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Acute toxicity assessment of lead nitrate on *Clarias Gariepinus* (Burchell, 1822)

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Abstract
Acute toxicity assessment of lead nitrate ($(PbNO_3)_2$) on post juvenile *Clarias gariepinus* under laboratory conditions were investigated. A total of 150 samples of the fish were acclimatized for two week. 6 aquaria tanks were set-up for determination of the 96hours LC_{50} with 6 samples of fish per tank. The lethal concentration values of lead nitrate used were 240mg/L, 260mg/L, 290mg/L, 320mg/L and 34mg/L with replicate in each case. The control setup was without the toxicant. The fish samples exhibited varying behavioural and physical changes which ranged from erratic swimming, poor feeding habit, frequent gasping for air at the surface of the tank, lacerations of the skin and secretion of mucus, and eventually death. The vigour and severity of these features were time and concentration dependent. The results showed that 96hours LC_{50} of *Clarias gariepinus* was 284.189mg/L. This indicated that greater quantity of the toxicant is needed to cause mortality in the fish and less deleterious at lower concentrations.

Keywords: lead nitrate, 96hours LC_{50}, *Clarias gariepinus*, mortality, behavioural and physical changes

1. Introduction
Fish is a rich source of animal protein throughout the world. Due to its nutritional value [1], the demand for fish food has been on the increase with increasing human population [2, 3]. Fish culture is an important source of protein and employment for many people [4]; and has been used to bridge the gap between demand and supply of fish from captured fisheries form the wild. *Clarias gariepinus* is a member of the Clarridae family. They occur naturally in South East Asia and in Africa and are sometimes called African catfish or mudfish. *C. gariepinus* is well appreciated in many African countries [5].
Lead (Pb) is one of the heavy metals that has contaminated water bodies on a global scale with adverse effects on human, environment health and aquatic life especially fishes [6]. Lead can be found in the environment, urban, industrial and agricultural waste waters and air, which is transported to the streams and rivers by runoffs where fish and other aquatic organisms take it up and incorporate it in their body. Several reports have indicated that Pb can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to Pb [7]. Lead as a metal has physical and chemical properties that make it extremely useful in industries especially in lead battery production, coloured inks and paint preparation. Lead thus constitutes an important constituent of wastes discharged from industries to which aquatic animals especially fishes are exposed. Lead and other trace metals have high affinity for animal tissues where they are concentrated to varying levels [8]. The up-take from the medium continues passively against a concentration gradient since backflow is limited by the excess high affinity binding sites within the body of the fish [9].
Heavy metals are non-biodegradable and form part of environmental pollutants in which elevated levels form threats to human health through food chain [10, 11]. Metals like lead are toxic and are known to present greater hazard when they are both persistent and bioaccumulative [12]. The quantity of metal accumulated has been reported to be directly related to the concentration to which the organisms are exposed and the period of exposure [13]. Consumption of fish with high amount of lead is a major route of human exposure to lead when contaminated fish are consumed [14, 15]. Fish accumulate toxic chemicals such as lead

nitrate directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediments and food [10, 16, 17].

Exposure and ingestion of lead nitrate can cause a myriad of physiological and neurological problems in both plants and animals and, ultimately deleterious effects in humans and other higher consumers. For instance, exposure to lead has been associated with reduced IQ, learning dissabilities, slow growth, hyper-activity, antisocial behaviours and impaired hearing [18]. Although Pb is a naturally occurring substance, its environmental concentrations are significantly increased by anthropogenic sources which include base metal mining, battery manufacturing, Pb-based paints and leaded gasoline [19]. Heavy metals have the potentials to bio-accumulate in aquatic organisms such as fish. Consumption of such staple food in contaminated forms calls for concern since lead has the potential to adversely affect human and animal health. It is known to cause physiological, biochemical and neurological dysfunctions in human [20]. Fresh water contamination with heavy metals such as lead is increasingly becoming a subject of great concern over the past decades not only because of their threat to pollute water supplies but also because of the damage caused to aquatic life especially fishes [21]. Therefore this study was carried-out to determine the effects of lead nitrate on *Clarias gariepinus* in an acute exposure.

2. Materials and Methods

2.1 Collection and Acclimatization of Post Juvenile *Clarias gariepinus*

A total of two hundred and fifty (250) samples of juvenile *Clarias gariepinus* were purchased from commercial Fish Farm and were transported to the Laboratory in a well aerated 25litres plastic bucket with water in it. The fish were distributed to 12 different plastic tanks (Aquaria) for a period of two weeks (14days) in which they were fed with vital (2mm) feed morning and night every day of the acclimatization period.

2.2 Lethal Concentration (96hours LC_{50}) determination

An initial trial on the determination of 96 hours LC_{50} of lead nitrate was carried –out.

The toxicant (lead nitrate) was introduced at varying concentrations as follows: control, 35mg/L, 55mg/L, 75mg/L, 95m/L, 115mg/L. Each concentration had its own replicate tank with 10 fishes per tank.

Another set of trial was conducted by doubling and tripling the highest concentrations from the initial set of concentrations giving: 230mg/L and 345mg/L with control set up as well. These various tests were used to determine the ranges of values to be used for the definitive test. The set-up for the definitive test of the LC_{50} were as follows: 240mg/L, 260mg/L, 290mg/L, 320mg/L, 340mg/L and 00mg/L as control. The exposure in each case lasted for 96 hours. The experiment was conducted using 6 fishes per tank. From the Experiment, LC_{50} value was determined using Probit analysis [22].

3. Results

3.1 Behavioural and Physical changes

When the samples were freshly exposed to lethal concentration of lead nitrate it was observed that there was an increased activity such as erratic swimming with the tendency to jump out of the tank for 2 hours after which they became adapted to the exposure. After 2 – 3 days their movement and swimming agility became very weak and slow. They stop feeding gradually and finally after 96 hours mortality rate was recorded from various tanks (Aquaria). There was severe weakness and side swimming in tanks with higher concentrations. After 12 hours of exposure opercula beat counts were lower than that of the control. There was also reduced feeding activity and mucus production from the mouth and skin especially in higher concentrations. There were also laceration of the skin and eventually, death.

3.2 Mortality Rate

The first trial did not yield any result as there were no mortality after 96 hours of exposure. They only exhibited lethargy in their activities and after three days of exposure they stopped feeding. They also engaged in frequent gasping for air at the surfaces of the tank. When the samples was exposed to lead nitrate at varying concentration it was observed that the tank (Aquaria) with the highest concentration caused 100% mortality within 24hours while the lowest concentration did not cause any mortality even after 96 hours (Table 3.1).

Table 3.1: The LC_{50} determination of *Clarias gariepinus* exposed to lead nitrate

Exposure period (hours)	Concentration (mg/L)	No. of Fish	Mortality (%)
24	350	6	100
48	320	6	66.7
72	290	6	50
96	260	6	0
	240	6	0

3.3 The Lethal points of lead nitrate on *Clarias gariepinus*

From the Probit analysis the points at which the lead nitrate began to have lethal effects on the fish varied significantly. The lethal concentration required to cause 10% mortality in the fish exposed to the toxicant was 276.457mg/L. This gradually increased from this point until it reached 284.189mg/L concentration that resulted in 50% mortality of the fish. As the level of bioaccumulation continued with increase in exposure period the concentration required to cause 95% mortality was 294.114mg/L. (Table 3.2).

Table 3.2: Lethal Concentration of lead nitrate on *Clarias gariepinus* Depending on Time of Exposure

Point	Concentration (mg/L)
LC_{10}	276.457
LC_{20}	279.111
LC_{30}	281.025
LC_{40}	282.661
LC_{50}	284.189
LC_{60}	285.718
LC_{70}	287.353
LC_{85}	290.443
LC_{95}	294.114

4. Discussions

The median lethal concentration (LC_{50}) of lead nitrate for *Clarias gariepinus* from the results of this research was 284.189mg/L. The mortality of the fish exposed to the toxicant increased with an increase in concentration. At the same time no mortality and behavioural changes were observed in the control groups. In addition to this, the results indicated that the mortality of the fish exposed to lead nitrate

was dose and time dependent and this reflects the regular mode of action which may be due to bio-accumulation and consequent magnification of lead nitrate up to detrimental levels that led to fish death. This agrees with the findings of Puvaneswari and Mohanambal (2013) [23] who observed similar abnormal behavioural changes in *Catla catla.* Other higher and lower LC_{50} values were obtained by various researchers. For instance, Askari *et al.* (2011) [24] have reported a 96 hours LC50 value of lead nitrate as 426.49 mg/L to the milk fish, *Chanos chanos;* Martinez *et al.* (2004) [8] have reported 95 mg/L to the neotropical fish, *Prochilodus lineatus*; Shamshun *et al.* (2010) [25] have reported 378 mg/L to the cat fish *C. batrachus*, 268.065 mg/L to the Sea kutum, *Rutilus frisii kutum* [26]; etc. Higher LC_{50} (as obtained in this research) are less toxic because greater concentrations are required to produce 50% mortality in organisms [27].

The test fish showed various behavioural changes at different lead concentrations. The type, rate and duration of the behavioural changes increased with increase in concentrations. In all of the treatments, fish were hyperactive and attempted to escape from the tank during the first two hours of the exposure. No behavioural changes or death occurred in the control group at any time during the experiment. All control fish were active and swam normally. At the same time, the treated fish tried to escape from the tank and mucus secretion was also observed. The behavioural and physical changes observed includes loss of balance, respiratory difficulty, slowness of motion, frequent surfacing activity, reduced feed consumption and mucus secretion after 24 hours of exposure. These toxic effects increased as the treatment concentration and exposure time increased. After 24 hours of exposure in higher concentration, the fish turned upside down in the water and became motionless, sideways swimming and loss of balance were also observed. These observations were in conformity with the findings of Puvaneswari and Mohanambal (2013) [23]. Similar observations were also reported by Puvaneswari and Karuppasamy [28]. They observed these abnormal behaviours in Indian catfish *Heteropneustes fossilis* exposed to cadmium toxicity.

5. Conclusions

The 96hours LC_{50} of *Clarias gariepinus* exposed to lethal concentrations of Lead nitrate ($(PbNO_3)_2$) under laboratory conditions was 284.189mg/L. This indicated that greater quantity of the toxicant is needed to cause mortality in the fish and less deleterious at lower concentrations.

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