Factors Affecting Acute Toxicity Dose of Lead Nitrate in Certain Indian Air-Breathing Fishes

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Abstract

The present work was conducted to determine an ideal median lethal concentration of lead nitrate in two air-breathing fishes namely *Channa punctatus* (Bloch) and *Clarias batrachus* (Linn.) using following methods and also influence of different factors to get at a logical conclusion. (1) Graphical method: The LC_{50} value was 12.02 and 165.0 mg/L for *Channa punctatus* while 350.0 and 380.0 mg/L for *Clarias batrachus*. (2) Probit Graphical method: The LC_{50} value was 11.48 and 177.8 mg/L for *Channa punctatus* while 315.0 and 346.7 mg/L for *Clarias batrachus*. (3) Regression method of Finney (1971): From this method LC_{50} value was calculated to be 11.75 and 208.0 mg/L for *Channa punctatus* while 320.0 and 348.0 mg/L for *Clarias batrachus*. (4) Behrens and Karber method: By this method, 96 h-LC_{50} was found 12.75 and 167.5 mg/L for *Channa punctatus* while 375.0 and 425.0 mg/L for *Clarias batrachus*.

From these values, the average median lethal concentration of 96 h-LC_{50} value was calculated to be 12.0 mg/L for *Channa punctatus* (body weight: 50-68 g) and 180.0 mg/L for *Channa punctatus* (body weight: 55-75 g). Similarly, 340.0 mg/L for *Clarias batrachus* (body weight: 85-110 g) and 375 mg/L for *Clarias batrachus* (body weight: 125-150 g). In this paper, factors such as intra-specific variation, physico-chemical features, spatial variation and methodological variation affecting the values of LC_{50} has also been studied.

Keywords: Channa punctatus; Clarias batrachus; Lead nitrate; Acute toxicity

Introduction

Aquatic life is strongly affected by physico-chemical properties of a water bodies. It has been reported that heavy metals had a negative impact on different physico-chemical parameters and caused various changes in animals. The heavy metal concentration in the body of fish depends upon feeding habits, trophic status, food availability, physico-chemical properties of water, metabolic rate of animal and toxicity of heavy metals [1]. Lead is a non-essential and non-beneficial element. It had added a problem of health hazard to human and experimental mammals.

It has also received the attention over the past few years as potentially important aquatic pollutant. Acute toxicity describes the adverse effect of a heavy metal/other chemical on an animal resulting from single exposure. Acute toxicity tests are rapid procedures used for measuring the concentration of heavy metal, expressed as the median lethal concentration (LC_{50}), which will affect the animal. It is the concentration of a substance in the solvent which kills 50% of the test batch of the fish or the other living organism within a stated continuous period of exposure.

Median lethal concentration of lead in various air-breathing fishes is reported [2-4]. However, effect of intra-specific variation, physico-chemical features, spatial variation and methodological variation on the value(s) of median lethal concentration in air-breathing fishes is lacking. Therefore, present work was conducted to study such factors on median lethal concentration of lead nitrate in two commercially important Indian air-breathing fishes namely, snake headed murrel, *Channa punctatus* (Bloch) and Indian catfish, *Clarias batrachus* (Linn.) to enhance reliability and to standardize the value.

Materials and Methods

In the present investigation, *Channa punctatus* (Body weight: 50-75 g) and *Clarias batrachus* (Body weight: 85-150 g) were collected from local fishermen of Arrah and adjacent localities during 2013-2015. The fishes were brought to the laboratory in polythene bags containing the pond water. The fishes were disinfected by washing properly with dilute $KMnO_4$ and then transferred to many large aquaria (90 × 60 × 45 cm). The fishes were fed with pieces of goat's liver and fish food available in the local market.

Fishes of each aquarium were thoroughly examined and unhealthy and injured specimens were rejected. To keep the aquaria free from fungal growth antifungal chemicals were used. The temperature of water of each aquarium was maintained at room temperate throughout the period of investigation and physico-chemical features of experimental water was determined. The experiments were conducted every time in between 11 AM to 1 PM.

The toxicity test of the lead nitrate [$Pb(NO_3)_2$] was conducted by employing the static bioassay method and recommendation for the toxicity test experiments [5,6]. Analytical Reagent grade chemicals (Merck India Co. Ltd) were used during experiments. Five different concentrations of lead nitrate were taken in separate aquarium. In each concentration, twenty fishes from the acclimatized fish stock were kept. The first set of experiment was conducted for 24 h, second for 48 h, third for 72 h and fourth for 96 h respectively. The experiments were monitored round the clock and numbers of fishes died during the experiments in each concentration of each set of experiment was recorded. The fishes were considered dead when they gave on response to touch with a glass rod. The whole sets of experiments were repeated thrice to get an average result and to minimize any error due to unavoidable reasons.

With the help of the records of dead fishes, 96 h-LC_{50} doses were determined by following methods:

(1)   Graphical method taking percent mortality

(2)   Graphical method taking log and probit mortality

(3)   Regression method [7] and

(4)   Behrens and Karber method

Statistical analysis was performed with the use of Graph Pad Prism 5.

Results

The physico-chemical features of experimental water have been given in TABLE 1. The data on determination of LC_{50} for different durations of lead nitrate on *Channa punctatus* (Bloch) and *Clarias batrachus (*Linn.*)* have been summarized in TABLES 2 to 6. The lethal range was determined to sequential concentrations from 0.1, 0.01, 10.0, 100.0 to 1000.0 mg/L of lead nitrate [8]. 20 specimens of fishes were released separately in a fish aquarium containing 20 liters of water and sequential concentrations of lead nitrate were added. The mortalities were recorded at 24, 48, 72 and 96 hours of commencement of the experiment and dead fishes were removed immediately.

TABLE 1. **Physico-chemical parameters of experimental water.**

Physico-Chemical Parameters	Values			
	Channa punctatus **(2013)**	*Channa punctatus* **(2015)**	*Clarias batrachus* **(2013)**	*Clarias batrachus* **(2014)**
Water temperature (°C)	25.0 ± 1.0	27.0 ± 1.0	25.0 ± 1.0	26.0 ± 1.0
pH	7.02 ± 0.5	7.41 ± 0.4	7.02 ± 0.5	7.08 ± 0.1
Dissolved oxygen (mg/L)	6.8 ± 0.4	6.1 ± 0.8	6.8 ± 0.4	6.5 ± 0.6
Free Carbon dioxide (mg/L)	-	-	-	-
Chloride (mg/L)	13.5 ± 2.8	12.95 ± 2.6	13.5 ± 2.8	13.8 ± 2.7
Hardness (mg/L)	107.2 ± 8.5	113.8 ± 10.4	107.2 ± 8.5	110.7 ± 5.8
Total alkalinity (mg/L)	113.5 ± 4.5	118.3 ± 8.4	113.5 ± 4.5	112.6 ± 5.2

TABLE 2. **Behren-Karber method for determination of 96 h-LC_{50} dose of lead nitrate in *Channa punctatus*.**

Group	Dose of lead nitrate (mg/L)	Difference between two consecutive dose (A)	No. of fish exposed	Mortality				Overall mortality at 96 hr	Mean mortality between two consecutive dose (B)	$A \times B$
				24 hr	**48 hr**	**72 hr**	**96 hr**			
1	0	0	20	0	0	0	0	0	0	0
2	5	5	20	0	3	4	5	5	2.5	12.5
3	10	5	20	0	6	7	8	8	6.5	32.5
4	15	5	20	0	7	9	12	12	10.0	50.0
5	20	5	20	3	10	13	16	16	14.0	70.0
6	25	5	20	5	12	17	20	20	18.0	90.0
-	-	-	-	-				-	-	255.0

$$96 \text{ h } LC_{50} = LC_{100} - \frac{\sum AB}{N}, = 25 - \frac{255.0}{20}, = 25 - 12.75, = 12.75 \text{ mg/L}$$

(1) Graphical method taking percent mortality: The LC_{50} value of lead nitrate was found 12.02 and 165.0 mg/L for *Channa punctatus* while 350.0 and 380.0 mg/L for *Clarias batrachus*.

(2) Graphical method taking log and probit mortality: From this method, the LC_{50} value of lead nitrate was found 11.48 and 177.8 mg/L for *Channa punctatus* while 315.0 and 346.7 mg/L for *Clarias batrachus*.

(3) Regression method: From this method LC_{50} value of lead nitrate was calculated to be 11.75 and 208.0 mg/L for *Channa punctatus* while 320.0 and 348.0 mg/L for *Clarias batrachus* [7].

 (4) Behrens and Karber method: In this method, a difference between two consecutive doses was determined and arithmetic mean of mortality caused by two consecutive doses was also calculated. Then product of difference between two consecutive doses and arithmetic mean of mortality caused by two consecutive doses was calculated. Finally, 96 h-LC$_{50}$ value of lead nitrate was found 12.75 and 167.5 mg/L for *Channa punctatus* while 375.0 and 425.0 mg/L for *Clarias batrachus*. From these values, the average median lethal concentration of 96 h-LC$_{50}$ of lead nitrate was calculated to be:

(a) 12.02+11.48+11.75+12.75=12.0 mg/L for *Channa punctatus* (body weight: 50-68 g) during 2013 at 25.0 ± 1.0°C.

(b) 165.0+177.8+208.0+167.5=180.0 mg/L for *Channa punctatus* (body weight: 55-75 g) during 2015 at 27.0 ± 1.0°C.

(c) 350.0+315.0+320.0+375.0=340.0 mg/L for *Clarias batrachus* (body weight: 85-110 g) during 2013 at 25.0 ± 1.0°C.

(d) 380.0+346.7+348.0+425.0=374.925 or 375 mg/L for *Clarias batrachus* (body weight: 125-150 g) during 2014 at 26.0 ± 1.0°C.

TABLE 3. **Behren-Karber method for determination of 96 h-LC$_{50}$ dose of lead nitrate in *Channa punctatus*.**

Group	Dose of lead nitrate (mg/L)	Difference between two consecutive dose (A)	No. of fish exposed	Mortality				Overall mortality at 96 hr	Mean mortality between two consecutive dose (B)	A × B
				24 hr	48 hr	72 hr	96 hr			
1	0	0	20	0	0	0	0	0	0	0
2	125	25	20	0	3	5	3	3	1.5	37.5
3	150	25	20	0	6	8	5	5	4.0	100.0
4	175	25	20	0	7	10	8	8	6.5	162.5
5	200	25	20	3	11	17	20	20	14.0	350.0
	--	--	--	--				--	--	650.0
96 h LC$_{50}$=LC$_{100}$ - $\frac{\sum AB}{N}$, =200– $\frac{650.0}{20}$, =200-32.5, =167.5 mg/L										

TABLE 4. **Behren-Karber method for determination of 96 h-LC$_{50}$ dose of lead nitrate in *Clarias batrachus*.**

Group	Dose of lead nitrate (mg/L)	Difference between two consecutive dose (A)	No. of fish exposed	Mortality				Overall mortality at 96 hr	Mean mortality between two consecutive dose (B)	A × B
				24 hr	48 hr	72 hr	96 hr			
1	0	0	20	0	0	0	0	0	0	0
2	250	250	20	4	6	8	8	8	4	1000
3	500	250	20	12	12	14	14	14	11	2750
4	750	250	20	16	18	18	18	18	16	4000
5	1000	250	20	16	20	20	20	20	19	4750
--	--	--	--	--				--	--	12500
96 h LC$_{50}$=LC$_{1000}$- $\frac{\sum AB}{N}$,=1000- $\frac{12500}{20}$, =1000-625, =375.0 mg/L										

TABLE 5. **Behren-Karber method for determination of 96 h-LC$_{50}$ dose of lead nitrate in *Clarias batrachus*.**

Group	Dose of lead nitrate (mg/L)	Difference between two consecutive dose (A)	No. of fish exposed	Mortality				Overall mortality at 96 hr	Mean mortality between two consecutive dose (B)	A × B
				24 hr	48 hr	72 hr	96 hr			
1	0	0	20	0	0	0	0	0	0	0
2	250	250	20	0	3	5	8	8	4	1000
3	500	250	20	0	6	8	12	12	10	2500
4	750	250	20	0	7	10	16	16	14	3500
5	1000	250	20	3	11	17	20	20	18	4500
--	--	--	--	--				--	--	11500
96 hr LC$_{50}$=LC100- $\dfrac{\sum AB}{N}$, = 1000 - $\dfrac{11500}{20}$, =1000-575, =425.0 mg/L										

TABLE 6. **Summary of 96 h-LC$_{50}$ dose of lead nitrate determined by different methods in *Channa punctatus* and *Clarias batrachus*.**

Name of fish	Body weight (g)	Water temperature (°C)	Year	Percent graphical method	Log-graphical method	Finney (1971) regression method	Behrens and Karber method	Average
Channa punctatus	50-68 g	25.0 ± 1.0°C	2013	12.02 mg/L	11.48 mg/L	11.75 mg/L	12.75 mg/L	12.0 mg/L
Channa punctatus	55-75 g	27.0 ± 1.0°C	2015	165.0 mg/L	177.8 mg/L	208.0 mg/L	167.5 mg/L	180.0 mg/L
Clarias batrachus	85-110 g	25.0 ± 1.0°C	2013	350.0 mg/L	315.0 mg/L	320.0 mg/L	375.0 mg/L	340.0 mg/L
Clarias batrachus	125-150 g	26.5 ± 1.0°C	2014	380.0 mg/L	346.7 mg/L	348.0 mg/L	425.0 mg/L	375.0 mg/L

Discussion

Studies on the median lethal concentrations usually provide measure of relative toxicity of different heavy metals and also the sensitivity of fish species. Fishes are generally used in the evaluation of aquatic system quality and some of their physiological changes can be considered as biological markers of environmental pollution [9].

Lead toxicity is mainly a function of water hardness and nature of species along with age of fishes. Increased water hardness reduces lead toxicity due to a significant inorganic complexion process that controls lead availability to fish [10,11]. LC$_{50}$ values differ from species to species for the same toxicant due to the mode of action and responses of the animals. The LC$_{50}$ has been modified several times since its development in the 1920s depending on the type of animal, nature of substance, environmental factor, etc., There are different methods used in the determination of LC$_{50}$ dose [7,12-15].

In this work, the average 96 h-LC_{50} dose of lead nitrate was calculated to be 12.0 mg/L and 180.0 mg/L during 2013 and 2015 respectively for *Channa puncattus* (TABLE 5). A dose of 96 h-LC_{50} was calculated 11.6 mg/L for lead nitrate in *Channa punctatus* [3]. In another work, this dose was reported to be 600.00 mg/L in *Channa punctatus* (body weight 50-90 g) [4].

However, all these works are in the similar lines. But, one of the works showed more than three times increase in LC_{50} dose of lead nitrate in *Channa punctatus* in comparison to the present work [4].

The molecular mass of lead nitrate is 331.2 g/mol. Therefore, 331.2 mg by part of lead nitrate contains 207.19 mg of lead or, 1 mg by part of lead nitrate will contain 207.19/321.2=0.645 mg of lead. It means that at least 50% of *Channa punctatus* can survive in a water body having 12.0 × 0.645=7.74 mg/L to 600.0 × 0.645=387.0 mg/L of lead in an inorganic or organic compound. The difference also depends on the phenotypic variations of fish. The statement is supported by a work in which they studied phenotypic variations in *Channa punctatus* and reported its three variants in India [16].

On the other hand, the average 96 h-LC_{50} dose of lead nitrate was calculated to be 340.0 and 375 mg/L during 2013 and 2014 respectively for *Clarias batrachus* (TABLE 5). During an experiment, it was calculated 96 h-LC50 value to be 378 mg/L in *Clarias batrachus* (body weight 80-100 g) [2]. In another work, it was also reported a 96 h-LC_{50} of lead nitrate as 300.45 mg/L in *Clarias batrachus* [17]. On the other hand, some other workers determined 48 h-LC_{50} of 500 mg/L lead as lead acetate in *Clarias batrachus*. It showed more than 1.25 times increase in LC_{50} dose of lead nitrate in *Clarias batrachus* [18]. Therefore, at least 50% of *Clarias batrachus* can survive in a water body having 300.45 × 0.645=194.0 mg/L to 500.0 × 0.645=323.0 mg/L of lead in an inorganic or organic compound.

The present work as well as earlier reports indicates some sort of spatial increment in LC_{50} dose of lead nitrate for the same fish of similar body weights. The increment may be due to increase in ambient temperature and adaptations shown by fishes. Further, the pattern of difference in LC_{50} dose of lead nitrate was related with the difference in the body weight of fishes. In this experiment, body weight ranged from 50-75 g and 85-150 g in *Channa punctatus* and *Clarias batrachus* respectively indicating a wider range in *Clarias batrachus*. It shows more active nature of *Channa puncataus* in comparison to *Clarias batrachus*. The active nature of *Channa puncataus* (b=0.802) and *Clarias batrachus* (b=0.763) was confirmed with their corresponding slope value of total oxygen uptake [19,20].

The values of various physico-chemical parameters of experimental water were more or less similar during this experiment. Therefore, physico-chemical parameters not seem to be major factors that influence LC_{50} dose of these fishes in this experiment. As far as determination of LC_{50} dose by different methods are concerned, they definitely affect the value of LC_{50} dose. Two-way ANOVA inferred that use of different methods determine has highly significant effect (p<0.001, F=186.2) on the value of LC_{50} dose. Therefore, it seems that selection of appropriate method to determine LC_{50} is one of the most important aspects in toxicological studies.

Conclusion

Therefore, it may be concluded that the variations in doses may be due to the differences in the age, body weight, nutrient supply and habitat as well as in the abiotic conditions. The values are different and dependent on the conditions under which

tests were performed. It also infers that the tolerance limit for a toxicant by a fish is increasing gradually due to increase in resistance power.

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