



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

Kishore Dhara^{1*}, Shubhajit Saha², Asish Kumar Panigrahi³, Nimai Chandra Saha⁴

¹ Office of the Deputy Director of Fisheries, Government of West Bengal, 9A, Esplanade East, Kolkata 700 069, West Bengal, India

² Assistant Professor, Department of Zoology, Sundarban Hazi Desarat College, South 24 Parganas, 743611, West Bengal, India

³ Department of Zoology, University of Kalyani, Nadia 741 235, West Bengal, India

⁴ Fisheries Ecotoxicology Research Laboratory (Vice-Chancellor's Research Group), Department of Zoology, University of Burdwan, Golapbagh, Bardhaman 713 104, West Bengal, India

*corresponding author, Email: kishordhara@gmail.com

Manuscript details:

Received: 01.02.2020
Accepted: 09.03.2020
Published: 05.04.2020

Cite this article as:

Kishore Dhara, Shubhajit Saha, Asish Kumar Panigrahi, Nimai Chandra Saha (2020) Sensitivity of the freshwater tropical oligochaete, *Branchiura sowerbyi* (Beddard, 1892) to the grey list metal, Zinc, *Int. J. of Life Sciences*, Volume 8(1): 93-101.

Copyright: © Author, This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Available online on
<http://www.ijlsci.in>
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

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 LC₅₀ 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.

Keywords: 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 trophic 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 (DWA, 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 aquatic 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 thus causes 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. sowerbyi* on 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.

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, $ZnSO_4 \cdot 7H_2O$ (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): (LC₅₀ at 24h/ LC₅₀ at any other exposure time)

The safe level estimation was calculated by multiplying the 96h LC₅₀ 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 LC₅₀ 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 *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	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 <i>Branchiurasowerbyi</i>			
	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 of exposure time

Name of the test organism	96h LC ₅₀ value (mg/l)	Method	Application factor (AF)	Safe level (mg/l)
<i>Branchiurasowerbyi</i>	45.479	Hart <i>et al.</i> (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 LC₅₀ X 0.03/S², where C is the presumable harmless concentration and S = 24h LC₅₀/48h LC₅₀)

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 *Branchiurusowerbyi* (Table 1,2,3 and 4) indicates that the metal is highly toxic to the worms probably it 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 LC₅₀ value for zinc to *Naiselinguis* was recorded as 0.91 mg/l by Shuhaimi-Othman et. el.(2012). Wurtz and Bridges (1961) and Bailey and Liu (1985) reported variable 96h LC₅₀ values for the aquatic oligochaete worm, *Lumbriculus variegates* (10.0 and 6.3 mg/l respectively). Khangarot (1991) found much lower 96h LC₅₀ value for zinc to another freshwater oligochaete, *Tubifex tubifex* (10.0 mg/l) at higher water hardness (225 mg/l as CaCO₃) than the present study. On the contrary, Qureshi et al. (1980) recorded much higher 48h LC₅₀ value (130 mg/l) for zinc to *T. tubifex* at higher water hardness (224 mg/l as CaCO₃) than the present observation (51.097 mg/l). Brkovic-Popovic and Popovic (1977) found higher sensitivity (2.98 mg/l as 48h LC₅₀ value) for the same species at lower water hardness (34 mg/l as CaCO₃). 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 LC₅₀ value for zinc to *B. sowerbyi* at the temperature of 25° C and total hardness of 40 mg/l as CaCO₃. Similar effects 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 LC₅₀ 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; McCahon and 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 LC₅₀ 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).

Acknowledgement

The authors are gratefully acknowledged the Department of Fisheries, Aquaculture, Aquatic Resources and Fishing Harbours, Govt. of West Bengal and the Principal, Barasat Government College for allowing them to undergo research activity.

Conflict of Interest

The author declares that there is no conflict of interest.

REFERENCES

- Abuo E, Naga EH, Moselhy KM, Mohamadein LI (2001) Effects of cadmium and copper on the digestive enzymes of the impet (*Patella* sp., Mollusca, Gastropoda). Egyptian Academy of Society and Environmental Development, 2: 29-36.
- APHA (2012) Standard methods for the examination of water and wastewater, 22nd ed. Washington, DC: American Public Health Association. AWWA, and WPCA.
- ATSDR (Agency for Toxic Substances and Disease Registry) (1989) Toxicological profile for zinc. Agency for Toxic Substances and Disease Registry, US Public Health Service, Atlanta, GA, ATSDR/TP-89-25: 121.
- ATSDR (Agency for Toxic Substances and Disease Registry) (1994) Toxicological profile for zinc. Agency for Toxic Substances and Disease Registry, US Public Health Service, Atlanta.
- Ayoola, SO, Kuton M.P, Idowu AA, Adekun AB (2011) Acute toxicity of Nile tilapia (*Oreochromis niloticus*) juveniles exposed to aqueous and ethanolic extracts of *Ipomoea aquatic* leaf. Nature and Science, 9(3): 91-99.
- Authman MMN, Zaki MS, Khallaf EA, Abbas HH (2015) Use of fish as bio-indicator of the effects of heavy metals pollution. Journal of Aquaculture Research & development, 6(4): 328 (doi: 10.4172/2155-9546.1000328).
- Bailey HC, Liu DHW (1980) *Lumbriculus variegates*, a benthic Oligochaete, as a bioassay organism. In: Aquatic Toxicology and Hazard Assessment, Eaton, J.C., Parrish, P.R. and Hendricks, A.C. (eds.) ASTM STP 707, Philadelphia, pp:205-215.
- Brkovic-Popovic I, Popovic M (1977) Effects of heavy metals on survival and respiration rate of tubificid worms: Part I- Effects on survival. Environ. Pollut., 13:65-72.
- Buikem JR, Naider-Lehner AI, Cairns JR (1982) Biological monitoring: Part IV, Toxicology Testing. Environmental Molecular Mutagen, 33: 239-262.
- Casellato S, Masiero L, Covre V (2013) Fluoride toxicity and its effects on gametogenesis in aquatic oligochaete *Branchiura sowerbyi* Beddard, Fluoride 46(1):7-18.
- Casellato S, Aiello R, Negrisol PA, Seno M (1992) Long-term experiment on *Branchiura sowerbyi* Beddard Oligochaeta, Tubificidae) using sediment treated with LAS (Linear Alkylbenzene Sulphonate). Hydrobiologia, 232,169-173.
- CCREM (1971) Canadian Water Quality Guidelines; Canadian Council of Resources and Environmental Ministry, Inland Waters Directorate, Environment Canada: Ottawa, ON, Canada.
- Chandra RK (1984) Excessive zinc impairs immune response. J. Am. Med. Assoc., 252: 1443-1446.
- Claesson P (2000) Undersökning av metal lsituationeni Kolbäcksanstillflödeni Fagersta. Uppsala universitet. Ekotoxikologiskaavdelningen Nr. 74.
- Committee on Water Quality Criteria (CWQC) (1972) A Report of the Committee on Water Quality. Ecological Research Series, EPA-R3-73-003, US Environmental Protection Agency Report, CWQC; Cincinnati, OH, USA.
- Del Piero S, Masiero L, Casellato S (2014) Toxicity and bioaccumulation of fluoride ion on *Branchiura sowerbyi* Beddard (Oligochaeta, Tubificidae). Zoosymposia, 9, 44-50.
- Dhara K, Mukherjee D, Saha NC (2014) Acute toxicity of cadmium to benthic oligochaete worm, *Branchiura sowerbyi* Beddard, 1892 and juvenile catfish, *Clarias batrachus* Linnaeus, 1758. Proceedings of the Zoological Society. (DOI: 10.1007/s12595-014-0106-7).
- Dhara K, Panigrahi AK, Saha NC (2018). Study on the acute toxicity of Chromium and Nickel to freshwater benthic oligochaete worm, *Branchiura sowerbyi* Beddard, 1892. In: Sustainable Management of Aquatic Resources, (Ed. B. K. Mahapatra, A. K. Roy and N. C. Pramanik), Part II, Section VI: Ecology, Climate Change, Pollution and Socio-economic Aspects, Ch. 45, Narendra Publishing House, Delhi, India, pp. 575-582.
- Dhara K, Saha NC, Panigrahi AK, Maity AK (2019) Assessment of mercury toxicity on sensitivity and behavioural response of freshwater tropical worm, *Branchiura sowerbyi* Beddard, 1892. Journal of Aquaculture in the Tropics, 34(1): 9-17.
- Dinkley KE, Scanlon JM, Kress GJ, Stout AK, Reynolds IJ (2000) Astrocytes are more resistant than neurons to the cytotoxic effects of increased Zn²⁺. Neurobiol. Dis., 7(4): 310-320.
- Doving KB (1992) Assessment of animal behaviour as method to indicate environmental toxicity, Comp Biochem Physiol, 100: 247-252.
- Ducrot V, Pèry ARR, Quèau H, Mons R, Lafont M, Garric J (2007) Rearing and estimation of lifecycle parameters of the tubificid worm *Branchiura sowerbyi*: application to ecotoxicity testing. Sci Total Environ., 384,252-263.
- DWAF (1996) South African water quality guidelines, 2nd ed., vol.6: Agriculture use: Aquaculture, Department of Water Affairs and Forestry: pp.46, 75, 183.
- Enuneku AA, Ezemonye LI (2012) Acute toxicity of cadmium and lead to adult toad *Bufo maculatus*. Asian Journal of Biological and Life Science. 1(3): 238-241.

- Fargasova A (1999) Ecotoxicology of Metals Related to Freshwater Benthos, Gen. Physiol. Biophys. 18 : 48–53.
- Finney DJ (1971) Probit analysis. London: Cambridge University Press.
- Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, John Wiley and Sons, New York, USA.
- Hamelink J, Landrum, PF Bergman, H Benson, WH (1994) Synopsis of Discussion Session on Physicochemical Factors Affecting Toxicity. In: Bioavailability: Physical, Chemical and Biological Interactions, CRS Press, pp 31-38.
- Hart WB, Weston RF, Dermann JG (1948) An apparatus for oxygenating test solution which fish are used as test animals for evaluating toxicity. Trans. Am. Fish Soc., 75: 288.
- Hopper PL, Visconti L, Garry PJ, Johnson GE (1980) Zinc lowers high density lipoprotein-cholesterol levels. J. Am. Med. Assoc., 244: 1960-1961.
- IJC (1977) New and Revised Great Lakes Water Quality Objectives. Great Lake Basin, Windsor, Ottawa, Canada, p.1.
- Jayakumar P, Paul VI (2006) Patterns of cadmium accumulation in selected tissues of the catfish *Clarias batrachus* (Linn.) exposed to sublethal concentration of cadmium chloride. Veterinarski Arhiv 76: 167–177.
- Kaviraj A, Konar S (1982) Acute toxicity of mercury, chromium and cadmium to fish, plankton and worm. Geobios 9: 97–100.
- Kenega EF (1979) Aquatic test organism and methods useful for assessment of chronic toxicity of chemicals, In: Dickson, K.L., Maki, A.W., Cairns, Jr., J. (eds.) Analyzing the Hazards Evaluation Process, American Fisheries Society, Washington DC, pp 101.
- Khargarot BS (1991) Toxicity of metals to a freshwater tubificid worm, *Tubifex tubifex* (Muller). Bulletin of Environmental Contamination and Toxicology 46: 906–912.
- Llyod TB (1984) Zinc compounds. In: Kirk-Othmer Encyclopedia of chemical Technology, 3rd ed., Ed. Mark, H.F., Othmer, D.F., Overberger, C.G. and Seaborg, G.T., John Wiley & Sons, New York: 851-863.
- Lobo H, Espindola ELG (2014) *Branchiurusowerbyi* Beddard, 1892 (Oligochaeta: Naididae) as a test species in ecotoxicology bioassays: a review. Zoosymposia, 9:59-69.
- Lobo H, Mendez-Fernandez L, Martinez-Madrid M, Daam MA, Espindola ELG (2016) Acute toxicity of zinc and arsenic to the warmwater aquatic oligochaete *Branchiurusowerbyi* as compared to its coldwater counterpart *Tubifex tubifex* (Annelida, Clitellata). J. Soils Sediments. (DOI 10.1007/s11368-016-1497.z).
- Lobner D, Canzoniero LM, Manzerra P, Gottron F, Ying H, Kaudson M, Tian M, Dugan LL, Kerchner GA, Shelin CT, Korsmer SJ, Choi DW (2000) Zinc induced neuronal death in cortical neurons. Cell Mol. Biol., 46(4): 797-806.
- Lucky TD, Venugopal B (1977) Physiological and chemical basis for metal toxicity. Metal toxicity in Mammals, 1: 1-238.
- McCahon CP, Pascoe D (1988) Use of *Gammaruspulex* (L.) in safety evaluation test: Culture and selection of a sensitive life stage. Ecotoxicol. Environ. Saf., 15: 245-252.
- Meyer JS, Boese CJ, Collyard SA (2002) Whole-body accumulation of copper predicts acute toxicity to an aquatic oligochaete (*Lumbriculus variegatus*) as pH and calcium are varied. Comp. Biochem. Physiol. C: Toxicol. Pharmacol., 133: 99-109.
- Mount DI, Stephan CE (1967) A method for stabilizing acceptable toxicant limits for fish: malathion and the butoxyethanol ester of 2,4-D. Trans American Fisheries Society, 96:185.
- NAS/NAE (1973) Water Quality Criteria, EPA-R3-033; US Government Printing Office: Washington, DC, USA.
- Öhlander J (2003) Sediment toxicity in River Kolbäckån-toxicity tests with *Chironomus riparius* and *Gammaruspulex* Master's Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK (2005) Acute toxicity bioassays of mercuric chloride and malathion on air breathing fish *Channapunctatus* (Bloch). Ecotoxicol. Environ. Saf., 61: 114-120.
- Phipps GL, Mattson VR, Ankley GT (1995) Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. Arch. Environ. Contam. Toxicol., 28:281-286.
- Pong K, Rong Y, Doctrow SR, Baudry M (2002) Attenuation of zinc induced intracellular dysfunction and neurotoxicity by a synthetic superoxide dismutase/ catalase mimetic, in cultured cortical neurons. Brain Res., 950(1-2): 218-230.
- Qureshi SA, Saksena AB, Singh VP (1980) Acute toxicity of four heavy metals to benthic fish food organisms from the River Khan, Ujjan. Intern. J. Environmental Studies, 15: 59-61.
- Rathore RS, Khargarot BS (2002) Effects of temperature on the sensitivity of sludge worm *Tubifex tubifex* (Muller) to selected heavy metals. Ecotox. Environ. Safe., 53,27-36.
- Rathore RS, Khargarot BS (2003) Effects of water hardness and metal concentration on a freshwater muller (*Tubifex tubifex*). Water Air Soil Pollut., 42,341-356.
- Schubauer-Berigan MK, Dierkes JR, Monson PD, Ankley GT (1993) pH dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca* and *Lumbriculus variegatus*, Environ. Toxicol. Chem., 12:1261-1266.
- Shelin CT, Behrens MM, Choi DW (2000) Zinc induced cortical neuronal death: contribution of energy failure attributable to loss of NAD (+) and inhibition of glycolysis. J. Neurosci., 20(9): 3139-3146.
- Soegianto A, Babang I, Hamami (2008). Bioaccumulation of Heavy metals in Aquatic animals collected from Coastal Waters of Gresik, Indonesia. Asian J. Water Environ. Pollut., 6(2), 95-100.

- Sparling DW (2016) Ecotoxicology Essentials: Environmental Contaminants and Their Biological Effects on Animals and Plants.
- Sprague JB (1971) Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. *Water Res.*, 3: 793-821.
- Srivastava N, Tyagi A (2006) Bioaccumulation of zinc in the gills and intestine of freshwater fish, *Channapunctatus*, after chronic exposure to zinc. *J. Environ. & Sociobiol.*, 3(1): 41-44.
- Strydom C, Robinson C, Pretorius E, Whitcut JM, Marx J, Bornman MS (2006) The effect of selected metals on the central metabolic pathways in biology: A review. *Water SA*, 32(4): 543-554.
- Taylor D, Maddock BG, Mance G (1985) The acute toxicity of nine grey list metals (Arsenic, Boron, Chromium, Copper, Lead, Nickel, Tin, Vanadium and Zinc) to two marine fish species: dab (*Limandalimanda*) and grey mullet (*Chelonlabrosus*). *Aquat. Toxicol.*, 7: 135-144.
- Tiwari M, Nagpure NS, Saksena DN, Kumar R, Singh SP, Kushwaha B, Lakra WS (2011) Evaluation of acute toxicity levels and ethological responses under heavy metal cadmium exposure in freshwater teleost, *Channapunctata* (Bloch). *International Journal of Aquatic Sciences*, 2: 36-47.
- US EPA (1999) Probit Program Version 1.5. Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268. <http://www.epa.gov/nerleerd/stat2.htm>. Accessed 14 Jan 15. 2012.
- Wang X, Matisoff G (1997) Solute transport in sediments by a large freshwater oligochaete, *Branchiura sowerbyi*. *Environmental Science and Technology* 31: 1926-1933.
- Weatherley AH, Lake PS, Rogers SC (1980) Zinc pollution and ecology of freshwater environment. In: Zinc in the environment (ed. Nriagu), Part 1: Ecological cycling. Wiley Interscience, New York: 337.
- Whitley LS (1967) The resistance of tubificid worms to three common pollutants *Hydrobiologia*, 32: 193-205.
- Zhang L, Wang W (2005) Effects of zinc pre-exposure on Cd and Zn bioaccumulation and metallothionein levels in two species of marine fish. *Aquatic Toxicology*, 73: 353-369.