



## Exploring Lethality of Lead Nitrate on *Channa Punctatus*

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### Abstract

According to WHO environmental health criteria No.180 (1996), fishes are excellent model for studying the effects of water and sediment-borne pollutants. The present study aims to test lethal concentration (LC50) and lethal time (LT50) of lead nitrate in our environmental conditions following semi-logarithmic graphical interpolation method. The study will enable us for further study like accumulation and effects on various systems of the fish. 10 large sized fishes were kept in each aquarium and different concentrations of lead nitrate were applied. 10 glass aquaria were taken and arranged them in a line on the laboratory table and then marked them number I, II, III, IV, V, VI, VII, VIII, IX and X. Each aquaria was filled up with 40 liters of tap water. Then toxicant was added respectively. Aquarium number 1 was kept free from toxicant that worked as control, while other aquaria were experimental ones. Then 10 fishes were released in each aquarium including the control and their mortality was observed up to 48 hours.

**Result:** The graph was plotted between concentration of lead nitrate and % mortality of *Channa punctatus*. The plot thus obtained is called as "Concentration- response relationship" curve. LC50 was found to be 500 ppm. Table. 2.1 outlines the results and figure 2 provides the concentration- response relationship. Similarly, a plot between time period and % mortality for a particular concentration of lead nitrate provided the LT50 value. This curve was called as "time-response relationship) curve.

**Keywords:** Lead toxicity; *Channa punctatus*; LC50; LT50; *Plumbum dulce*.

### 1. Introduction

Life is diverse, and this diversity is now affected by pollutions. Pollution of air, water and soil has led to difficulties in conducting life activities. Many organisms have become extinct and many are in the verge of extinction. The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Canli 1998; Voegborlo et al., 1999; Dirilgen, 2001; Vutukuru, 2005), and is getting extensively contaminated due to the release of metals from domestic, industrial, and other anthropogenic activities (Velez and Montoro, 1998; Conacher et al., 1993). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006; Farombi et al., 2007). It poses serious risks to many aquatic organisms by changing genetic, physiological, biochemical and behavioural parameters (Scott and Sloman, 2004). Lead (Pb) is one among the heavy metal and its contamination in

the water body has occurred on a global scale with adverse effects to human, environment health and damage caused to aquatic life especially fishes (Markus and Mc Bratney, 2001). Lead found in the environment, urban, industrial and agricultural waste waters and its occurrence in the air, which is transported to the streams and rivers by runoffs, are consumed by fish and other aquatic organisms (Weis and Weis, 1998; Chen and Folt, 2000). Several reports have indicated that Pb can cause neurological, haematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes aquatic bodies depending on the dose and time of exposure to Pb (Reglero et al., 2009; Abdallah Mirhashemi, et al., 2010 Rout and Naik 2013a). Fish are largely used in evaluation of aquatic systems quality and some of their physiologic changes can be considered as biologic markers of environmental pollution (Dautremepuits et al., 2004 Rout and Naik 2013c). It has a great potential to serve as sensitive indicators, signaling exposure and

understanding the toxic mechanisms of stressors in aquatic ecosystems (Vutukuru, 2005). The impact of metals, are to be evaluated by toxicity tests, which are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms. The 48hrs LC50 test paradigm is used to measure the susceptibility and survival potential of organisms exposed to particular toxic substances, such as heavy metals. Higher LC because greater concentrations are required to produce 50% mortality in organisms (Eaton et al., 2005). Behavior is a selective response that is constantly adapting through direct interaction with physical, chemical, social, and physiological aspects of the environment. Behavioral endpoints that integrate endogenous and exogenous factors can link biochemical entities thus providing insights into individual environmental contamination (Brewer et al., 2001, Vogl et. al. 1999). Little is known about the lethal effects of lead (Pb) on fishes (Pickering and Henderson, 1966; Martinez al., 2008; Tawari-fufeyin et al., 2008; Ramesh Khan et al., 2011; Askari Hesni et al.). Lethal effects of lead acetate on freshwater fish are scanty. Hence, the present work is aimed to investigate the acute toxicity of lead nitrate  $[(Pb(NO_3)_2)]$  responses of the freshwater fish *Channa punctatus*. [1-10]

## 2. Material and Methods

### 2.1. Selection of *Channa Punctatus* as a Model

According to WHO environmental health criteria No.180 (1996) fishes are excellent model for studying the effects of water and sediment-borne pollutants. There are several other good reasons for studying immunotoxicity in fish:

- as many of the diseases are related to environmental quality.
- various pollutants have immunotoxic potential, and
- many of the diseases have an immune components

Fishes are easy to obtain, there is an extensive body of knowledge, and their economic interest facilitates the finding of research resources. At present, immunotoxicology in fish is not as sophisticated as that in mammals. Screening and functional tests are being developed in the laboratory but can not yet be applied to field and laboratory studies. Our model

fish *Channa punctatus* (Bloc), fulfils all the criteria as out lined above. It is easy to available and acclimatize due to its air breathing habit and hardly nature. The fish lives generally in oxygen-poor, polluted fresh water bodies and can be cultured for its economic and food value [11-20]

### 2.2. Procurement and Acclimatization

Fishes of both sexes with varying weight, were collected from Rengali Dam of village Nalaban in Deogarh district of Orissa. After collection, the fishes were maintained in the laboratory aquaria for about 10 days for acclimatization, following Dehadrai(1971). They were kept in large sized aquaria (size 2.5' X 1' X1'). Containing 80 litres of water each. Live earthworms and fish food were supplied daily with water was 1/10th of their body weight. The water was changed daily with aeration. The Physico-chemical profile of the aquaria water was monitored following APHA (1989) which was constant for control and experimental aquaria.

### 2.3. The Compound Used

The compound of lead used for present piece of work is "Plumbum dulce" or, Lead Nitrate  $Pb(NO_3)_2$ , procured from Jhonson and Sons, London. It has a relative molecular mass of 331.2. it has a good solubility index i.e. 597gm/ltr of normal water. Selection of lead nitrate for experimental work was due to its following physical and chemical properties.

- A white crystalline solid, soluble readily in cold water. [21-30]
- Anhydrous with relative density 4.53 and melting point  $470^\circ C$ .
- It forms a variety of complex in solution and therefore reacts with different biomaterials.
- The biological availability of lead nitrate is highest in comparison to other compounds (Dieter et.al.1993). [61-66]

Hence for treatment, simply weighing it at desired dose and releasing to aquaria water is easy.

#### 2.3.1. Calculations for lead content in Aquaria water.

Compound used: Lead Nitrate:  $Pb(NO_3)_2$ : mol wt.207  
 $+(14 + 16 \times 3) \times 2 \Rightarrow 331.21$  mole of lead nitrate has 331gms of wt. or, 331 gm by part of lead nitrate contains 207 gm of lead, Or, 1ltr of water contain 1PPM lead Nitrate or,0.62 mg or 620  $\mu g$  of lead.



### 2.3.2. Determination of LC50 and LT50

LC50 is that concentration of a toxicant at which 50 % of the test animal is killed in a specified time. For example, 48hours LC50 for a toxicant refers to that concentration of the toxicant at which 50 % of the test animals are killed. LC refers to lethal concentration and 50 to 50 % population. However, lethal concentration is used for aquatic media only. Another related term LT50 refers to lethal time required to kill 50 % population of the test animal. The lethal concentration LC50 was determined following trial and error method of Omkar(1994). At the beginning of the experimental work, 10 large sized fishes were kept in each aquarium and different concentrations of lead Nitrate were applied. Taken 10 glass aquaria. Arranged them in a line on the laboratory table and then marked them number I, II, III, IV, V, VI, VII, VIII, IX and X. Filled up each aquaria with 40 liters of tap water. Then added toxicant at the rate of 17.5 mg/L, 15.5 mg/L, 31.5 mg/L, 62.5 mg/L, 125 mg/L, 250mg/L, 500mg/L, 1000mg/L and 2000mg/L in aquaria marked number II, III, IV, V, VI, VII, VIII, IX and X respectively. Kept aquarium number 1 free

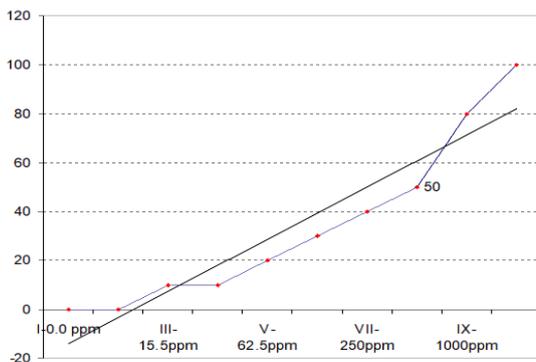
from toxicant that works as control, while other aquaria are experimental ones. Then released 10 fishes in each aquarium including the control and observe mortality up to 48 hours The concentrations increased logarithmic value and % mortality was calculated after 48 hrs for each concentration. The graph was plotted between concentration of lead nitrate and % mortality of *Channa punctatus*. The plot thus obtained is called as “Concentration-response relationship” curve LC50 was found to be 500 ppm. Table. 2.1 outlines the results and figure 2 provides the concentration- response relationship. Similarly, a plot between time period and % mortality for a particular concentration of lead nitrate provided the LT50 value. This curve was called as “time-response relationship curve. The LT50 was 45 days for 25 ppm, 40 days for 50 ppm, 37 days for 75 ppm, 35 days for 100 ppm, 30 days for 125 ppm, and 28 days for 150 ppm, the chronic 15 ppm treatment has a very long LT50 i.e. 150 days for the fishes weight for 200-250 gms. [31-40]

**Table 1** Determination of LC50 of Lead Nitrate on *Channa punctatus*, Bloc. by Semi logarithmic interpolation method for 48 hours

Sl.No. of Aquarium	No.of test Animals	Concentration of Lead Nitrate in PPM	No of Death	% of death
I	10	Control – 0	0	0
II	10	7.5	0	0
III	10	15.5	1	10
IV	10	31.5	1	10
V	10	62.5	2	20
VI	10	125	3	30
VII	10	250	4	40
VIII	10	500	5	50
IX	10	1000	8	80
X	10	2000	10	100

**Table 2** LT50 Of Used Concentrations of Lead Nitrate on Channa Punctatus, Bloc. During Experimental Plumbism

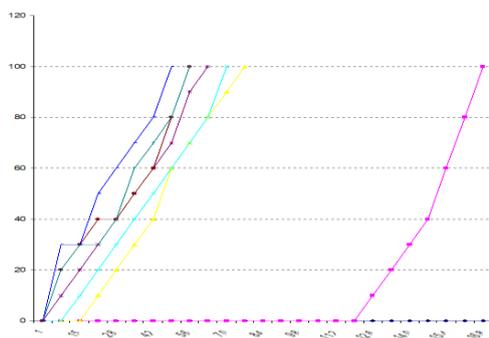
Concentrations of Lead Nitrate (ppm)	50% Lethal period of the chemical(days)
15	150
25	45
50	42
75	35
100	35
125	30
150	21



Different concentrations of Lead Nitrate

—●— % Mortality — Linear (% Mortality)

**Figure 1** Determination of LC<sub>50</sub> By Semi Logarithmic Interpolation Method



Time of exposure of different concentration of Lead Nitrate in days

—●— 0 —■— 15 —▲— 25 —✕— 50 —\*— 75 —●— 100 —+— 125 —■— 150

**Figure 2** Determination of LT<sub>50</sub> lead Nitrate on Channa punctatus by semi logarithmic interpolation method

### 3. Results and Discussion

#### 3.1. Acute Toxicity on Mortalities

The pollution of aquatic environment by toxicants adversely affects the survival of aquatic organism including the commercially important fish species which form the dominant group of aquatic system (Somaraj et al., 2005; Radhakrishnan Nair, 2006). The toxic effects of heavy metal on fish are multidirectional and manifested by numerous changes in the physiological and chemical processes of their body systems (Dimitrova et al., 1994). The median lethal concentration (LC<sub>50</sub>) of [(Pb(NO<sub>3</sub>)<sub>2</sub>)] for Channa punctatus was derived for 48 hrs. The mortality data was subjected to semi-logarithmic interpolation method and plotted against log dose concentrations, resulting in almost a straight line (Fig.1). The LC<sub>50</sub> values and 95% upper and lower confidence limits of Pb on Channa punctatus are given in Table - 1. [51-60] The LC<sub>50</sub> values for 48 hrs of exposures was estimated as 500- mgPbL-1 (PPM) . The present result clearly indicates that mortality increased with an increase in concentration and required the decrease of exposure time to bring about 50 percent mortality of fish. At the same time no mortality and behavioral changes were observed in the control groups. Further, the present findings indicate that, the mortality of the test fish to [(Pb(NO<sub>3</sub>)<sub>2</sub>)] was dose and time dependent and this reflects the regular mode of action which may be due to accumulation and subsequent magnification of [(Pb(NO<sub>3</sub>)<sub>2</sub>)] up to dangerous level that caused deaths of fish. Shah and Altindu (2005) have also suggested that the accumulation of a heavy metal has a direct effect on the LC<sub>50</sub> values of the respective metal in fish. The result of the present work is strongly concurrent with the findings of Guven et al. (1999), Shyang and Chen (2000), Karuppasamy (2001), Kanabur and Sannadurgappa (2001), Subathra and Karuppasamy (2003), Martinez et al. (2004), Puvaneswari and Karuppasamy (2007), Askari Hesni et al. (2011) and Nekoubin et al. (2012). Askari Hesni et al. (2011) reported a 96 hrs LC<sub>50</sub> value of Pb(CHCOO)<sub>2</sub> as 426.49 mgL-1 to the milk fish Chanos chanos. At the same time, Martinez et al. (2004) found out the 96 hrs LC<sub>50</sub> value of the same metal salt as 95 mgPbL-1 to the neotropical fish



*Prochilodus lineatus*, 300.45 mgL<sup>-1</sup> in *Clarias batrachus* (Ahmad khan et al., 2011), 378 mgL<sup>-1</sup> to the cat fish *C. batrachus* (Shamshun Nehar et al., 2010), 268.065 mgL<sup>-1</sup> to the Sea kutum *Rutilus frisiiikutum* (Gharedaashi et al., 2013) and 2.624 mgL<sup>-1</sup> to the juvenile common carp (Nekoubin et al., 2012). Srivastava and Mishra (1979) recorded the 96 hrs LC50 of Pb as 19 ppm to the test fish *Colisa fasciatus*. However, Hodson et al. (1978) found 2.4 ppm Pb for the 21-day LC50 of *Salmo gairdneri*. Shah and Altindü (2005) recorded the 96 hrs LC50 for *Tinca tinca* as 6.5 ppm for Cd and 300.0 ppm for Pb. Braaich and Kaur (2015) have reported that in *clarias* the value is between 1-50 mg for *Catla*. In contrast with these results, the present study determined a 48 hrs LC50 of 500 mgPbL<sup>-1</sup> for *Channa punctatus*. The above mentioned 96 hrs LC50 values disagree with the present investigations, this may be due to the differences in the test species, age and also the difference in the abiotic factors. The values obtained by toxicity testing (LC50 value) vary and are dependent on the conditions under which tests were performed so that interpretation of LC50 values needs to be done with caution (Walker et al., 1996). Amongst fish species, considerable differences in sensitivity to lead have been reported (Salmerón-Flores et al., 1990). According to Demayo et al. (1981), lead toxicity is a function of water hardness, species tested, and fish age. Increased water hardness reduces lead toxicity to fish due to a significant inorganic complexation process that controls Pb availability to fish (Hodson et al., 1984). Pickering and Henderson (1966) showed that in soft water (20 mg CaCO<sub>3</sub>L<sup>-1</sup>) the 96 hrs LC50 for *Pimephalespromelas* and *Lepomis macrochirus* was 5.6 and 23.8 mg Pb L<sup>-1</sup>, whereas in hard water (360 mg CaCO<sub>3</sub>L<sup>-1</sup>) 96 hrs LC50 was 482442 mg PbL<sup>-1</sup>, respectively. Darmayati and Hindarti (1994) found that young juvenile milkfish are more sensitive to hexavalent Cr than to Cd and they obtained 96 hrs LC50 values for Cr and Cd of 22.45 mgL<sup>-1</sup> and 38.9 mgL<sup>-1</sup> respectively. Diaz (1994) reported that the approximate 96 hrs LC50 for Cd in juvenile *Chanos chanos* is 27.3 mgL<sup>-1</sup>. From the previous report of Pb toxicity on various fish species, it can be inferred that the toxicity of Pb to aquatic organisms varies with life

stages of organism, test water criteria and duration of exposure. The wide difference in LC50 values of Pb to various species might be due to the mode of toxic potentiality and responses of animals under static conditions. Thus, the test employing the single species may provide information about the environmental risks of a toxicant (Taylor et al., 1991). [41-50]

### 3.2. Behavioral Abnormalities

The test fish showed various behavioral changes at different lead concentrations. The type, rate and duration of the behavioral changes increased with increase in concentrations. In all of the treatments, fish were hyperactive and attempted to escape from the tank during the first hours at which movement occurred. No behavioral changes or death occurred in the control group at any time during the experiment. All control fish were active and swam normally. Abnormal behavior was not expected to occur spontaneously in the control group. At the same time, the treated fish tried to escape from the tank and increased mucus secretion was also observed. The behavioural disorders included loss of balance, respiratory difficulty, slowness of motion, frequent surfacing activity and increased mucus secretion were observed after 48 hrs of exposure. The present observations were concurrent with the reports of Puvaneswari and Karuppasamy (2007). They observed these abnormal behaviours in Indian catfish *Heteropneustes fossilis* exposed to cadmium toxicity. Relatively increased breathing rate at the beginning and reduced rate as later revealed by opercular movements. Finally after prolonged period of exposure, the decrease in opercular movement and corresponding increase in frequency of surfacing of test fish clearly indicates the adaptivity shifts towards aerial respiration and the fish tries to avoid contact with the metal through gill chamber (Karuppasamy, 2001; Gharedaashi et al., 2013). The hyper activities in the test fish, which have higher metabolic activity could require higher levels of oxygen and thus could have a higher respiration or breathing rate (Canli and Kargin, 1995). Heavy extrudation of mucus over the body and discoloration are attributed to the endocrine/pituitary gland under toxic stress, causing changes in the number and area of mucus glands and



chromatophores (Pandey et al., 1990). The accumulation and increased secretion of mucus in the fish exposed to lead nitrate may be an adoptive response perhaps providing additional protection against corrosive nature of the metal and to avoid the absorption of the toxicant by the general body surface. This is in agreement with the earlier findings of Das and Mukherjee, (2003), Yilmaz et al. (2004), Prashanth et al. (2005) and Subathra and Karuppasamy (2003). Further, the test chemical produces effects on the skin at the site of absorption and is then transported systemically to produce its typical effects on the central nervous system and other organs (Askari hesni et al., 2011). The site of the highest concentration of the chemical is not often, the target organ of toxicity. Lead is concentrated in bone, (Rout and Naik 2013b) but its toxicity is due to its effects in soft tissues, particularly the brain. The target organ most frequently involved in systemic toxicity is the CNS (brain and spinal cord) (Klaassen, 2008), resulting in loss of coordination and locomotion, instability followed by hyper excitability, tremors and convulsions (Wouters and Vanden Brecken, 1978). For this reason, exposure to lead can affect the normal behavior of the test fish. Thus, the results of this study clearly illustrate that the toxic effects (mortality and behavioral changes) of lead nitrate on *Channa punctatus* varied with increasing heavy metal concentrations and in response to such water conditions as temperature, pH and dissolved O<sub>2</sub>. The study demonstrated that the test fish *C. punctatus* can be used as an effective bio-indicator for acute pollutants such as lead nitrate. Finally, death resulting from acute lead nitrate, in the test fish might be due to increased gastric hemorrhage, convulsion and suffocation (Valee and Ulmer, 1972). In conclusion, acute toxicity test constitute only one of the many tools available to the aquatic toxicologists but they are the basic means of provoking a quick, relatively inexpensive and reproducible estimate of the toxic effects of a test material. The assessment of toxicity on fish exposed to a particular toxicant will reveal facts regarding the health of given ecosystem and would eventually help us to propose policies to protect the ecosystem. It will also reveal the organisms sensitivity to a particular

toxicant that would help us to determine the permissible limit of a toxicant in an ecosystem.

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