ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 3(2): 196-198

www.fisheriesjournal.com
Received: 15-08-2015
Accepted: 16-09-2015

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Lead nitrate toxicity on haematological changes in a live fish species *Channa punctatus* (Bloch)

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Abstract
The present investigation deals with the effect of lead nitrate on the haematological parameters on the model fish organism *Channa punctatus*. Most of the physicochemical parameters of experimental aquarium are maintained at optimal level. Although the concentration of FCO_2 exceed beyond 15 $mg.l^{-1}$ in certain time of experimental period, but is not act as a toxic substance under high concentration of DO (7.27 ± 0.88 $mg.l^{-1}$). Moreover, the effect of high concentration of FCO_2 is minimal as the model organism is a live fish. The LC_{50} concentration where 50% death of the fishes occur is experimentally carried out which is found to be 600 $mg.l^{-1}$. The fishes were treated at sub lethal concentration of LC_{50} ($1/5^{th}$ of LC_{50} concentration i.e. 120 $mg.l^{-1}$) of lead nitrate for a period of 24hrs, 48hrs, 72hrs, and 96hrs to get haematological variations. The result shows that RBC and Hb% is decreased significantly following the exposure of lead nitrate in connection with time of exposure. Unlike RBC and Hb%, WBC is found to be increased with time. The examination of DLC shows that lymphocytes, basophil and eosinophil are increased as a function of time of exposure at sub lethal concentration; however, in neutrophil and monocytes the reversed phenomenon is discerned with significant variation.

Keywords: Lead nitrate, haematological changes, *Channa punctatus*, physicochemical parameters

1. Introduction
Pollution has become a serious threat due to urbanization and industrialization worldwide. Among all pollutions, heavy metal pollution considered as the burning issue due to its high solubility, persistence and biomagnifications in water which cause serious threat to aquatic ecosystem (Shukla and Tripathi, 2012) [17]. Aquatic environment is constantly polluted from heavy metals by various natural processes as well as due to anthropogenic interferences (Kumar, 1989; Seth, 2000; Kar *et al.*, 2008; Begum *et al.*, 2009) [4, 9, 10, 16]. In reality heavy metals cause harmful effect on in RBC's life span, hemoglobin surface area, alterations in haematological parameters in different fish species. Fishes are one of the most potent victims of such contaminated water among the aquatic fauna and serve as a good indicator of aquatic pollution (Shukla and Tripthi, 2012) [17].

2. Materials and methods
2.1 Model organism: The study has been carried on *Channa punctatus*, a common murrel species, which is distributed in the wetlands of Assam. *Channa punctatus* has a wide natural distribution and commonly found in the wetlands, freshwater ponds and tanks in the plains.

2.2. Procedure: Live, healthy and disease free fishes (weight 50-90 g and length 13-15 cm) were collected from Deepor Beel (wetland) located at $91^0 36^/$-$91^0 42^/$ East longitude and $26^0 6^/$ to $26^0 09^/$ North latitude and bought to the laboratory without any mechanical injury. Fishes were treated with 0.1% $KMnO_4$ solution to avoid any dermal infection before keeping them into the experimental aquarium. The fishes were acclimatized in laboratory for a period of 10 days. The fishes were fed daily with commercial food during the period of acclimatization. The faecal matters and other wastes are washed off daily to reduce ammonia content in water. After 10 days the acclimatized fishes were separated into two groups. One of the groups was served as control which was kept in tap water and the remaining was used as treated which was kept in lead nitrate water to determine the LC_{50} value. 60 fishes were exposed to different concentration of lead nitrate for 96 hrs to get the concentration where 50% deaths of the fishes do occur. After the determination of LC_{50}, the fishes were treated at sub lethal concentration of

LC_{50} (1/5th of LC_{50} concentration). Fish samples were collected after the exposure of lead nitrate from the aquarium in the interval of 24hrs, 48hrs, 72hrs, and 96hrs for haematological studies. After exposure in sub lethal concentration of lead nitrate, blood was collected from the heart and caudal peduncle of anesthetized fish. TLC was studied by using Neubauer's chamber (Daice and Lewis, 1977) [5]; DLC by use of Leishmann stain and the method given by Daice and Lewis, 1977 [5]. Hb% was studied by using a haemometer. The physicochemical parameters were examined as per A.P.H.A., 1988 [1] and N.E.E.R.I., 1988 [13] at Department of Zoology, Pandu College, Guwahati.

3. Result

3.1. Determination of LC_{50}: The concentration at which 50% death of fish occurs was considered as LC_{50} value. After 96 hour of exposure with lead nitrate it has been found that 600 mg.l^{-1} is the concentration of lead nitrate where 50% death occurs (Table-1).

Table 1: Different concentration of lead nitrate with its mortality

Number exposed	Number survived	Number respond	Concentration(mg.l^{-1})
10	10	0	00
10	9	1	200
10	7	3	400
10	5	5	600
10	2	8	800
10	0	10	1000

3.2. Haematological parameters: The treatment with lead nitrate at sub lethal concentration, the total count of RBC is reduced drastically. The study also reveals that the reduction of total count of RBC is time dependent; as time increased the RBC level declines (Table-2). However, the results of total count of white blood cells (WBC) are reversed as compared to total count of RBC i.e. increased with the time of exposure at sub lethal concentration. Unlike WBC, there is a sequential decline in Hb% in the treated group observed 24 hrs time intervals. Variation on DLC was also detected after sequential exposure of lead nitrate on the basis of duration of time. Lymphocytes, basophil and eosinophil are increased as the time of exposure at sub lethal concentration is increased unlike neutrophil and monocytes, where reversed phenomenon is noticed in the present study.

3.3. The physicochemical parameters of the experimental aquarium were maintained at the range as shown in Table-4. The most important physicochemical parameters like DO and pH are found at average 7.27±0.88 mg.l^{-1} and 6.57±0.208 mg.l^{-1} respectively in all experimental aquarium. However, FCO_2 is attaining 15 mg.l^{-1} in certain time of experiment.

Table 2: Effect of sub-lethal concentration of lead nitrate on haematological parameters of *Channa punctatus* at different hours of exposure.

Parameters	Control	24hr(trt)	48hr(trt)	72hr(trt)	96hr(trt)
Hb percentage (g/100ml)	8.2±0.28	7.65±0.21	6.55±0.35	6.1±0.14	5.25±0.353
Total RBC count(10^6mm^3)	3.52±0.028	3.31±0.127	3.195±0.007	2.98±0.028	2.81±0.84
Total WBC count(10^3mm^3)	4.15±0.14	4.38±0.106	4.55±0.070	4.78±0.035	5.07±0.39

Trt=treatment, RBC=Red blood cell, WBC=white blood cell, Hb=Hemoglobin

Table 3: Differential count of WBC (%) (Trt=treatment)

Parameters	Control	24hrs(trt)	48hrs(trt)	72hrs(trt)	96hrs(trt)
Basophil	2.33±0.58	2.33±1.15	2.66±0.58	3.33±0.58	3.67±0.577
Eosinophil	3.66±1.523	4.33±0.57	4.67±2.08	5.00±1.00	5.66±0.57
Neutrophil	50.66±4.04	43±2.65	36±4.00	29.33±2.52	26.67±2.08
Monocytes	6.33±1.15	5.33±0.58	4.66±0.577	2.67±1.53	1.67±0.58
Lymphocytes	31.67±1.53	32.33±1.53	35.33±2.52	37±3.605	42.33±3.511

Table 4: Physicochemical parameters of water in experimental aquarium

Parameters	Average±SD
P^H	6.57±0.208
Total Hardness (mg.l^{-1})	41.67±1.53
Total Alkalinity (mg.l^{-1})	171.67±18.56
Free CO_2 (mg.l^{-1})	15.67±4.04
DO (mg.l^{-1})	7.27±0.88

4. Discussion

The present study reveals that exposure of fish to sub-lethal concentration of lead nitrate for 24 hours, 48 hours, 72 hours and 96 hours caused significant alteration in haematological parameters of Indian fresh water fish *Channa punctatus*. The lead nitrate treatments inflict a reduction in the total count of RBC which is found to be time dependent. Panigrahi and Mishra, 1978 [14] also observed reduction in hemoglobin percentage and RBC count of the fish *Anabas scandens* treated with mercury. The exposure of *Channa punctatus* to sub lethal concentration of lead nitrate also significantly decreases the Hb%, which may leads to anemic condition of the fish. Similar results with significant reduction of RBC and hemoglobin content in fish exposed to different heavy metals have also been reported previously by Goel and Sharma, 1987 [8]. White blood cell count was found to be increased following lead nitrate exposure (Table-2). The increase in WBC, particularly lymphocytes observed in the present study could be attributed to stimulation in the immune system in response to tissue damage (Gill and Pant, 1985) [7] which may be the effect of lead nitrate. Misra and Shrivastava, 1980 also reported an increase in leucocyte count when they exposed fishes to heavy metals.

Most of the physicochemical parameters of the experimental aquarium is maintaining at the productive range (Table-4); except FCO_2, where in certain time of experiment it exceed 12 $mg.l^{-1}$, which is detrimental for the growth of fishes (Klontz, 1973). However, as the model fish organism is a live fish so the effect is minimal in this case. Moreover, high concentration of FCO_2 is not always toxic (Das and Das, 1994) [6]; but retards the uptake of DO under lower concentration of the same by fish (Alabaster *et al.,* 1957; Basu, 1959 and Saunders, 1962) [2, 3, 15] which is not evident in the present study as DO level is optimum (Table-4). DO and pH are very important parameters for survival of fish and is found to be in optimal level in experimental aquarium.

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