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Effects of Lead Chloride on Growth Performance of Clarias gariepinus (Burchell, 1822)

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Abstract: The study determined the median Lethal Concentration (LC50), investigated the growth and behavioral response of *Clarias gariepinus* to sub-lethal concentration of lead chloride in a static renewal bioassay. Acute toxicity test was carried out using different concentrations of lead (350, 400, 450, 500, 550 mg/L) in a statics bioassay for 96 hrs to assess median lethal concentration of lead on the fish. The concentrations used for the sub-chronic toxicity test were based on the results obtained from the acute test. A static renewal bioassay procedure was adopted in which the test culture was regularly renewed every 48 hrs at the set concentrations of $PbCl_2$ (0, 20, 40, 60, 80, 100, mg L^{-1}). The growth and behavioral response and as well as the mortality response were monitored for the 21 days of exposure. Data obtained were analysed using Analysis of Variance (ANOVA). The results showed an LC50 of 426 mg/L. Sub-lethal exposure showed a decrease in growth rate with an increase in $PbCl_2$ concentration. The value of specific growth rate (1.95 ± 0.015 mg/L) of fish in the control tank was significantly ($p<0.05$) higher than the other level of $PbCl_2$ concentrations. The fish behavior was normal through-out the period of experiment (21 days) with some exceptions where there was erratic movement in high concentration (80 mg/L and 100 mg/L), lethargy and vertical positioning majorly after 48 hrs prior to changing of the water. The study concluded that lead metal (heavy metal) has negatively impacted on *Clarias gariepinus* with a reduction on growth performances and behavioral response.

Keywords: Sub-lethal, *Clarias gariepinus,* Heavy metal, Lead, Toxicity, Growth

Introduction

In the most recent decades, most of the countries are undergoing a rapid industrial development, urbanization, construction, mining activities and deforestation [1]. These activities may lead to the environmental problem such as land, air and water pollution [2]. Water pollution is a major problem across the globe with the presence of harmful contaminants in the environment that had increased much concern because of the green revolution [3]. Water bodies around the world are increasingly getting polluted, more than ever in history due to human perturbation [4,5].

Heavy metals such as lead (Pb) bioaccumulate and distribute to tissues in aquatic life especially fishes and causes serious damage which eventually get into the food chain [6,7]. It is a cumulative poison and a possible human carcinogen [8]. Its toxicity results in mental disturbance and impairment of speech, hearing, vision and movement [9]. Lead (Pb) is persistent in nature and causes serious threat to the aquatic environment. It has the potential to adversely affect the human and animal health and reduced rates of growth [10]. At low level of exposure during the early development may result to long-lasting cognitive and neurobehavioral dysfunctions in humans. Exposure to high or chronic lead levels (40-60 µg/dl) can severely damage the brain and kidneys and ultimately cause death [11]. These effects are not reversed or improved even by chelation therapy [12,13]. At high concentration, lead metal, are known to have negative effect on the survival, growth, reproduction potential and high mortality rate of fish [14].

Moreover, bioaccumulation of lead metal in fish critically influences the physiological, neurological, gastrointestinal, reproductive, circulatory, biochemical and histological alterations in survived fish [15,16]. Also, it consequently affects the meat quality of fish [17].

Clarias gariepinus generally referred to as African catfish, belongs to the Phylum: Chordata, Class: Osteichthyes. It is commonly found throughout Africa, It inhabit varieties of fresh water including ponds, lakes and pools [18]. The African catfish is referred to as a bottom dweller and an obligate air breather. The species can live in very poorly oxygenated water and also, is able to secrete mucus to avoid drying conditions [19].

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Clarias gariepinus is commonly cultured in fish farms in Nigeria and of great economic interest [20]. It is major part of the human diet due to high protein content, low saturated fat and sufficient omega fatty acids which are known to support good health. *Clarias gariepinus* has been identified as the best choice for aquaculture due to its handiness, large size attainable and fast growth rate [21]. Fish are considered as biomonitors of aquatic ecosystems for estimation of heavy metal pollution and risk potential for human consumption [22]. It can therefore be a good model to study responses of fresh water fishes to various environmental pollutants especially heavy metals.

Toxicology testing is a test conducted to determine the degree to which a pollutant or contaminant can damage a living or non-living organism by analyzing the actual chemical in the samples or the used laboratory animals in studies [23]. The measure of a chemicals toxicity which is its Median Lethal Dosage (LD50/LC50) value is the concentration that can cause average kills of 50 percent of a test population of animals on trial from a single or limited exposure [24].

According to Adedeji et al. [25] acute toxicity is usually caused by exposure of an organism to a large dose of a toxic compound for a short period of time with a rapid effect being produced usually causing mortality. This test may equally be used to determine the median lethal concentration of a compound over a given period of time.

Chronic toxicity which may either be lethal or sub lethal is caused by very low doses of a toxic compound or effluent over a long period of time [26]. Sub-lethal effects can occur at the biochemical, physiological or behavioral level, genotoxicity and change in rate of growth [27]. This study evaluated lead-induce toxicity in *Clarias gariepinus* for 21 days under controlled experimental conditions by determining the median Lethal Concentration (LC50) of lead on the fish and investigating the growth and behavioral response of catfish to sub-lethal concentration of lead.

Materials and Methods

Experimental design

Sixty (60) healthy and active juveniles *Clarias ganiepinus* (average weight of 30-36 g and length range of 15-20 cm) were obtained from hatchery units of Department of Fisheries, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. The fish were transferred to the culture room in the Institute of Ecology and Environmental Studies prior to toxicity testing, where the fish were acclimatized for one week under controlled conditions. Twelve (12) stock tanks were used for the toxicity test, five (5) juvenile *Clarias gariepinus* was introduced into each tank with about 20 L of water containing the different lead concentrations in duplicates.

Toxicity testing

During determination of acute toxicity test, lead (Pb) toxicity experiments were performed using different concentrations of Pb (350, 400, 450, 500, 550 mg L^{-1}) with a control in duplicate in static bioassay. A 96 hrs test was carried out using juveniles *Clarias gariepinus* to assess the toxicity of various concentration of lead on the fish. Sub-lethal toxicity in a renewal static renewal bioassay [28] procedure was adopted. The definitive concentration used for the sub-lethal toxicity test was determined based on the results obtained from the acute test. The solutions were renewed every 48 hrs at the set concentrations for 21 days. This allowed monitoring of behavioral responses and mortality of the test organism to different concentrations of lead chloride (Pb).

Growth performance indices

The fish were fed with 5% of their body weight twice per day for 21 days [29]. A top-loading weighing balance was used to take the weight of the test fish in g. Data collection started from the day of stocking till end of the experiment. Weighing exercise was carried out weekly for each treatment tank and data collected were used to calculate total feed Intake, growth performance and other indices as follows:

Daily Feed Intake (DFI)-Calculated according to the method of Pitcher and Hart [30].

$$DFI = \frac{\text{Total feeding intake (g)}}{\text{Number of fish per tank}} \quad (1)$$

Where,

$$TFI = \frac{\text{Total feeding intake (g)}}{\text{Time}}$$

Mean Weight Gain (MWG)-Calculated according to the method of Pitcher and Hart [30].

$$MWG = W_f - W_i \quad (2)$$

Where,

W_f =Final mean weight.

W_i=Initial mean weight.

t=Rearing period.

Daily Weight Gain (DGW)-Calculated according to the method of Pitcher and Hart [30].

$$DGW(g) = \frac{MWG}{t} \quad (3)$$

Percentage Weight Gain-Calculated according to the method of Pitcher and Hart [30].

$$SGR = \frac{100(Log W_f - Log W_i}{t} \quad (4)$$

Where,

W_i=Mean Initial Weight.

MWG=Mean Weight Gain.

Specific Growth Rate (SGR)-This was estimated from the logarithm differences between final and initial mean weight gain [31].

$$SGR = \frac{100(Log W_f - Log W_i}{t} \quad (5)$$

Where,

W_f=Final Mean Weight.

W_i=Initial Mean Weight.

t=Rearing Period.

Feed Conversion Ratio (FRC)-The FCR was calculated according to the methods of Burel et al. [32].

$$FCR = \frac{\text{Total feed intake}}{\text{Mean weight gain}} \quad (6)$$

Survival Rate-The mortality rate was observed by monitoring the experimental tanks every six hrs and mortality were removed and recorded.

$$\%\,Survival = \frac{N_f \times 100}{N_i} \quad (7)$$

Where,

N_f=Final number of fish at end of experiment.

N_i=Initial number of fish at the beginning of the experiment.

Statistical analysis-Data collected were subjected to statistical analyses using one-way ANOVA at α=0.05 different level of significance. Followed by the Duncan Multiple Range Test (DMRT) to analyze the data for statistical significance, while graphical representations were constructed using Microsoft Excel.

Results

Acute mortality and time of death of *Clarias gariepinus* exposed to varying concentration of lead metal (Table 1). During the 96 hrs acute toxicity test, no fish mortality was recorded in the control (Figure 1). Number of dead fishes increased with an increase in concentration and noticeable changes in behavioral patterns. These changes includes; loss of balance, skin bleaching and weakness. Reduced

Table 1: Acute mortality and time of death of *Clarias gariepinus* exposed to varying concentration of lead metal.

Conc	12 hrs	24 hrs	48 hrs	72 hrs	98 hrs	Number	Mortality (%)
Control	0	0	0	0	0	5	0
350 mg/L	0	0	0	0	1	5	20
400 mg/L	0	0	0	1	1	5	40
450 mg/L	0	0	0	1	2	5	60
500 mg/L	0	0	1	1	2	5	80
550 mg/L	0	1	1	2	2	5	100

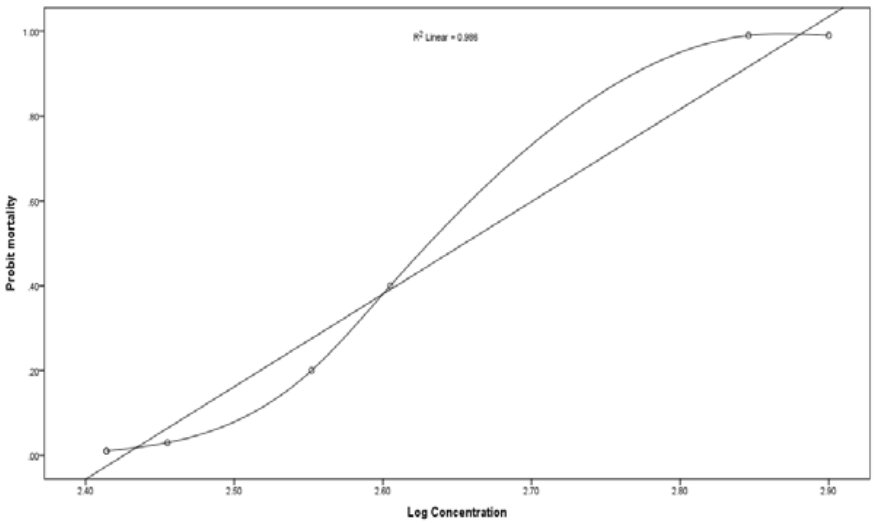

Figure1: Relationship between response and log concentration of *C. gariepinus* juvenile exposed to acute concentration of lead chloride for 96 hrs.

activity evidenced by erratic movement to obtain oxygen at the surface of water, less mobility and lethargy. 100% fish mortality was recorded for the highest concentration (550 mg/L) after 96 hrs with layer of mucus covering the skin of the dead fish (Table 1). The values of LC50 for acute toxicity were recorded at 426 mg/L with 333.4 LCL and 497.1 UCL (Table 2).

Growth Performance-The mean weight gained by the *C. gariepinus* juveniles was found to be highest in the third week of exposure. Control was found to have the highest mean weight gained which ranged from 6.56 ± 3.122 mg/L (wk 1) and 45.5 ± 3.12 mg/L (wk 3) while, 100 mg/L lead concentration was recorded to have the lowest weight gained through the period of exposure. Mean values ranged from 3.20 ± 2.00 mg/L (wk 1) and 32.0 ± 3.12 mg/L (wk 3) (Table 3).

The Specific Growth Rate (SGR) in the fish juveniles cultured in the control tank was significantly higher ($p<0.05$) than juveniles exposed to varying concentrations of lead (Table 4). The values ranged between 1.14 ± 0.04 mg/L (80 mg/L lead concentration) and 1.95 ± 0.015 control. Comparative analysis showed that Feed Conversion Ratio (FCR) was highest for fish exposure to lead at a concentration of 40 mg/L (Table 4). Daily Feed Intake (DFI) (Table 4) of the juveniles ranged between 1.10 ± 0.11 mg/L (100 mg/L lead concentration) and 1.43 ± 0.09 control.

The Total Feed Intake (TFI) of fish juveniles cultured in the control tank was significantly higher ($p<0.05$) than for the juveniles exposed to varying concentrations of lead. Values ranged between 29.12 ± 0.57 mg/L (40 mg/L lead concentration) and 38.38 ± 2.08 control.

The Mean Daily Weight Gain (MDWG) values in the control were statistically higher ($p<0.05$) than in tanks expose to lead. The MDWG of 20 mg/L and 40 mg/L were not significantly different ($p<0.05$) from each other (Figure 2).

The Percentage Weight Gain (PWG) of juveniles ranged between 54.90 ± 17.1 mg/L (100 mg/L lead concentration) and 92.65 ± 0.85 in control tank. The mean value of PWG was statistically lower ($p<0.05$) in the 80 mg/L and 100mg/L exposed tanks (Table 4). No mortality was recorded in the control culture tank, 10% and 20 % mortality were recorded in tanks exposed to 60 mg/L and 80 mg/L lead concentration respectively.

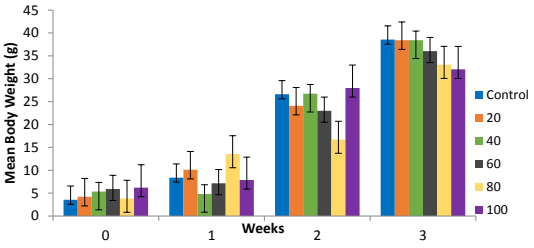

Figure 2: Mean body weight gain of catfish to sub-lethal concentration. Bars represent standard errors.

Table 2: Toxicity Indices for Acute Toxicity Test.

Index	Concentration mg/L	95% LCL	95% UCL
LC50	426.5	333.4	497.1
LC90	560.9	486.4	1495.3

Table 3: Mean weekly growth performance indices (Mean Weight Gain) of *C. gariepinus* juveniles exposed to lead concentration.

Lead Concentration	Weeks of Exposure			
	Wk 0	Wk 1	Wk 2	Wk 3
Control	6.56 ± 3.122	13.37 ± 3.12	30.60 ± 3.12	45.5 ± 3.12
20 mg/L	5.210 ± 3.20	10.11 ± 2.06	24.0 ± 2.56	37.4 ±3 .22
40 mg/L	4.35 ± 2.12	9.83 ± 2.22	26.73 ± 3.12	36.4 ± 3.12
60 mg/L	4.90 ± 3.12	7.150 ± 2.33	22.98 ± 3.24	36.03 ± 3.00
80 mg/L	3.81 ± 3.22	8.54 ± 2.12	16.3 ± 2.15	34.07 ± 3.22
100 mg/L	3.20 ± 2.00	7.87 ± 2.11	26.9 ± 3.12	32.0 ± 3.12

Table 4: Mean growth performance indices of *C. gariepinus* juveniles exposed to lead concentration.

Lead Concentration	Lead Concentration (mg/L)					
	Control	20	40	60	80	100
DFI	1.43 ± 0.09	1.24 ± 0.21	1.27 ± 0.025	1.22 ± 0.15	1.11 ± 0.13	1.10 ± 0.11
MDWG	1.82 ± 0.25	1.78 ± 0.22	1.75 ± 0.55	1.62 ± 0.021	1.35 ± 0.05	1.29 ± 0.045
TFI	38.38 ± 2.08	33.19 ± 4.48	29.12 ± 0.57	30.20 ± 3.27	32.02 ± 2.65	29.82 ± 2.32
PWG	92.65 ± 0.85	74.75 ± 9.65	62.00 ± 7.20	60.95 ± 15.85	59.95 ± 1.95	54.90 ± 17.1
FCR	0.76 ± 0.080	0.67 ± 0.07	0.86 ± 0.07	0.69 ± 0.36	1.03 ± 0.075	0.67 ± 0.08
SGR	1.95 ± 0.015	1.78 ± 0.19	1.64 ± 0.09	1.28 ± 0.33	1.14 ± 0.04	1.255 ± 0.48
% Mortality	0	0	0	10	20	0

Discussion and Conclusion

The LC50 recorded at 96 hrs was 426.5 mg/L with lower and upper limit values (333.4 mg/L and 497.1 mg/L) respectively. This result was higher than the one obtained by Otitoloju [33] who recorded 370.77mg/L of $Pb(NO_3)_2$ against *Tympanotonus fuscatus* (periwinkle) and Falayi and Amatosero [23] who obtained LC50 of 161.07mg/L of anhydrous Lead Chloride ($PbCl_2$) on *Clarias gariepinus* fingerlings. This can be a possible explanation of the low toxicity, species tested, fish age and undefined enzymatic defense response of fishes exposed to the lead metal [26,34]. Toxicity also varied with respect to size of fish and duration of exposure [34]. Death of the fishes during the study could be due to increased heart failure, hypertension, gastric hemorrhage or suffocation as result of prolong exposure to lead metal [35].

Fish mortality was not recorded in all groups during the sub-lethal experimental period except for 60 mg/L and 80 mg/L in first week of experiment with 10% and 20% mortality respectively. These could be due to invasion of bacteria, the test organism been stressed or newly acclimatized to the environment according to USEPA, 2000 [24] and Nubi et al. [36] who suggested that exposure of fish to metals give room for the growth or presence of some microorganisms which may impair the health of the water and thus affect the organisms

The behavioral changes observed in this study include curling of spine, erratic swimming and vertical or motionlessness movement of the fish and finally settling down before death. All these changes may be due to loss of equilibrium at high intoxication, impaired metabolism, nervous disorder which makes the fish to turn upside down and finally died [37]. Mucous secretion on the skin and its coagulation all over the body surface also observed in this study may be due to dysfunction of pituitary endocrine gland under the toxic stress causing changes in the number and area of mucous glands. Similar observations was made by Lal et al. who reported vertical erect orientation of the fish with head up and tail down(motionless movement) before death with exudation of mucous over the body of *Channa punctatus* exposed to lead chloride [38]. TFI and SGR in the fish juveniles cultured in the control tank was significantly higher ($p<0.05$) than those exposed to varying concentrations of lead, the result was in line with the study of Alkahemal-Balawi et al. [39] who recorded a significant increase in SGR. Lead exposure produced a gradual decline in the growth rate of the exposed group relative to controls, although, all the fishes continued to gain weight during the experiment. The findings on effect of varying concentrations of lead chloride on the growth performance of African catfish *Clarias gariepinus* (Burchell, 1822) was studied with respect to its effect on growth performance. The result of this study revealed that the exposure of the test organism to sub-chronic concentrations of lead chloride significantly affected the survival and growth performance, specific growth rate and food conversion efficiency. Therefore, the concentration of lead chloride introduced to the experimental culture tanks negatively impacted the water and the fish in the culture system.

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