RESEARCH PAPER
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Bioassay evaluation of acute toxicity levels of lead chloride to *Channa punctatus* Bloch

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The present study deals with the acute toxicity of lead chloride on the behaviour and mortality of *Channa punctatus*. The LC $_{50}$ values for 24, 48, 72 and 96h have been determined. The results indicate that the fish exposed to different concentrations of lead chloride exhibited slow abnormal behaviour, skin depigmentation and a dose and dose-time dependent mortality rate.

Key words : *Channa punctatus*, Lead chloride, Acute toxicity, Behavioural changes, Mortality

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INTRODUCTION

The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005). The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.*,2007; Vosylinene and Jankaite, 2006). Heavy metals play an important role among numerous factors contributing to pollution into a fish body from the environment either through direct uptake form water or with food. In consequence, they become substantially accumulated in fish tissues, their concentration increases greatly than that of water. These heavy metals affect their behaviour, growth and reproductive capacity. These metals include mercury, nickel, lead, arsenic, cadmium, aluminium, platinum and copper (metallic and ionic form). Most of the heavy metals are highly toxic. Lead is biologically non essential metal and can be toxic to biota even at very low levels. Studies have shown that lead is accumulating in the fish at alarming levels (Senarathne and Pathiratne, 2007). Lead is known to cause the disease called plubism. It accumulates in aquatic biomass, gets concentrated and passed up the food chain to human consumers. Lead is also known to damage the brain, the central nervous system, kidneys, liver and the reproductive system (Ademoroti, 1966). In the present research *Channa punctatus* Bloch was selected due to its adoption in polluted aquatic environment. The purpose of this research is to quantify the LC $_{50}$ for different time interval and its effect on the mortality and behaviour of the test fish.

RESEARCH METHODOLOGY

The study was carried out at department of Zoology, R.B.S. College, Agra, Uttar Pradesh. Test fish *C. punctatus* (Bloch) (14-16 cm length and 60-70 g) were collected from the local ponds and pools. Test fishes were acclimated to the laboratory condition for one month in large plastic tubs containing 50 litres of tap water (having dissolved oxygen 8 mg/l, hardness 23.25mg/l and temperature 22 ± 2^0C) prior to the commencement of the experiment. During their confinement the fish were regularly fed on every alternate day with minced goat liver. Water was renewed every 24 hours along with the removal of unconsumed food and fecal matters.

Determination of LC $_{50}$:

Acute toxicity assays:

Laboratory bio assays were conducted to determine the 24 hrs, 48 hrs, 72 hrs and 96 hrs LC_{50} values for *C. punctatus* exposed to lead chloride. The experimental design and calculations for the acute toxicity were based on well known procedures given by Finney (1971). The test was carried out in 10 litres water capacity aquaria filled with well aerated tap water (pH 6.5-7.0). The fish selected for the test were visibly free of any deformities, lesions or diseases. The 96 hrs static bio-assay was conducted to assess acute toxicity of lead chloride as described in standard methods (APHA *et al.*, 1980). The food was not provided 24h before and during the acute toxicity test.

After determining the exploratory range of lead chloride concentration the definite acute toxicity test was conducted by placing a set of ten fishes in each of eight aquaria as having different concentrations of lead chloride *i.e.* 150.00, 143.75, 125.00, 112.50, 106.25, 100.00, 93.75 and 87.5 mg/l. A control aquarium was allowed to run simultaneously. Mortality rate of the fish was recorded after 24, 48, 72 and 96h and observations were made on their behavioural response to the toxic substance during the exposure period. A fish was considered dead when observed to be totally immobile with no opercula movement seen when probed with a glass rod. The experimental setup was constantly monitored to observe the changes in the behaviour of the fishes while the dead fishes were removed from the aquaria. The control and each test concentration of lead chloride were tested in duplicate.

LC_{50} value of lead chloride for *C. punctatus* was determined by arthmatical method and also for the graphical interpolation taking logarithms of lead chloride concentration on x-axis and probit value of percentage mortality on y-axis (Finney, 1971). The fiducial limits of LC_{50} values were calculated by the probit computation at 95 per cent confidence at normal variate (Mead and Curnow, 1983; Lewis, 1984).

RESEARCH FINDINGS AND ANALYSIS

In the present study LC_{50} values of lead chloride for the *C.punctatus* at 24, 48, 72 and 96h were found to be same by graphical interpolation and arthmatical method *i.e.* 122.5378, 107.5418, 106.3162 and 98.2695 mg/L respectively (Figs. 1, 2, 3 and 4). The fiducial limits calculated for LC_{50} values have been shown in Table 1.The data on percentage mortality clearly revealed that with the increase in the time period, the mortality of the test organism increased. The probable reason for such finding may be that the toxicant has regular mode of action and due to its accumulation and subsequent magnification leads to the death of the fish. Lead chloride has lethal action due to its toxic action on the bio-chemical processes. Lead is also known to affect brain, the central nervous system, kidneys, liver and the reproductive system (Ademoroti,1996) ultimately leading to death of animal (Agarwal,1991).

During the experimentation the test fishes in the aquaria exposed to different graded concentrations of lead chloride did not exhibit abnormal behaviour immediately. When compared to the fishes in the control aquaria the fishes in the treated aquaria showed abnormal behaviour after 17-18 hours.

Table 1: Percentage mortality of *Channa punctatus* Bloch at different concentrations of lead chloride over period upto 96 hours (Number of fish in each case was ten)

Exposure time in hours	Control	87.5 mg/l	93.75 mg/l	100.0 mg/l	106.25 mg/l	112.50 mg/l	125.0 mg/l	143.75 mg/l	150.0 mg/l	LC_{50} (with 95% confidence limits
24 hours	-	-	10	20	30	30	50	90	100	122.5378 (118.3473-126.8477)
48 hours	-	-	20	30	50	50	70	90	100	107.5418 (104.7450-110.6987)
72 hours	-	20	40	30	50	70	80	100	100	106.3162 (104.1000-108.5796)
96 hours	-	20	40	50	80	80	80	100	100	98.2695 (93.3134-102.3084)

- = No mortality

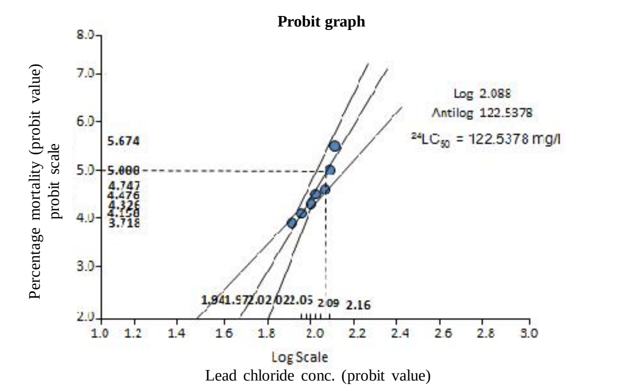

Fig. 1: Relation between probit of mortality of *Channa punctatus* at 24h and dose of lead chloride

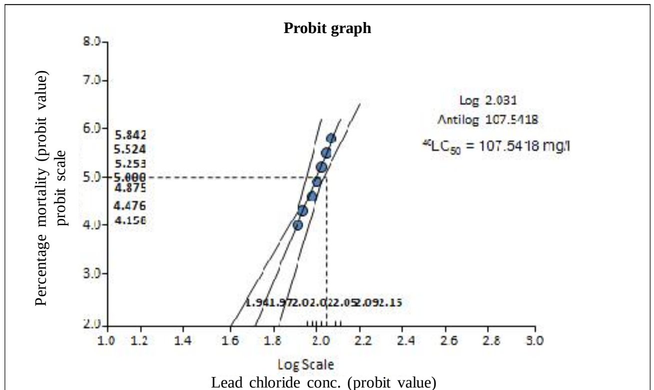

Fig. 2: Relation between probit of mortality of *Channa punctatus* at 48h and dose of lead chloride

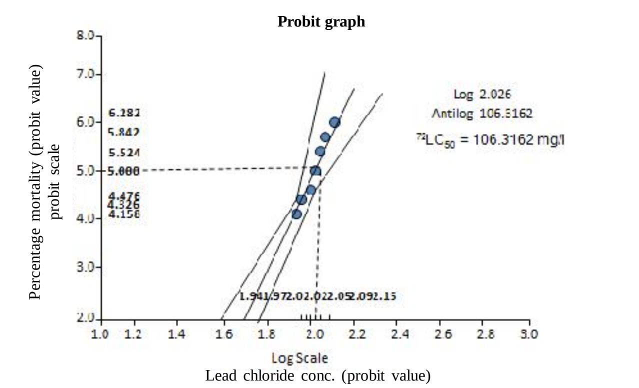

Fig. 3: Relation between probit of mortality of *Channa punctatus* at 72h and dose of lead chloride

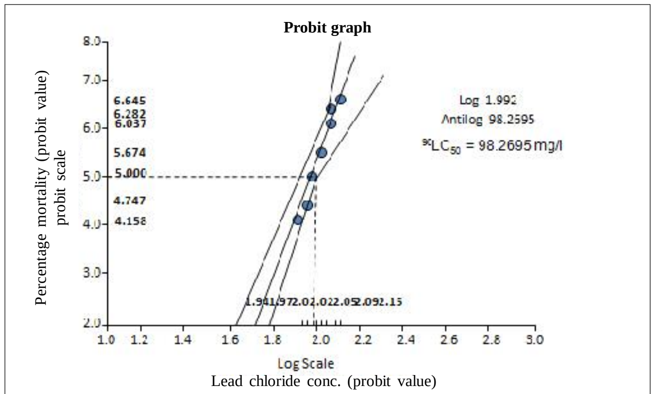

Fig. 4: Relation between probit of mortality of *Channa punctatus* at 96h and dose of lead chloride

The fishes gradually showed suffocation, frequent jerky movements and tried to jump out of the aquaria. Mucous secretion on the skin was less and its coagulation all over the body surface was observed. The fishes showed vertical erect orientation with head up and tail down before finally settling down before death (Agarwal, 1991; Pragatheeswaran *et al.*, 1987). Behavioural changes observed in the treated fishes may be due to skin irritation, respiratory rate impairment and coughing induced by the toxicant on the fishes. Death might be due to increased heart failure, hypertension, gastric hemorrhage, heart failure, suffocation etc (Tawari-Fufeyin *et al.*, 2008). Severe or prolonged exposure to lead may also cause chronic nephropathy, hypertension and reproductive impairment. Lead inhibits enzymes, alters cellular calcium metabolism and slows nerve conduction (Lockitch,1993). Exudation of mucous over the body may be due to dysfunction of endocrine/pituitary gland under the toxic stress causing changes in the number and area of mucous glands and chromatophores (Pandey *et al.*, 1990).

The above cited behavioural abnormalities of the fish and subsequent death implies that the toxic effect is mediated through the distributed nervous/cellular enzyme system affecting the respiratory function and nervous system, which are involved in controlling almost all the vital activities. Fish are considered as biomonitors of aquatic ecosystems for estimation of heavy metal pollution and risk potential for human consumption (Agarwal *et al.*, 2007). Continuous monitoring of the heavy metal accumulation in the fishes is very important as the food fishes may become unfit for human consumption if the level of heavy metals cross the safety limits. Thus, it is concluded from the present study that the fishes are highly sensitive to lead chloride toxicity and their mortality rate is dose and dose-time dependent.

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