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Acute toxicity test of lead on *Clarias gariepinus* juveniles

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Abstract
The acute toxicity test of Lead to *Clarias gariepinus* juveniles and the rate of bioaccumulation absorbed in the muscles and intestine of *Clarias gariepinus* juveniles was investigated using the Standard Methods Bioassay by APHA. In this study, the LC_{50} values of *Clarias gariepinus* at 96h period was determined to be 50.12mg/ L. Results obtained from this study showed that lead ($Pb(NO_3)_2$) was bioaccumulated at slower rate in the organs of the *Clarias gariepinus* juveniles because of the time of exposure (96hrs) but it shows extensive histological changes in the muscles rather than the gastrointestinal tract exposed to the various lead concentrations of $100mgL^{-1}$, $75mgL^{-1}$ and $50mgL^{-1}$.The lead concentration disturbed the homeostasis and led to physiological disorders in functions of opercular ventilation, tail bat movement and general behavior and subsequently led to the death of the juveniles *Clarias gariepinus*.

Keywords: *Clarias gariepinus*, Lead, acute toxicity, juveniles, opercular ventilation, tail fin movement

Introduction
The contamination of freshwater with a wide range of pollutants has become a matter of concern over the last few decades (1) [1]. Metal contamination in the environment is an ongoing problem, particularly in aquatic environments, and there has been extensive investigation of metal effects on aquatic organisms (Niyogi and Wood, 2004) [2]. A great variety of pollutants affect the majority of water course which receive domestic, industrial and agricultural effluents (Reglero *et al.,* 2009, Abdallah *et al.,* 2010, and Mirhashemi *et al,* 2010) [3, 4, 5].

The sensitivity of organisms to pollutants varies among species, population and life stage (Martinez *et al*., 2004) [6]. Therefore, it is important to choose the most suitable test organism. Fish have been the most popular choice as test organism because they are presumably the best-understood organisms in the aquatic environment and also due to their importance to man as a protein source (Schlenk and Benson, 2005) [7]. As a result, aquatic pollution influences human indirectly through the ingestion of fish due to bioaccumulation. It is therefore of great significance to evaluate pollution effects on fish for both environmental protection and socioeconomic reasons (Osman *et al.,* 2007) [8].

Lead is found in nature as a component of various minerals (McCulley *et al*., 1991) [9]. Some, such as galena (PbS), cerussite ($PbCO_3$), and anglesite ($PbSO_4$), are economically important sources of lead (Chandravathy and Reddy, 199) [10]. Lead (Pb) is an immune toxicant which through human exposure results in immune function changes and has the potential to adversely affect human health (WHO, 2015) [11]. Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. Bioassay technique has been the cornerstone of programs on environmental health and chemical safety (Ward and Parrish, 1982) [12]. It has many uses in industry including pipes, paints, enamels, glazes, motor industry, lead-acid batteries, bullets and shot. The major hazard in industry arises from the inhalation of dust and fume but the organic compounds may also be absorbed through the skin (Tranel and Kimmel, 2009) [13].

The natural waters are continuously being contaminated by lead due to increased anthropogenic activities and industrial exploitation of this metal (Chandravathy and Reddy, 1996) [10]. Several reports have indicated that Lead can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to Lead (Reglero *et al*., 2009, Abdallah *et al.,* 2010 and Mirhashemi *et al.,* 2010) [3-5].

Objective of the Study
The Objective of the study is determine the effect of lead on gastrointestinal tract of the Clarias gariepinus Juveniles

Materials and Methods
The Study was carried out at Zoological garden of the department of Biological Sciences, Kaduna State University. The method employed in this experiment is based on recommended method for the test of acute toxicity of pollutant to fish described by Standard Methods of Examination of Water and Waste Water (APHA, 2000) [14].

Selection of fish
It has been suggested by Fish Toxicity Testing Framework, [15] that economically local fish should be used in toxicity assay. *Clarias gariepinus* also called African catfish belongs to the Phylum; Chordata, Class; Osteichthyes, Order; Siluriformes, Family; Clariidae (air breathing fishes). They are black on the dorsal surface with dark green or olive color and white on the ventral surface. The head is dorso-ventrally flattened, with skin usually smooth in the young and coarsely granulated in adult. African catfish is of high commercial value and are very common in Nigerian fresh waters. They are of high nutritive value and data of their nutritive value have been well documented by (Otitoloju, 2001) [17]. People show much interest in fish farming and farmers are being encouraged to set up both small and large scale fish farms the most common fish which can be kept in captivity and grow to table size within a short period of time is the African catfish. On the basis of availability, commercial and ecological important *Clarias gariepinus* was chosen for this study.

Site determination
This Experiment was carried out in the zoology garden of the Department of Biological Sciences, Faculty of Science, Kaduna State University, (Coordinates: 10º31N; 7º26E) and 6.14m above sea level, Nigeria.

Collection of fish
Two hundred live active juveniles (8 weeks old) of *Clarias gariepinus* were purchased from united Patry fish farm in Barakalahu, Kaduna East, Kaduna Nigeria and transported in plastic bucket containing half filled with pond water to local fish pond (length: 84.00cm, height: 115.00cm, bottom diameter: 95.00cm and top diameter: 102.00cm) in the Zoological garden of the Department of Biological Sciences Kaduna State University, Kaduna. The fish were kept in the transparent (plastic) aquaria to observe any visible pathological symptoms. Before introducing in the pond fish were treated with 0.1% of $KMnO_4$ solution to serve any dermal infection.

Acclimatization
The juveniles fish were kept in the fish pond, containing dechlorinated water for a period of fourteen days to acclimatize to the environment before they were used in the bioassays.

Feeding
The juveniles were fed with fish feed (Coppens 2mm) in broadcasting method at 3% of body weight twice daily, and the water was changed once every 48hours to enhance oxygen content in the water. Feeding was stopped one day prior to the bioassay to avoid contamination of the toxicity of the water due to their excretory product.

Experimental Water
Underground water (borehole) was used for the experiment since it is known to be the best form of water for fish farming. The physico–chemical parameters of the water used were examined after exposure to air to lose chlorine if any. These parameters include temperature, dissolved oxygen (D.O.), water hardness and the hydrogen ion concentration (pH).

Lead Test and Preparation
Lead as $Pb(NO3)_2$ with molecular weight 331.21g, and purity of 99.5%. The metal was of analytical grade and manufactured by BDH laboratory supplies Poole, a division of BH15 1TD England. Stock solutions of lead metal was prepared by taking computed amount (2g) and made up to the desired volume (1 liter) using distilled water to achieve a stock of 2g L^{-1}. The stock solutions were serially diluted to obtain solutions with desired concentrations selected after range finding experiments. The different concentrations required were calculated as follows:

$$\frac{\text{Weight of lead required x molecular weight of lead}}{\text{Atomic weight of lead}}$$

$$\frac{2 \times 331.21\text{g}}{207.2} = 3.179\text{g}$$

Preliminary screening (pilot test)
Thirty fish of 8.70-9.60cm size and weight 5.15-6.02g, ten to each aquarium were exposed to higher lead and lower concentration of 5g L^{-1}, 3g L^{-1} and 2g L^{-1} respectively to determine the appropriate concentration range for testing chemical before the start of actual experiment.

General Bioassay Techniques
Bioassay containers
Eight rectangular plastic bowls (volume: 30.00 liters, height: 25cm, bottom diameter:
24.00cm and top diameter: 34.00cm) were used as bioassay containers.

Selection of animal for bioassay
Hundred and ten active juveniles of *Clarias gariepinus* of similar age and size (age: 8 weeks old, mean snout to tail length: 8.70-9.60cm, mean weight: 5.15-6.02g) were taken from ponds and randomly assigned to experimental containers.

Randomization
The juveniles were properly distributed into the eight aquariums at random as described Bano (1999) and Babatunde (2008) [17, 18].

Bioassays
Acute Toxicity Test of Lead
About 10 juveniles of *Clarias gariepinus* of similar age and size were taken from the pond, using a scoop net and randomly assigned to bioassay containers already with test media or untreated control. Each treatment was replicated twice, giving a total of 80 juveniles exposed per treatment. The fish sample and replicate were exposed to three lead concentrations; $Pb^{3}+$ at; 100mg L^{-1}, 75mg L^{-1}, 50mg L^{-1} with an untreated control (0.00).

Quantal Response
After the introduction of lead, the fish were observed at pre-determined intervals; (0,10,20.30,40,50,60 minutes, 2,4,8,16,24,32,36,48,72 and 96 hours) validity period. Juveniles were taken to be dead if no body movements including the operculum were observed, even when prodded with a blunt glass rod. Mortality was assessed once every 24hrs for a period of 4 days as recommended by Babatunde *et al*., 2014 [19].

Tissue Preparation
At the end of the 96hours Acute toxicity test, live *Clarias gariepinus* per replicate was randomly selected, dissected keeping the structure intact, the gastrointestinal tract were rinsed in normal saline, fixed in 10% formalin and preserved for about 24 hours at 4°C, till further analysis. (APHA, 2000)

Statistical Analysis
The dose-response data of the acute toxicity test of the test lead metal against the test species *Clarias gariepinus* were analyzed using by probit analysis after Finney (1980) [20]. One way analysis of variance, ANOVA were used to test for significant difference (5% level) for 96hrs, the indices of toxicity measurement derived from the analysis were:
LC_{95}- lethal concentration that will kill 95% of the exposed population of test animals
LC_{50}- lethal concentration that will kill 50% of the exposed population of test animals TF = Toxicity factor for relative potency measurements.

Aquaria Conc. mg/l		Log conc.	24hrs		48hrs		72hrs		96hrs		Percentage mortality	Probit Kill value
			R1	R2	R1	R2	R1	R2	R1	R2		
1	Control	0.000	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0%	0.000
2	50	1.6989	2	1	1	2	1	1	1	1	50%	5.000
3	75	1.8751	2	2	2	2	1	1	1	1	60%	5.2533
4	100	2.000	3	2	2	2	2	3	2	2	90%	6.2816

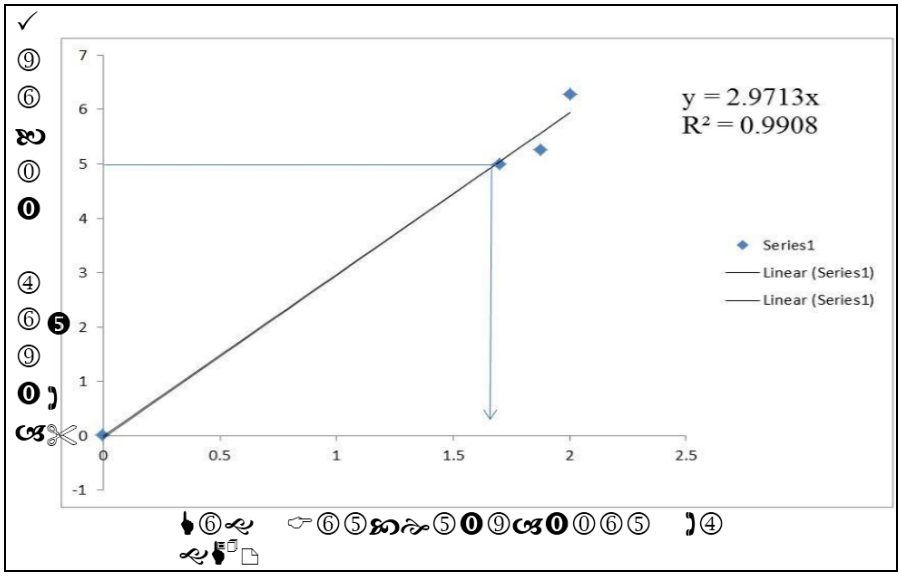

Fig 1: 96hrs LC_{50} linear relationship between probit mortality (%) and log. Concentration (mgL^{-1}) of *Clarias gariepinus* exposed to various concentrations of lead.

Log_{10} of Concentration (Mgl^{-1})
LC_{50} 1.7 → antilog =50.118MgL^{-1}

Determination of the Physico-Chemical Water Parameters
The temperature was measured with mercury-in-glass thermometer. The dissolved oxygen of the water was measured with a Griffin oxygen meter.
The D.O. was calculated as shown below:

$$D.O = \frac{S \times P\ (mg/l)}{100}$$

Where
S = Conversion of percentage saturation to parts per million (ppm).
P = Reading on the oxygen meter.

Table 1: Average Physicochemical Parameters

Parameters	Average Range
Temperature	12.5 – 14^0C
Ph	7.4 – 8.5
Dissolved Oxygen	4.0 – 4.6mgL^{-1}
Water Hardness	20 – 22mgL^{-1} $CaCO_3$
Alkalinity1	18 – 21mg $CaCO_3$ L^{-1}

Acute Toxicity Test
On exposure of the fish to the toxicant, fishes were observed to swim very rapidly in jerky and erratic movement. There is also sign of restlessness and agitation. The fish was also observed to move from one end of the aquaria another. Within 24 hours of this activity, about 80% of the fishes were also observed to settle at the bottom of the aquaria, some were positioned vertically with their head upward and their tail tip just on the aquaria. Reduced respiration rate, convulsive

movement and finally death occurred in some fishes. Dead fishes were observed to be covered with slimy and furry materials on their body; this was seen clearly on the side of the aquaria.

Effect of Lead on the Opercular beat rate of *Clarias gariepinus* juveniles

The opercular beat rate of juveniles of *Clarias gariepinus* exposed to the toxicant is shown in Table 2 and Figure 1. The period with the highest opercular rate was at the 12 hours in the aquaria containing the highest concentration of the toxicant (100mgL^{-1}). Then the opercular beat fell with time in aquaria 100mgL^{-1}, 75mgL^{-1} and 50mgL^{-1} in the order presented on the table below. The control aquaria recorded the lowest average opercular beat at the onset of exposure which later rose gradually at the 12th and 24th hour to about 79 and 80 beats per minutes respectively due to increased activity and aggression which later fell to about 78 beats at the 48 hours and 77 beats at the 72 hours of the bioassay and later rose to about 80 beats at the end of the 96 hours bioassay.

Table 2: Average Opercular Rate of Juveniles of *Clarias gariepinus* Exposed to Various Concentrations of Lead (Test sample).

Aquaria	Conc mg L^{-1}	12Hrs	24Hrs	48Hrs	72Hrs	96Hrs
1	Control	79	80	78	77	80
2	50	118	112	110	95	85
3	75	130	125	120	95	95
4	100	150	135	130	115	100

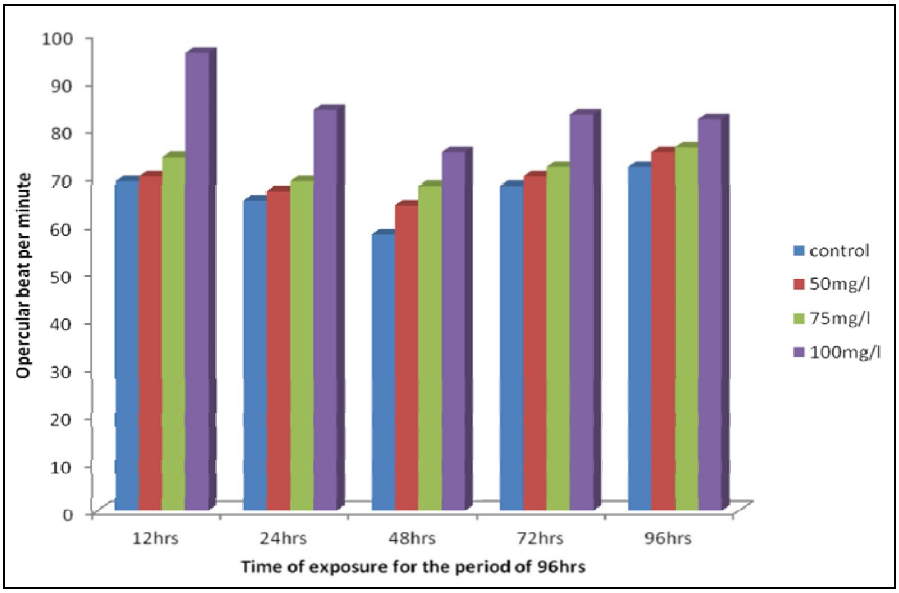

Fig 2: opercular ventilation rate of clarias gariepinus juveniles exposed to various concentrations of ph $(NO_3)_2$

Effect of Lead on the Caudal Fin Movement of *Clarias gariepinus* juveniles

The test fishes in the aquaria containing the highest concentration of the toxicant 100mgL^{-1} recorded the highest tail fin beats of about 115 per minutes. An average beat of 96 per minute was recorded in the aquaria containing 75mgL^{-1}. The number of beats rose in the 24th hour and fell slightly in the 48th hour. The number of fell continues till the end of the bioassay. However, the control recorded average beat of 70 per minute at the onset of the experiment and the number of beats rose gradually with time due to increase activity of the fishes in the aquaria in the order presented below.

Table 4: Average Caudal Fin Movement of Juveniles of *Clarias* gariepinus Exposed to Various Concentrations of Lead (Test sample).

Aquaria	Concentrations mg L^{-1}	12Hrs	24Hrs	48Hrs	72Hrs	96Hrs
1	Control	70	70	72	68	72
2	50	86	82	79	76	74
3	75	96	96	82	78	76
4	100	115	103	95	85	80

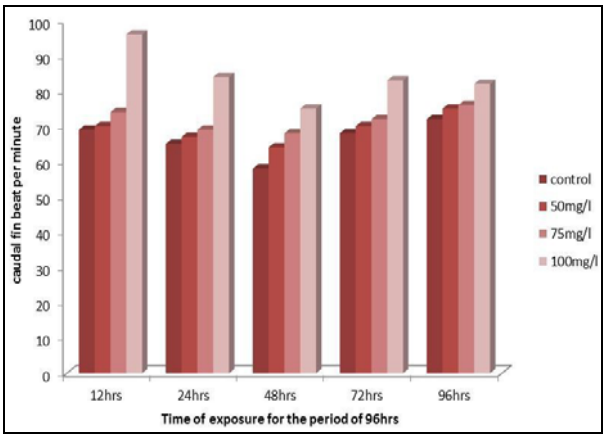

Fig 3: Caudal Fin beat of *Clarias gariepinus* juveniles exposed to various concentrations of $Pb(NO_3)_2$

Behavioral Changes and Mortalities of Fish

The results showed that lead affected the behavioral characteristics of *Clarias gariepinus*. The control specimens were not hyperactive and showed normal swimming patterns and fin movements throughout the exposure period. However, with increasing $Pb(NO_3)_2$ concentrations and exposure duration time, hyperactivity and jerky movements increased. In contrast, the swimming rate, fin movement, and equilibrium status decreased. Fish mortalities increased as the concentration of $Pb(NO_3)_2$ increased (Table 1). After 96 hours exposure, the control group has 0% mortality and the group exposed to50mg L^{-1}, 75mg L^{-1} of lead had mortalities of 40% and, 60% respectively. Whereas the group exposed to100mg $L^{-1}Pb(NO_3)_2$ had 90% mortality

Statistical analysis of variance

Tests of Between-Subjects Effects

Dependent Variable: Average opercular rate

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	4270.300[a]	2	2135.150	88.037	.000
Intercept	19616.001	1	19616.001	808.815	.000
TIME	3060.300	1	3060.300	126.183	.000
CONC	1210.000	1	1210.000	49.891	.000
Error	291.033	12	24.253		
Total	200643.000	15			
Corrected Total	4561.333	14			

a. R Squared = .936 (Adjusted R Squared = .926)

From the above table, the result shows that there is a significant difference in the time of the average opercular rate per minute, since the p-value (0.00) is < (0.05) which implies that it is significant. It also shows that there are significant differences in the concentration of the average opercular rate per minute, since the p-value (0.00) is < (0.05) which implies that it is significant. Also the R Squared is 0.936 which implies that 93.6% of the variability is explained by the independent variables.

Parameter Estimates

Table 8: Dependent Variable: Average opercular rate

Parameter		B	Std. Error	T	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
dimension1	Intercept	122.633	4.312	28.440	.000	113.238	132.028
	TIME	-10.100	.899	-11.233	.000	-12.059	-8.141
	CONC	11.000	1.557	7.063	.000	7.607	14.393

Average opercular rate = 122.633 – 10.10*Time + 11.00* Conc

The above regression model shows that the average opercular rate is depending on time and Concentration which is significant at 0.05 level of significance, since p-value (0.00) is < (0.05) Significance value.

Tests of Between-Subjects Effects

Table 9: Dependent Variable: Average tail fin Beat

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1688.633[a]	2	844.317	42.732	.000
Intercept	10162.180	1	10162.180	514.324	.000
CONC	656.100	1	656.100	33.206	.000
TIME	1032.533	1	1032.533	52.258	.000
Error	237.100	12	19.758		
Total	115113.000	15			
Corrected	1925.733	14			
Total					

a. R Squared = .877 (Adjusted R Squared = .856)

From the above table, the result shows that there is significant differences in the time of the average Tail Fin Beat per minute, since the p-value (0.00) is < (0.05) which implies that it is significant. It also shows that there is significant differences in the concentration of the average Tail Fin Beat per minute, since the p-value (0.00) is < (0.05) which implies that it is significant. Also the R Squared is 0.877 which implies that 87.7% of the variability is explained by the independent variables.

Parameter Estimates

Table 10: Dependent Variable: Average tail fin Beat

Parameter	Standard	95% Confidence interval				
B	error	T	Sig		Lower bound	Upper bound
Intercept	88.267	3.892	22.679	0.000	79.787	96.747
Conc.	8.100	1.406	5.762	0.000	5.037	11.163
Time	-5.867	0.812	-7.229	0.000	-7.635	-4.098

Average Tail Fin Beat = 88.267 + 8.10*Time - 5.867* Conc

The above regression model shows that the average Tail Fin Beat is depending on time and Concentration which is significant at 0.05 level of significance, since the p-value (0.00) is < (0.05) Significance value.

Acute toxicity test

The results presented here revealed that lead exposure resulted in histopathological changes in the gastrointestinal tract and muscles of *Clarias gariepinus* juveniles. The 24, 48, 72 and 96h LC_{50} were calculated using probit analysis according to Litchfield and Wilcoxon (1949) [21]. Expressing the result in Log- probit transformation were first used by Fry *et al*. (1946) [22] for testing lethal temperatures and concentrations and has since been used for assays of pollutants and toxicants. It is used in Paraquat toxicity to *O. niloticus* by Babatunde *et al*., (2014) [20].

In this study the LC_{50} value of *Clarias gariepinus* 24, 48, 72 and 96h period was determined to be 50.118ppm or 50.12mg/ L. It is a high value that shows the concentration of the metal that will kill 50% of the population of the juveniles *Clarias gariepinus*. The acute toxicity test showed that the higher the concentration the higher the percentage mortality of the test organisms. In this study the control fish showed normal constant health tail fin and opercular ventilation movement. However, in the test organisms the higher the concentration the higher the tail fin and opercular movements. This shows a sign of stress in the bid of the organism to avoid or get rid of the obnoxious environment; indicating that the lead is toxic to the fish. The tail fin and opercular movement started decreasing due to fatigue and some eventually died (Saito, 2009) [23].Lead is known to be a contaminant in water bodies and also known from research work to Affect human children and aquatic organisms most especially fish This study is relevant to add to knowledge Since it that can affect life generally as it also bioaccumulates and passes through food web.

Behavior

These include lying on the bottom of aquaria, jumping out of test water, erratic swimming, gulping of air, stillness and finally death which are dose dependent .However the control fish showed normal behavior. Our

Conclusion

In this study, it has shown that lead can cause damage to the health of organisms in water and indirectly on humans when they consume these fish due to the accumulation of toxic substances. Acute lead toxicity causes anemia, ataxia, appetite loss, and behavioral changes, often resulting in sudden death. Both acute and Chronic lead toxicity results in gastrointestinal stasis and causes anemia, liver, kidney, and nervous system dysfunction. Avoiding contaminating our water bodies with lead is very important.

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