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Acute Toxicity of Arsenic and Mercury to Fresh Water Climbing Perch, *Anabas testudineus* (Bloch)

[1]Mosammat S. Akter, [1]Md.K. Ahmed[1], [2]Md.A.A. Akhand and [1]Md.M. Islam

[1]Department of Fisheries, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh

Abstract: The objective of this work was to examine the toxicological effects of two major heavy metal pollutants sodium arsenite ($NaAsO_2$) and mercuric chloride ($HgCl_2$), in fresh water climbing perch, *Anabas testudineus* (Bloch). Static bioassays were conducted in the laboratory for 96 hours to determine the median lethal concentrations (LC_{50}) of $NaAsO_2$ and $HgCl_2$ to *A. testudineus*. Two preliminary trials were conducted to figure out the suitable ranges to be used in the final trials of lethality test. In final trials, mortality of fish was recorded at 6, 12, 24, 48, 72 and 96 hours 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 hours of exposure the LC_{50} value of $NaAsO_2$ and $HgCl_2$ were 18.211 ppm (95% confidence limit, 5.962 to 53.724) and 0.606 ppm (95% confidence limit, 0.228 to 1.293), respectively.

Key words: Arsenic · mercury · acute toxicity · *Anabas testudineus*

INTRODUCTION

Industrial pollution and deteriorating water quality is a growing environmental concern in Bangladesh and most of the industries are located along the bank of the main rivers, leading to high level of inorganic contaminants into the water bodies [1]. Among different toxicants of the river water, heavy metals are getting importance for their non-degradable nature and often accumulate through tropic level [2]. Thus, contamination of a river with heavy metals may have devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organisms becomes limited with the extent of contamination [3].

Mercury, lead and arsenic are the top three toxic pollutants of environmental concern [4, 5]. Arsenic and mercury may be introduced into aquatic systems through geogenic processes and anthropogenic pathways including effluent containing dyes, fungicides, mining, industrial wastes and combustion of fossil fuels, municipal and medical wastes [5-9]. In aquatic environments, several species of microorganisms make arsenic and mercury biologically available to organisms including fish [5, 10]. Fish tissues can accumulate heavy metals and serves as a sensitive indicator of aquatic pollution. 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 [11, 12]. In general, acute toxicity tests are conducted by exposing organisms to several concentrations of toxicants and allow us to establish a dose-response relationship which is required for the handling and monitoring of a toxicant in the environment.

In Bangladesh, high arsenic concentrations have been found in the range of 1 to 1500 μg/L in ground water of Bangladesh [13] whereas mercury contamination is found to be profound in industrialized area of the state. However, no toxicity data of arsenic and mercury for fishes are available in Bangladesh. It is, therefore, thought that investigation of the toxic effects of mercury and arsenic to local fishes would be of importance. In the present study we exposed *Anabas testudineus* to different concentrations of $NaAsO_2$ and $HgCl_2$ to observe their mortality. *A. testudineus* was selected as an experimental model because of its hardness, availability round the year, adaptability to laboratory. The behavioral changes in the exposed fishes were monitored and on the basis of observed mortality the median lethal concentrations of arsenic and mercury were determined.

Corresponding Author: Dr. Mosammat Salma Akter, Department of Fisheries, University of Dhaka, Dhaka-1000, Bangladesh

MATERIALS AND METHODS

Place of the study: The present experiments were conducted in the aquatic laboratory of the Department of Fisheries, University of Dhaka. The analytical and other laboratory works were conducted in same laboratory.

Collection of experimental fishes: Fishes with almost same sizes (Length 12.05± 0.51cm, Weight 19.05± 0.75g) were collected from a fish farm and used for the experiment. Fishes were transported to the laboratory in large buckets with proper covering and frequent agitation.

Acclimation of the test fishes: On arrival at the laboratory, the fishes were immediately released into three big tanks containing tap water and then maintained there for about 6-7 days in a static condition. Fish were fed on artificial feed twice daily. Any debris or unwanted particles were removed from the tank after feeding. The water medium was changed at 24 hours interval to remove the metabolic-pollutants. 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 fishes were used for experiment and the length and weight of the fishes were noted.

Preparation of heavy metal solution: Sodium arsenite ($NaAsO_2$) and mercuric chloride ($HgCl_2$) were collected from the BDH laboratory (England) in original package form. By mixing with tap water five to seven different concentrations of both metals were used as stock solution. Different test doses were prepared making dilution of the stock concentration.

Doses of heavy metals that induced fish mortality: Fishes were acclimated to the test water for 24-48 hours before adding the chemicals. During this final acclimation and test periods fish were not fed. Fishes were exposed to heavy metals in glass aquaria containing 10-20L water. Tap water stored in the tank for two months confirming the settlement of Iron, were used for the experiment. The water was aerated for one day before starting the experiment. Stone aerators connected to a compressed air supply were used to maintain an adequate level of dissolved oxygen in each aquarium. For preliminary trial fishes were exposed to 10 different concentrations of $NaAsO_2$ (0.02, 0.04, 0.08, 0.4, 2, 10, 50, 250, 500 and 1000 ppm) and $HgCl_2$ (0.0025, 0.005, 0.01, 0.25, 0.5, 1, 2, 4, 6 and 8 ppm). Fish mortality induced by those concentrations was noted. On the basis of observed mortality in the preliminary trial, a series of closely spaced concentrations were selected to be used in the final trial. The concentrations were selected as such for final trial so that complete mortality occurred in the highest concentration used and no mortality occurred in the lowest concentration [12, 14]. The selected doses for final trial were 0.4, 2, 10, 50, 250 and 500 ppm for arsenic whereas 0.10, 0.25, 0.5, 1, 2 and 4 ppm for mercury.

Determination of LC_{50}: Fishes transferred to each aquarium and exposed to different concentrations of $NaAsO_2$ and $HgCl_2$. In all cases, control groups of fish were maintained. Each experimental trial was carried out for a period of 96 hours. The mortality of the fish was recorded at logarithmic time intervals that is, after 6, 12, 24, 48, 72 and 96 hours of exposure. The test media was renewed daily during the experimental period. The physicochemical characteristics test of the water such as temperature, pH, alkalinity, hardness, oxygen concentration were conducted frequently following the standard procedures described in APHA [14]. The temperature was measured with a N-filled mercury thermometer in°C whereas dissolved oxygen, pH, total hardness, carbon dioxide, ammonia concentration were measured using a portable water kit (Mettler-Toledo Ltd. USA). The effect of each concentration was tested at least in duplicate to verify reproducibility. The data obtained in course of the investigation were analyzed statistically to see whether there is any influence of different treatments (concentrations) on the mortality of fish. The median lethal concentration (LC_{50}) values and their 95% confidence limits for different exposure time were calculated by using the computer software "Probit Analysis", EPA version 1.5, USA.

RESULTS AND DISCUSSION

The physico-chemical properties (temperature, dissolved oxygen (DO), pH, carbon dioxide, total hardness, ammonia concentration) of the tap water were monitored during the acclimation period, preliminary trial and final trial with fishes exposed to $NaAsO_2$ and $HgCl_2$ (Table 1). As evident from the data in Table 1, the water quality parameters did not fluctuate greatly not only among the different treatment aquarium but also between different experimental trials and remained within the normal ranges. Similarly, there were no noticeable

Table 1: Physico-chemical properties of experimental medium

Physico-chemical properties	Mean±SD
Temperature (°C)	27.50±0.19
Dissolved oxygen (mg/L)	5.70±0.30
pH	7.65±0.20
Carbon dioxide (mg/L)	10.50±0.23
Total Hardness (mg/L)	132.00±2.10
Ammonia (mg/L)	0.15±0.002

differences between the results of water quality parameters measured with pond water (Data not shown). This observation was also supported by an earlier report [11]. According to APHA [14], fluctuation in temperature should not exceed 4°C and similarly oxygen content must not fall below 4 mg/L for the warm water fish. In the present experiment fluctuations in temperature was found between the range 26°C-29°C and dissolved oxygen was within the normal range that is 4.87 to 6.3 mg/L. However, aeration of water ensured adequate supply of oxygen. During the experimental trials the fish were not fed which helped to avoid large fluctuations in their metabolic wastes and fouling of test solutions. The fish density in the test solution was kept low enough that prevented the build up of high metabolic pollutants. Water exchange twice daily ensures a removal of toxic NH_3 formed due to metabolic wastes.

In Table 2 the data on the cumulative mortality (%) of the preliminary trials are shown for $NaAsO_2$ treatment. No mortality was observed at 0.01and 0.05 ppm concentrations of $NaAsO_2$ after 96 hours exposure. Very little mortality occurred in the fish groups exposed up to 0.08 and 0.4 ppm of $NaAsO_2$. As the concentration increased, percent mortality was increased gradually. 60% and 80% mortalities were observed in fish at 50 ppm by 72-h and 96-h of exposure, respectively. However, a complete mortality (100%) occurred in the fish above 250 ppm of $NaAsO_2$ within 6-h or more of exposure. The data on the cumulative mortality (%) of the preliminary trial of $HgCl_2$ are shown in Table 3. No mortality was observed at 0.0025 and 0.005 ppm concentrations of $HgCl_2$ after 96 hours exposure. Less than 10% mortality occurred in the fish groups exposed up to 0.01 and 0.25 ppm of $HgCl_2$ within 96-h exposure period. 60% and 80% mortalities were observed in fish at 1.00 and 2.00 ppm within 96-h of exposure respectively. However, a complete mortality (100%) occurred in the fish at 4.00 or more ppm within 96-h of exposure.

The data presented in Fig. 1 and 2 showed the average percentages of the cumulative mortality of final trial in different concentrations of $NaAsO_2$ and $HgCl_2$ respectively after three replications and 96 hours exposure period. It was observed that 10 percent mortality was occurred in 0.4 and 2.00 ppm of $NaAsO_2$ whereas 30, 50, 80 and 100 percent mortality were observed in 10, 50, 250 and 500 ppm, respectively (Fig. 1). However, in case of $HgCl_2$, data showed 10% mortality at 0.01 ppm as well as 30% and 60% mortality at 0.5 and 1ppm, however, a complete mortality was observed at 4ppm (Fig. 2).The "Probit" calculation gave a plot of adjusted probit values of different lethal concentrations and predicted regression line (Fig. 3 and 4). After 96h exposure the LC_{50} value of $NaAsO_2$ was 18.211ppm (95% confidence limit, 5.962 to 53.724) as observed in Fig. 3 and of $HgCl_2$ was 0.606 ppm (95% confidence limit, 0.228 to 1.293) as showed in Fig. 4. The acute sensitivity of *A. testudineus* found in the present study was about two times lower than that reported by Rajan and Banerjee [15] for fresh water cat fish (96-h LC_{50} of $HgCl_2$ was 0.300 ppm) and by Khangarot [16] for fresh water snakehead (96-h LC_{50} of $HgCl_2$ was 0.314 ppm). This difference in the toxicity might be due to the differences in fish species. Moreover, fresh water perch is hardier than fresh water cat fish and snakeheads. We observed that arsenic was found to be toxic at 18.11 ppm or 18000°g/L as 96-hr LC_{50} value. In salmonids the estimated LC_{50} values of arsenic was found between 3,000 and 167,000°g/L [17] whereas estimates of LC_{50} for arctic grayling were above 8940 °g/L of arsenic [17] that was two times lower than the present findings. This variation in arsenic toxicity might be due to the difference in species and environment condition.

Various behavioral anomalies were observed in the experimental fish during $HgCl_2$ and $NaAsO_2$ exposure. However, the degree and extent of such behavioral observations were clearly dependent upon metal's concentrations in the exposure media. The first visible reactions of the treated fish at the higher concentrations were observed within few-minutes of exposure, especially at higher concentrations (4.00ppm of $HgCl_2$ and 250ppm of $NaAsO_2$). However, the fish exposed to lower concentrations i.e., below 0.01ppm of $HgCl_2$ and 0.08ppm of $NaAsO_2$ showed no or little behavioral changes depending on the concentrations in the exposure media. Available evidences indicate that a minute amount of some toxicants has the ability to cause abnormal behavior performances in fish through impaired perceptive acuity [18]. In the present study, exposure of $HgCl_2$ and $NaAsO_2$ caused various abnormal behaviors such as erratic movement, rapid movement of operculum, jumping out of

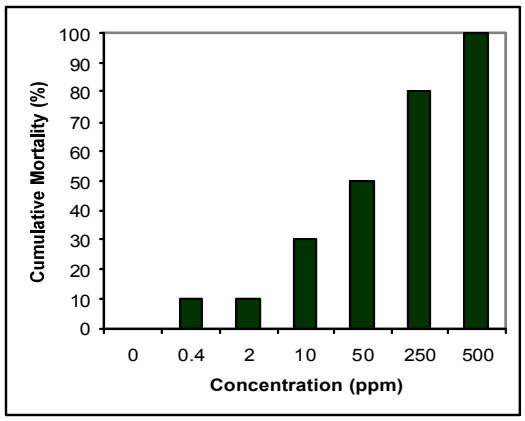

Fig. 1: Cumulative mortality (%) of *Anabas testudineus* at different concentration of $NaAsO_2$ after 96 hours exposure time (Final trial). Each column denotes the average of cumulative mortality of three replicas

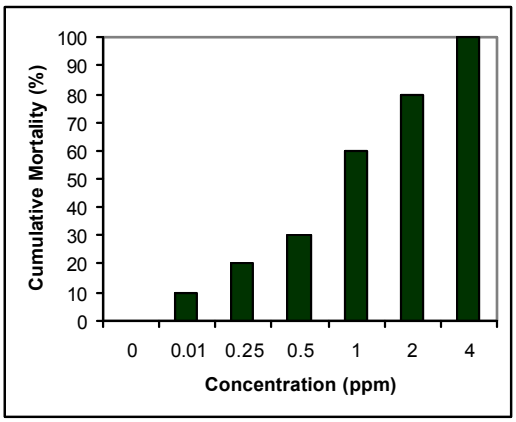

Fig. 2: Cumulative mortality (%) of *Anabas testudineus* at different concentration of $HgCl_2$ within 96 hours exposure time (Final trial). Each column denotes the average of cumulative mortality of three replicas

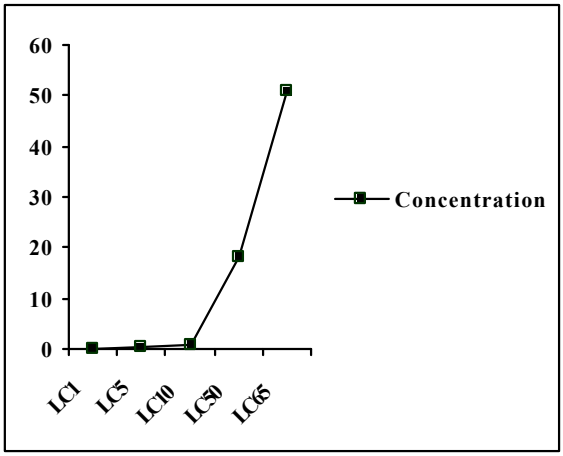

Fig. 3: The LC_{50} value of $NaAsO_2$ after 96h exposure was 18.211ppm (95% confidence limit, 5.962 to 53.724)

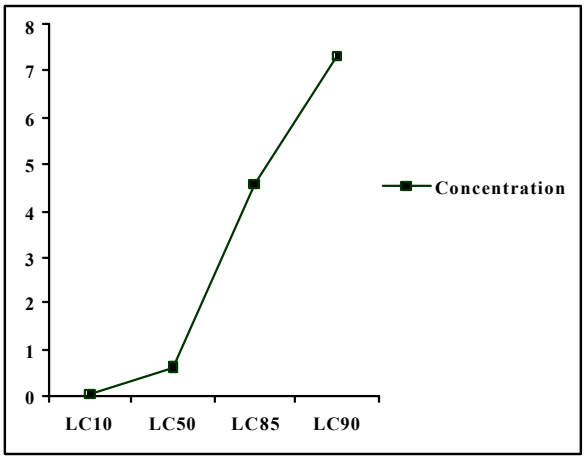

Fig. 4: The LC_{50} value of $HgCl_2$ after 96h exposure was determined by probit analysis which was 0.606 ppm (95% confidence limit, 0.228 to 1.293)

Table 2: Cumulative mortality (%) of *Anabas testudineus* at different concentration of $NaAsO_2$ within 96 hours exposure time (Preliminary trial)

		Exposure time (hours) Cumulative mortality (%)					
Treatment No	Concentration of $NaAsO_2$ (ppm)	6	12	24	48	72	96
Control	0.00	0	0	0	0	0	0
1	0.01	0	0	0	0	0	0
2	0.05	0	0	0	0	0	0
3	0.08	0	0	0	5	10	10
4	0.40	0	0	0	0	10	10
5	2.00	0	0	0	0	20	30
6	10.00	0	0	10	20	30	50
7	50.00	0	0	30	50	60	80
8	250.00	40	40	40	40	60	80
9	500.00	80	80	90	90	100	100
10	1000.00	100	100	100	100	100	100

Table 3: Cumulative mortality (%) of Anabas testudineus at different concentration of $HgCl_2$ within 96 hours exposure time (Preliminary trial)

		Exposure time (hours) Cumulative mortality (%)					
Treatment No	Concentration of $HgCl_2$ (ppm)	6	12	24	48	72	96
Control	0.00	0	0	0	0	0	0
1	0.0025	0	0	0	0	0	0
2	0.005	0	0	0	0	0	0
3	0.01	0	0	0	5	10	10
4	0.25	0	0	0	10	10	20
5	0.50	0	0	0	20	20	30
6	1.00	0	0	10	20	30	60
7	2.00	0	0	10	40	60	80
8	4.00	80	90	100	100	100	100
9	6.00	80	80	90	100	100	100
10	8.00	100	100	100	100	100	100

the test media, lateral swimming, loss of equilibrium etc. Abnormal behaviors were also observed in different fishes treated with various heavy metals [19-22]. The abnormal behaviors were probably caused by the neurotoxic effects and also by the irritation to perceptive system of the body. Jumping out and to and fro movement signified the avoidance reaction of the fishes 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 the impairment of nervous system [20]. Finally the fish were found dead scattered at the bottom of the aquarium with their mouth wide open. The control fishes i.e., untreated with heavy metals remained alive and active throughout the experimental period. A recent study characterized the toxic effect of arsenic and mercury, especially at the molecular level, which postulated that both arsenic and mercury induce fish death and reduce liver cell viability involving DNA fragmentation and induction of a particular protein expression [23].

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. LC_{50} values help to monitor the water quality and to take initiative against the particular pollutant. However, toxicity values of other heavy metals could be estimated by following this methodology. Thus toxicity of different heavy metals can be compared and we could get an idea of their relative adverse effects on fishes and other aquatic animals, which in terns serve the Ichthyologists to select the proper environment for any aquaculture practice.

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