World Journal of Zoology 7 (3): 174-177, 2012
ISSN 1817-3098

DOI: 10.5829/idosi.wjz.2012.7.3.648

Preliminary Evaluation of the Acute Toxicity of $Pb(NO_3)_2$ in Catfish, *Clarias batrachus* (Linnaeus)

[1]*Suchismita Das and* [2]*Anima Das*

[1]Department of Life Science and Bioinformatics, Assam University, Silchar (India-788011)
[2]Department of Zoology, Haflong Government College, Haflong (India-788819)

Abstract: Acute toxicities, in terms of median lethal concentrations (LC_{50}), of $Pb(NO_3)_2$ on *Clarias batrachus*, at 24, 48, 72 and 96 hours were 1.74, 1.55, 1.35 and 1.19 g L^{-1} respectively at 125 mgL^{-1} $CaCO_3$ hardness and pH 6.8. Severely altered behavioural pattern due to acute doses of lead treatment was observed.

Key words: $LC_{50,}$ Lead · Acute Toxicity · Behaviour

INTRODUCTION

Lead (Pb) is a non-essential heavy metal that is largely used in the production of storage batteries, paints, pigments and coloured inks; however, natural sources such as erosion also contribute to its high concentration in soil and water [1]. Lead has been shown to have toxic effects on a variety of freshwater organisms with sensitivity as low as 4 µg L^{-1} [2]. Acute toxicity of Pb is mainly due to a mucous-induced respiratory asphyxiation and the disruption of Ca^{2+} and Na^{+} homeostasis [3]. Lead exposure has been associated with behavioural anomalies, learning impairment, memory loss, damaged cognitive functions in human and experimental animals [4]. In a metal accumulation study, heavy metals, including Pb, were detected in tissues of aquatic invertebrates, *Angulyagra oxytropis,* as well as in wetlands like floodplain lakes, marshes and swamps of Barak Valley (valley situated by the river Barak), Assam, India [5]. In the present study, the acute effects of lead, measured as mortality and behavioural changes, has been evaluated in catfish *Clarias batrachus* (Linnaeus).

MATERIALS AND METHODS

Fishes of similar length (24.25±1.94 cm) and weight (97±19.46 g) were collected from unpolluted, freshwater pond, near Haflong Govt. College in Assam state of India. They were acclimatized under laboratory conditions seven days prior to experimentation and commercially available fish feed was given *ad libitum* twice daily. Temperature, dissolved oxygen, hardness (as $CaCO_3$) and pH under laboratory condition were 29°C, 5.5 mg L^{-1}, 125 mg L^{-1} and 6.8 respectively. Stock solution of $Pb(NO_3)_2$ (Merck, Germany) was prepared using double distilled water. Serial dilutions of stock solutions were prepared using chlorine free tap water as per dilution techniques [6]. $Pb(NO_3)_2$ was slightly insoluble in distilled water but readily mixed on mechanical agitation. Static-with-renewal acute toxicity tests were conducted with ten fish in each graded concentration. Fish were placed in five glass aquaria containing dechlorinated tap water. Thereafter, lead was added as per the following concentrations: 0.5, 0.75, 1, 1.25, 1.5, 1.75 2 and 2.25 g L^{1}. The fish kept in chlorine free tap water served as a control. Feeding was withheld 24 hours prior to acute toxicity tests. The test solution was replaced and mortality monitored at 24, 48, 72 and 96 hours. Dead fish were removed as the test proceeded. The number of dead fish per group was recorded against the time of their death in a tabular form [7]. The entire experiment was repeated thrice. The 24, 48, 72 and 96 h LC_{50} values of lead were calculated by Probit method [8] using SYSTAT 13 for Windows. The behavioural pattern of fish was monitored regularly under above treatment conditions.

RESULTS

The fish in the control aquarium were observed to be healthy and normal and no mortality was recorded in it. In $Pb(NO_3)_2$ treated set no mortality was observed at 0.5 gL^{-1} concentrations after 96 h exposure.

Corresponding Author: Suchismita Das, Department of Life Science and Bioinformatics, Assam University, Silchar (India- 788011). Tel: +91-9435173898.

Table 1: Acute toxicity of $Pb(NO_3)_2$ to *Clarias batrachus*

	No. of alive fish					
Conc. (g L^{-1})	24 hrs	48 hrs	72 hrs	96 hrs	% survival at 96 hrs	% mortality at 96 hrs
0	10	10	10	10	100	-
0.5	10	10	10	10	100	-
0.75	10	10	10	9	90	10
1.0	10	9	8	8	80	20
1.25	9	8	7	5	50	50
1.5	8	6	3	2	20	80
1.75	6	4	3	1	10	90
2.0	3	1	-	-	0	100
2.25	-	-	-	-	0	100

Table 2: LC_{50} values of $Pb(NO_3)_2$ on *Clarias batrachus*

Hours	LC_{50} (g L^{-1})	Standard Error	95 % Confidence interval (Lower, Upper)
24	1.74	0.07	1.59-1.91
48	1.55	0.074	1.39-1.71
72	1.35	0.07	1.2-1.49
96	1.19	0.068	1.04-1.33

Table 3: Impact of $Pb(NO_3)_2$ on the behavioural pattern of *Clarias batrachus* up to 96 h of exposure

		$Pb(NO_3)_2$			
Parameters	Control	24-h	48-h	72-h	96-h
Hyperactivity	-	++++	+++	++	+
Loss of balance	-	+	++	+++	++++
Rate of swimming	-	++++	+++	++	+
Rate of opercular activity	-	++++	+++	++	+
Mucous filled gill	-	+	++	+++	++++

The increase or decrease in the level of behavioural parameters is shown by numbers of (+) sign.

The (-) sign indicate normal behavioural conditions

However, at 0.75, 1, 1.25, 1.5, 1.75 and 2 gL^{-1} concentrations, the percent mortality was found to be 10%, 20%, 50%, 80%, 90% and 100% respectively (Table 1).

The median lethal concentrations (LC_{50}) of $Pb(NO_3)_2$ for *Clarias batrachus* at 24, 48, 72 and 96 hours were 1.74, 1.55, 1.35 and 1.19 g L^{-1} respectively (Table 2).

During acute toxicity studies, fish showed severe nervous responses. Exposed fish showed erratic swimming pattern, sudden spurts of speedy swimming followed by lethargy and finally, reduced swimming performance. Fish shed fin and secreted mucous. Scar and ulceration of the gill lamellae was observed. Ultimately the fish died of suffocation. The opercular movement rate was high up to 24 hours, but slowed down thereafter (Table 3).

DISCUSSION

Amongst fish species, considerable differences in sensitivity to Pb have been reported by many workers. Pb toxicity is a function of water hardness, species tested, pH and fish age [9]. Increased water hardness reduces Pb toxicity to fish due to a significant inorganic complexation process that controls lead availability to fish [10]. The present investigation shows relatively low Pb toxicity at hard water (125 mg $CaCO_3$ L^{-1}). In tune with the present investigation, 96 h-LC_{50} on *Pimephales promelas* and *Lepomis macrochirus* in hard water (360 mg $CaCO_3$ $^{-1}$) was found to be 482 and 442 mg Pb $^{-1}$, respectively whereas with soft water (20 mg $CaCO_3$ L^{-1}), the 96 h-LC_{50} was much lower at 5.6 and 23.8 mg Pb L^{-1}, respectively for the same fish species [11]. In a study on juveniles

Prochilodus lineatus, 24 h LC_{50} was found to be 126 mg Pb L^{-1} and 96 h LC_{50} was 95 mg Pb L^{-1} in water of 82 mg L^{-1} $CaCO_3$ hardness [12]. But in the air breathing fish *Clarias batrachus,* LC_{50} values were found to be much higher than other fishes. This was due to the presence of accessory respiratory organs in this fish that quickly adjust them towards aerial respiration when water becomes contaminated [13]. The 96-hr LC_{50} value for fingerlings of African catfish (*Clarias gariepinus*) was 0.6 mg L^{-1} but the fingerlings were only 6g in weight and 5cm in length [14]. In contrast, the size of the fish in the present investigation was relatively large (length: 24.25 ± 1.94 cm and weight: 97±19.46 g), which might be the reason for higher toxicity tolerance. Fish also show varied range of Pb toxicity based on pH of ambient water. At pH 6.8, the present investigation found 96-h LC_{50} to be 1.19 g L^{-1} which is much higher than that for *Pimephales promelas* at pH 6.4 (169 mg L^{-1}). For the same fish, at pH 5.5, 7.5 and 8.3, 96-h LC_{50} values varied at 188, 293 and 790 mg L^{-1} respectively [15].

Behavioural study is another rewarding tool in toxicity analysis. In this study, fish showed initial jerky movements which might be due to unexpected stressful chemical environment of heavy metal Pb. Such behavioural anomaly is supported with the increased production of lipid peroxides in the brain by Pb toxicity in minor carp [16]. Later, fish showed impaired swimming performance which could also arise from Pb induced haematological effects that cause a reduction in oxygen carrying capacity [17]. Pb injury to nervous system of fish have been reported including the disruption of various neurotransmitter systems in rainbow trout, *Oncorhynchus mykiss* [18], increased brain endocannabinoid levels in fathead minnows, *Pimephales promelas* [19] and injury to the hippocampus and optic tetum, regions of the brain controlling memory and visuomotor function in other teleosts [20]. As seen in this study, initial high opercular rate during first 24 hours was due to sudden impending toxic environment but a decline thereafter helped in reducing entry of more toxicants. Further, scar and ulceration of gill as well as mucous filled gill surface are the key symptoms of Pb toxicity, mainly manifested in respiratory asphyxiation later on [3]. Besides Pb specific behavioural changes, certain generalised symptoms such mucous secretion, loss of equilibrium and erratic swimming are reported in the present study and well supported in *Cirrhinus mrigala* due to $Zn(CN)_2$ [21] and in *Anabas testudineus* due to As and Hg [22].These abnormal behaviour can be due to neurotoxic effects of toxicants while; mucous in gill is also a protective measure that traps toxicants and prevents entry inside the epithelium.

Thus, although Pb is a toxic metal, the catfish in the present study conditions was quite tolerant in terms of acute toxicity. But further laboratory as well as field studies is required to ascertain long term, chronic toxicities of this metal on fish using some suitable biomarkers.

REFERENCES

1. World Health Organization (WHO), 1995. Environmental Health Criteria 165. International Programme on Chemical Safety, Geneva.
2. Grosell, M., R.M. Gerdes and K.V. Brix, 2006. Chronic toxicity of lead to three freshwater invertebrates-*Brachionus calyciflorus*, *Chironomus tentans* and *Lymnaea stagnalis*. Environmental Toxicology and Chemistry, 25(1): 97-104.
3. Birceanu, O., M.J. Chowdhury, P.L. Gillis, J.C. McGeer, C.M. Wood and M.P. Wilkie, 2008. Modes of metal toxicity and impaired branchial ionoregulation in rainbow trout exposed to mixtures of Pb and Cd in soft water. Aquatic Toxicology, 89(4): 222-231.
4. Lockitch, G., 1993. Perspective on lead toxicity. Clin. Biochem., 26(5): 371-381.
5. Gupta, A., 1998. Metal accumulation by riverine and lacustrine populations of *Angulyagra oxytropis* (Benson) (Gastropoda: Viviparidae) from Barak Valley, Assam, India. Environ. Monit. Assess., 50(3): 249-254.
6. APHA, 2005. Standard methods for the examination of water and wastewater. 21st Edn. American Public Health Association, AWWA, WPCP, Washington, DC.
7. Sprague, J.B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Research, 3(11): 793-821.
8. Finney, D.J., 1971. Probit Analysis. Cambridge University Press, London.
9. Demayo, A., M.C. Taylor, K.W. Taylor and P.V. Hodson, 1981. Toxic effects of lead and lead compounds on human health, aquatic life, wildlife plants and livestock. Critical Reviews in Environmental Control, 12(4): 257-305.

10. Hodson, P.V., D.M. Whittle, P.T.S. Wong, U. Borgmann, R.L. Thomas, Y.K. Chau, J.O. Nriagu and D.J. Hallet, 1984. Lead contamination of the Great Lakes and its potential effects on aquatic biota. In Toxic contaminants in the Great Lakes, Eds., J.O. Nriagu and M.S. Simmons. John Wiley and Sons, Indianapolis, In.
11. Pickering, Q.H. and P. Henderson, 1966. The acute toxicity of some heavy metals to different species of warm water fishes. International Journal of Air and Water Pollution, 10: 453-463.
12. Martinez, C.B.R., M.Y. Nagae, C.T.B.V. Zaia and D.A.M. Zaia, 2004. Acute morphological and physiological effects of lead in the neo tropical Fish *Prochilodus lineatus*. Brazilian Journal of Biology, 64(4): 797-807.
13. Singh, R.N., R.K. Pandey, N.N. Singh and V.K. Das, 2009. Acute toxicity and behavioural responses of Common Carp *Cyprinus carpio* (Linn.) to an organophosphate (Dimethoate). World Journal of Zoology, 4(2): 70-75.
14. Olaifa, F.E., A.K. Olaifa and O.O. Lewis, 2003. Toxic stress of lead on *Clarias gariepinus* (african catfish) fingerlings. African Journal of Biomedical Research, 6(2): 101-104.
15. Mager, E.M., A.J. Esbaugh, K.V. Brix, A.C. Ryan and M. Grosell, 2011. Influences of water chemistry on the acute toxicity of lead to *Pimephales promelas* and *Ceriodaphnia dubia*. Comparative Biochemistry and Physiology, Part C, 153(1): 82-90.
16. Shafiq-ur-Rehman, 2003. Lead-exposed increase in movement behaviour and brain lipid peroxidation in fish. Journal of environmental science and health, Part A, 38(4): 631-643.
17. Mager, E.M. and M. Grosell, 2011. Effects of acute and chronic waterborne lead exposure on the swimming performance and aerobic scope of fathead minnows (*Pimephales promelas*). Comparative Biochemistry and Physiology, Part C, 154(1): 7-13.
18. Sloman, K.A., O. Lepage, J.T. Rogers, C.M. Wood and S. Winberg, 2005. Socially-mediated differences in brain monoamines in rainbow trout: effects of trace metal contaminants. Aquatic Toxicology, 71(3): 237-247.
19. Rademacher, D.J., D.N. Weber and C.J. Hillard, 2005. Waterborne lead exposure affects brain endocannabinoid content in male but not female fathead minnows (*Pimephales promelas*). Neurotoxicology, 26(1): 9-15.
20. Giusi, G., R. Alo, M. Crudo, R.M. Facciolo and M. Canonaco, 2008. Specific cerebral heat shock proteins and histamine receptor cross-talking mechanisms promote distinct lead-dependent neurotoxic responses in teleosts. Toxicology and Applied Pharmacology, 227(2): 248-256.
21. Shwetha, A. and B.B. Hosetti, 2009. Acute effects of zinc cyanide on the behaviour and oxygen consumption of the Indian major carp, *Cirrhinus mrigala* (Hamilton). World Journal of Zoology, 4(3): 238-246.
22. Akter, M.S., Md. K. Ahmed, Md. A.A. Akhand and Md. M. Islam, 2008. Acute toxicity of As and Hg to freshwater climbing perch, *Anabas testudineus* (Bloch). World journal of Zoology, 3(1): 13-18.