zinc increases with the advancement of temperature. Again Rathore and Khangarot (2003) demonstrated that acute toxicity value of zinc was higher in hard water than soft water. Such variation in 96h LC_{50} values for a particular metal to the same or different test organisms were probably due to their age and size differences, variation in physico-chemical parameters like pH, temperature, water hardness, alkalinity etc. of the culture medium, presence of sediment, test chemicals used., design of the experiment and also due to species variation (Kaviraj and Konar, 1982; McCahonand Pascoe, 1988; Casellato *et al*., 1992; Hamelink *et al*., 1994; Phipps *et al*., 1995; Rathore and Khangarot, 2003; Meyer *et al*., 2004; Del Piero *et al*., 2014; Lobo *et al*., 2016; Sparling, 2016).

Tolerance is an important mechanism of the organism by which they react to their surrounding adverse environment (Enuneku and Ezemonye, 2012). In the present study, the degree of tolerance of *B. sowerbyi*to zinc was determined by the toxicity factor (TF) at different time of exposure (Table 6). With the progress of time, it increases gradually probably in accordance with the degree of decreased uptake, increased excretion or redistribution of the metal to less sensitive target sites (Enuneku and Ezemonye, 2012).
The estimated possible safe level for zinc recorded in the present study (Table 7) showed large variation and thus made controversy over its acceptability (Buikema *et al*., 1982; Pandey *et al*., 2005). The major weakness in calculation of application factor (AF) is its dependence on LC_{50} value (Kennega, 1979).So it is difficult to extrapolate laboratory data to the field as acceptable concentration as "safe" for the toxicant (Mount and Stephan, 1967; Abou *et al*., 2001).

The changes in behaviour of the treated worms in the present study (Table 8) were probably an early indication of their avoidance reaction from the toxicant.The avoidance reaction may be related to narcotic effects or to change in sensitivity of chemo receptors (Suterlin, 1974). The behavioural changes of the worms may also be considered as the neurotoxic effects of the heavy metals (Doving, 1992 and Tiwari *et al*., 2011).Excess mucus secretion in the organisms exposed to different metals probably prevents the entry of metal ions into the body as the –SH groups present in the mucus acts as protective ion trap (Jayakumar and Paul, 2006). The concentration of specific differences in response to metal observed in the present study may be due to the variation in the formation of mucus-metal complex which precipitates over the body wall of worms that blocks the exchange

of oxygen and carbon dioxide at different degrees (Whitley, 1967).

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Conflict of Interest

The author declares that there is no conflict of interest.

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