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Preliminary Acute Toxicity Bioassays of Lead and Cadmium on Fresh Water Climbing Perch, *Anabas testudineus* (Bloch)

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ABSTRACT

The aim of this study was to examine the toxicological effects of two major heavy metal pollutants, lead and cadmium, on fresh water climbing perch, *Anabas testudineus* (Bloch). Acute toxicity tests were conducted in the laboratory for 96 h to determine the median lethal concentrations (LC_{50}) of lead chloride ($PbCl_2$) and cadmium chloride ($CdCl_2$) on *A. testudineus* and to study their behavior. Two preliminary trials were conducted to figure out the suitable ranges to be used in the final trials of a lethality test. In the final trials, the mortality of fish was recorded at 6, 12, 24, 48, 72 and 96 h of exposure. The LC_{50} values and their 95% confidence limits for different exposure time were calculated by using computer software 'Probit Analysis'. After 96 h of exposure the LC_{50} value of $PbCl_2$ and $CdCl_2$ were 1.015 ppm (95% confidence limit, 0.586-1.780) and 191.491 ppm (95% confidence limit, 124.005-316.841), respectively. These results indicate that $PbCl_2$ is more toxic than $CdCl_2$ to the fish species under study. Dose-dependent mortality was also observed in response to both test chemicals.

Keywords: freshwater fish, heavy metal, lethal concentrations, pollution monitoring, toxicity test

INTRODUCTION

Increased industrialization, urbanization, population growth and overall man's greed to overexploit mother nature has created a serious threat to all kinds of life in the form of pollution which has now become a global problem. Among all types of pollution, aquatic pollution is of greater concern as every kind of life depends on water. Among all types of aquatic pollutants, heavy metals are gaining importance for their non-biodegradability, bioaccumulation and biomagnification. Lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) are the top most toxic pollutants of environmental concern (ATSDR 2001) entering into aquatic ecosystems through diverse sources including both natural and anthropogenic activities (Leland *et al.* 1978; Mance 1987). The major sources of contamination include paper PVC plastic, pigments and ceramic industries, battery, mining and smoldering units and many other modern industries (Gupta *et al.* 2003). These enter into aquatic bodies through sewage sludge and with the run off from agricultural lands as they are one of the major components of phosphate fertilizers, where they produce deleterious effects on aquatic flora and fauna by affecting various physiological, biochemical and cellular processes (Gill *et al.* 1988; Venugopal *et al.* 1992; Faverney *et al.* 2001; Drastichova *et al.* 2004; Horacio *et al.* 2006; Patro 2006).

Bangladesh, as a developing country, is at a high risk of aquatic pollution, especially inland water bodies, which face the highest pollution problems. The river systems of Bangladesh may be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents. Pb and Cd were reported to vary from 41.24-63.15 and 7.12-10.11 μg/L, respectively in the water of the Shitalakhya River at different sampling stations during pre-monsoon, monsoon and post-monsoon (Ahmed *et al.* 2010). In the Buriganga River these two heavy metals varied seasonally and spatially from 58.17-72.45 and 7.08-12.33 μg/L, respectively (Ahmed *et al.* 2009). Fishes and other aquatic organisms are exposed to such metals in both dissolved and dietary phases (Khalaf *et al.* 1985). Concentrations of heavy metals in some fresh water and marine water fishes as well as in mollusks and oysters have been reported in Bangladesh waters (Sharif *et al.* 1991, 1993a, 1993b, 1993c, 1993d; Haque *et al.* 2006, 2007; Ahmed *et al.* 2009). However, no toxicity data of Pb and Cd for fishes are available in Bangladesh. It is, therefore, thought that investigation of the toxic effects of Pb and Cd to local fishes would be of importance.

The harmful effects on aquatic organisms due to environmental contamination can be detected by performing toxicity tests and most of the toxicological studies are mainly concerned with acute lethality tests (Sprague 1969; Alabaster and Lloyd 1982). Acute toxicity tests allow us to establish a dose-response relationship which is required for the handling and monitoring a toxicant in the environment. The present study was conducted to determine the acute toxicity of lead chloride ($PbCl_2$) and cadmium chloride ($CdCl_2$) to the freshwater climbing perch *Anabas testudineus* (Bloch). We exposed *A. testudineus* to different concentrations of $PbCl_2$ and $CdCl_2$ to observe their behavioral change and mortality. The median lethal concentrations of Pb and Cd were determined on the basis of observed mortality. This species was selected for bioassays because it can be easily raised under laboratory conditions. It fulfills most of the requirements of a model species and is available throughout the year.

MATERIALS AND METHODS

The present study was conducted in the aquatic laboratory of the Department of Fisheries, University of Dhaka. Healthy specimens of freshwater fish *A. testudineus* (Bloch), collected from a fish farm, of almost the same size (length 11.70 ± 0.29 cm, weight 19.5 ± 0.35 g) were used for the experiment. Fish were transported to the laboratory in large buckets with proper covering and frequent agitation. On arrival at the laboratory, the fish were given prophylactic treatment by bathing twice in 0.05% potassium permanganate solution for 2 min to avoid any dermal infections and then

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immediately released into a circular tank containing pond water and maintained there for about 6-7 days in a static condition. Fish were fed on commercial feed produced by Sabinco Ltd. twice daily with water change at 24-h intervals. An air compressor with air stones was used for oxygenation of water. The water quality parameters of the acclimation tank were studied at times. However, after acclimation, only healthy fish were used for the experiment and the length and weight of the fishes were noted.

$CdCl_2$ (MERCK, India) and $PbCl_2$ (RDH, Germany) stock solutions were prepared by mixing with experimental water and from those different test doses were prepared. Fishes were exposed to heavy metals in glass aquaria containing 50 L of water. The concentration of test solution was calculated on the basis of active ingredients. For preliminary trial fishes were exposed to 10 different concentrations of $PbCl_2$ (0.035, 0.07, 0.15, 0.3, 0.6, 1.2, 2.4, 4.8, 9.6 and 19.2 ppm) and $CdCl_2$ (6, 12, 24, 48, 96, 144, 216, 324, 486 and 729 ppm). On the basis of observed mortality in a preliminary trial, a series of closely spaced concentrations were selected to be used in the final trial according to Sprague (1969) and APHA (1980). The selected doses for final trial were 0.3, 0.6, 1.2, 2.4, 4.8 and 9.6 ppm for Pb and 48, 96, 144, 216, 324, 486 and 729 ppm for Cd.

The acute toxicity bioassay to determine the LC_{50}-96 h value of $PbCl_2$ and $CdCl_2$ was conducted in the semi-static system with a change of test water on every alternate day. In all cases, control groups of fish were maintained. The mortality of the fish was recorded at logarithmic time intervals. The physicochemical characteristics of the test water such as temperature, dissolved oxygen, pH, alkalinity, hardness, carbon dioxide (CO_2) and ammonia concentration were measured using a portable water kit (Mettler-Toledo Ltd., Ohio, USA). The data obtained were analyzed statistically to observe whether there is any influence of different treatments (concentrations) on the mortality of fish. Statistical software SPSS version 12 was used at $P < 0.05$ to analyse the data using one-way ANOVA. The median lethal concentration (LC_{50}) values and there 95% confidence limits for different exposure time were calculated by using the computer software "Probit Analysis", EPA version 1.5, USA.

Table 1 Physico-chemical properties of experimental medium.

Physico-chemical properties	Mean ± SD
Temperature (°C)	26.20 ± 0.2
Dissolved oxygen (mg/L)	5.61 ± 0.17
pH	7.03 ± 0.04
Carbon dioxide (mg/L)	10.81 ± 0.37
Total hardness (mg/L)	132.25 ± 2.10
Ammonia (mg/L)	0.15 ± 0.002

Table 2 Cumulative mortality (%) of *Anabas testudineus* at different concentrations of $PbCl_2$ within 96-h exposure time (preliminary trial).

Concentration of	Exposure time (h)					
$PbCl_2$ (ppm)	6	12	24	48	72	96
0 (control)	0	0	0	0	0	0
0.035	0	0	0	0	0	0
0.07	0	0	0	0	0	0
0.15	0	0	0	0	0	10
0.3	0	0	0	0	10	20
0.6	0	10	20	20	30	40
1.2	0	10	20	30	40	40
2.4	10	20	30	30	50	50
4.8	10	30	40	60	70	80
9.6	50	80	100	100	100	100
19.2	90	100	100	100	100	100

Table 3 Cumulative mortality (%) of *Anabas testudineus* at different concentrations of $PbCl_2$ within 96-h exposure time (preliminary trial).

Concentration of	Exposure time (h)					
$CdCl_2$ (ppm)	6	12	24	48	72	96
0 (control)	0	0	0	0	0	0
6	0	0	0	0	0	0
12	0	0	0	0	0	0
24	0	0	0	0	10	10
48	0	0	0	0	10	20
96	0	0	0	10	10	20
144	0	0	10	10	20	30
216	0	10	20	20	40	50
324	10	20	20	30	50	50
486	30	40	40	50	70	80
729	90	100	100	100	100	100

RESULTS AND DISCUSSION

The physico-chemical properties (temperature, dissolved oxygen, pH, CO_2, total hardness, ammonia concentration) of the pond water did not fluctuate greatly not only among the different treatment aquarium but also between different experimental trials and remained within the normal ranges during the acclimation period, preliminary trial and final trial with fishes exposed to $PbCl_2$ and $CdCl_2$ (**Table 1**). This observation was also supported by an earlier report (Alabaster and Lloyd 1982). Fluctuation in temperature should not exceed 4°C and similarly oxygen (O_2) content must not fall below 4 mg/L for the warm water fish (APHA 1980). In the present experiment, fluctuations in temperature was found between the range 26°C-29°C and dissolved O_2 was within the normal range that is 4.67 to 6.81 mg/L. However, aeration of water ensured adequate supply of O_2. During the experimental trials the fish were not fed which helped to avoid large fluctuations in their metabolic wastes and fouling of test solutions. Water exchange daily ensures a removal of toxic NH_3 formed due to metabolic wastes.

Exposed fish to different concentrations of metals showed behavioral anomalies. At the start of exposure, fish were alert, and stopped swimming and remained static in position. Faster opercular activity was observed as surfacing and gulping of air. However, the degree and extent of such behavioral observations were clearly dependent upon each metal's concentrations in the exposure media. The first visible reactions of the treated fish at the higher concentrations were observed within a few minutes of exposure, especially at higher concentrations (19.2 ppm of $PbCl_2$ and 729 ppm of $CdCl_2$). However, the fish exposed to lower concentrations i.e., below 0.15 ppm of $PbCl_2$ and 12 ppm of $CdCl_2$ showed no or little behavioral changes. Available evidence indicates that a minute amount of some toxicants has the ability to cause abnormal behavior performances in fish through impaired perceptive acuity (Kabir and Begum 1978; Akter *et al.* 2009). In the aquarium with higher concentrations of test chemicals, the fish swam erratically with jerky movements and hyperexcitability. Their fins became hard and stretched due to stretching of body muscles. They secreted copious amounts of mucus from the whole body continuously, and soon a thick layer of mucus was deposited in the buccal cavity and gills. Ultimately, fish lost their balance, became exhausted, lost consciousness, and became lethargic within 6 hrs of exposure period. Finally, they remained in a vertical position for a few minutes with the anterior side or terminal mouth up near the surface of the water, trying to gulp air, and the tail turned downwards. Soon they settled on the bottom of the aquarium; and their bellies turned upward and the fish died within 12 hrs of exposure period. Abnormal behaviors were also observed in fish treated with various heavy metals (Armstrong 1979; Sinha and Kumar 1992; Ramamoorthy and Baddaloo 1995; Santha *et al.* 2000). The abnormal behaviors were probably caused by the neurotoxic effects and also by the irritation to the perceptive system of the body. Jumping out and to-and-fro movement signified the avoidance reaction of the fish to the toxicants. Secretion of excessive mucus was probably due to irritation of the skin because of direct contact with the heavy metals. Lateral swimming and loss of equilibrium were probably due to impairment of the nervous system (Sinha and Kumar 1992). The control fish i.e., untreated with heavy metals remained alive and active throughout the experimental period.

In **Table 2** the data on the cumulative mortality (%) of the preliminary trials are shown for $PbCl_2$ treatment. No

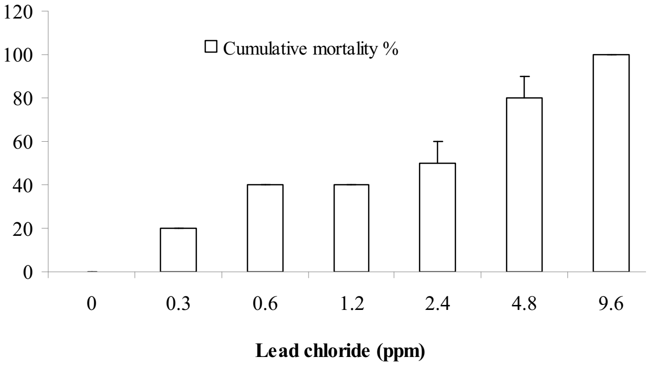

Fig. 1 Cumulative mortality (%) of *Anabas testudineus* at different $PbCl_2$ concentrations after 96-h exposure (final trial). Each column denotes the average of cumulative mortality of three replicas.

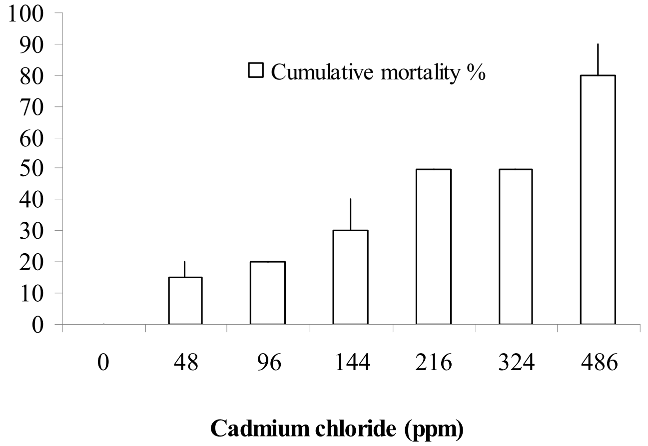

Fig. 2 Cumulative mortality (%) of *Anabas testudineus* at different $CdCl_2$ concentrations after 96-h exposure (final trial). Each column denotes the average of cumulative mortality of three replicas.

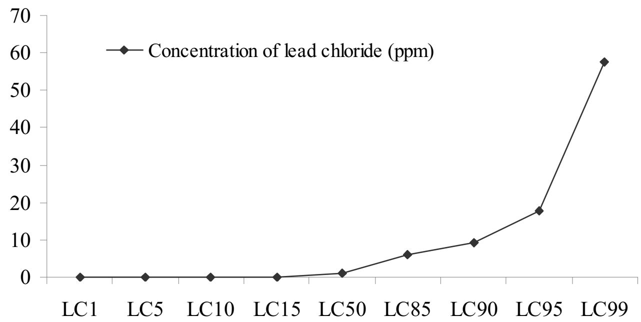

Fig. 3 The estimated LC_{50} value of $PbCl_2$ after 96-h exposure was 1.015 ppm (95% confidence limit, 0.586 to 1.780).

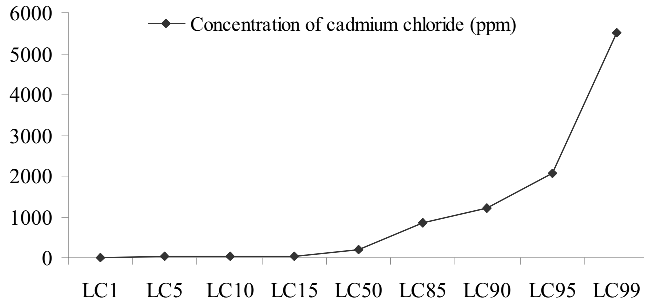

Fig. 4 The estimated LC_{50} value of $CdCl_2$ after 96-h exposure was 191.491 ppm (95% confidence limit, 24.005 to 316.841).

mortality was observed at 0.035 and 0.07 ppm of $PbCl_2$ after 96-h exposure. As the concentration increased, mortality increased gradually, 50 and 80% mortalities were observed at 2.4 ppm by 72 h and at 4.8 ppm by 96 h of exposure, respectively. However, complete mortality (100%) occurred at 19.2 ppm of $PbCl_2$ within 12 h. The data on the cumulative mortality (%) of the preliminary trial of $CdCl_2$ is shown in **Table 3**. No mortality was observed at 6 and 12 ppm of $CdCl_2$ after 96-h exposure. However, 50 and 80% mortalities were observed at 324 and 486 ppm within 96-h of exposure, respectively. However, complete mortality (100%) occurred at 729 ppm within 96-h of exposure.

The data presented in **Figs. 1** and **2** show average percentages of cumulative mortality of the final trial in different concentrations of $PbCl_2$ and $CdCl_2$, respectively after three replications and 96-h exposure period. 20% mortality occurred in 0.3 ppm $PbCl_2$ whereas 40, 50, 80 and 100% mortality were recorded at 1.2, 2.4, 4.8 and 9.6 ppm, respectively (**Fig. 1**). However, for $CdCl_2$, data showed 15% mortality at 48 ppm, 20% mortality at 96 ppm, 30 and 50% mortality at 144 and 216 ppm, while 80% mortality was observed at 486 ppm (**Fig. 2**). A smaller concentration of $PbCl_2$ is required than $CdCl_2$ to cause the same percentage mortality of the exposed fish under study. The "Probit" calculation gave a plot of adjusted probit values of different lethal concentrations and predicted regression lines (**Figs. 3** and **4**). After 96-h exposure, the LC_{50} value of $PbCl_2$ was 1.015 ppm (95% confidence limit, 0.586-1.780) (**Fig. 3**) and that of $CdCl_2$ was 191.491 ppm (95% confidence limit, 124.005-316.841) (**Fig. 4**). The acute sensitivity of $PbCl_2$ to *A. testudineus* found in the present study was about two times greater than that reported by Olaifa *et al.* (2003) for fresh water cat fish *Clarius gariepinus*. This difference in the toxicity might be due to the differences in fish species. In the cat fish, *Heteropneustes fossilis*, the estimated LC_{50} values of Cd was 392.92 ppm (Kasherwani *et al.* 2009), 2-fold greater than the present findings. Sobha *et al.* (2007) calculated the average 96-h LC_{50} value of $CdCl_2$ as 4.533 ppm in *Catla catla*. This variation in Cd toxicity might be due to the difference in species and environmental condition. Various factors may influence bioassay techniques like differences in fish (e.g., species, weight, size) and other environmental factors (viz. temperature, variations in pH of the water, total hardness of water, dissolved O_2). Akter *et al.* (2008) reported that the variation in acute toxicity of heavy metals may be due to differences in species and environmental condition. Jindal *et al.* (2005) also reported that the acute toxicity of Cd, Cu, Hg, Zn and Cr showed a direct correlation with temperature, and an inverse correlation with hardness of water. Therefore, the 96 hours LC_{50} value obtained in the present study may be used as incipient LC_{50} or lethal threshold concentration. The lethal concentration values imply the toxicity strength of the pollutants hence it may be used as a measure of indication of pollution in the aquatic environment.

CONCLUSION

Chemical determination of any persistent toxicant concentration in water may not provide information on the severity of contamination, especially in the case of sub-lethal levels. Biological monitoring using a series of assays having different endpoints in a “key species” could allow a sensitive approach to predict the potential risk of persistent contaminants like heavy metals, which is helpful in formulating the “safe levels” of such bioaccumulative chemicals having genotoxic potential. Acute toxicity studies are the very first step in determining the water quality requirements of fish. This study reveals the toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. Thus, it can be concluded from the present study that *Anabas testudineus* (Bloch) are highly sensitive to $PbCl_2$ and $CdCl_2$ and their mortality rate is dose dependent.

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