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Acute Toxicity and Bioaccumulation Patterns of Lead and Zinc in Juveniles of Clarias Gariepinus

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Abstract: *Acute toxicity of lead and zinc salts (Pb(NO3)2 and ZnCl2) and their bioaccumulation patterns in juveniles of Clarias gariepinus was investigated. ZnCl2 with a 96hr LC50 value of 15.301mg/l was found to be more toxic than Pb(NO3)2 with a 96hr LC50 value of 51.516mg/l. ZnCl2 was bio-accumulated at a faster rate in the gills and flesh of the fish than Pb(NO3)2 . The amount of ZnCl2 and Pb(NO3)2 bio-accumulated reduced as the experiment proceeded. Higher levels of ZnCl2 were recorded in the gills than in the flesh of the juvenile fishes. Essential heavy metals can be more toxic to aquatic organisms than non essential heavy metals when the former is present in high enough concentrations. Water chemistry, speciation and bio-availability of heavy metals in surrounding media are major factors that determine rate of accumulation in aquatic organisms.*
Keywords: *Acute toxicity, Bioaccumulation, Clarias gariepinu, Heavy Metals, Juvenile.*

I. Introduction

The contamination of freshwater with a wide range of pollutants has become a matter of concern over the last few decades [1].

The most common categories of pollutants entering freshwaters include sewage, nutrients, heavy metals, detergents, pathogens, pesticides, heat gases and many others [2] ,with the main route of entry into aquatic ecosystems being through the discharge of metal-laden effluents directly into water bodies or indirectly via drainages and canals [3,4].

Freshwater has become an increasingly scarce resource with an alarming decrease in the pelagic and benthic animal species including fishes inhabiting it. Previous reports of acute toxicity of different heavy metals on both pelagic and benthic aquatic organisms include those of [5] on *Tympanotonus fuscatus*, *Clibanarius africanus* and *Sesarma hurzadi*, [6] and [7] on *Clarias gariepinus*. Information also exists on the bioaccumulation of heavy metals in freshwater fishes. [8] studied the bioaccumulation pattern of zinc in freshwater fish *Channa puncatatus* after chronic exposure, they noted that organ wise distribution of residual zinc revealed that liver is the prime site of accumulation with highest persistence which was followed by kidney, gills and intestine in the test fish throughout the exposure period. [8] observed that higher concentrations of heavy metals (Mn, Cu, Zn, Fe, Cr) occurred in fish tissues than surrounding water in *Clarias gariepinus* from a lake in Ibadan and that generally lower concentrations of heavy metals occurred in gills and bones than intestines and muscles.

The African catfish *Clarias gariepinus* is generally considered to be one of the most important tropical catfish species for aquaculture; it has a pan African distribution ranging from the Nile to West Africa and from Algeria to South Africa. *Clarias gariepinus* is commonly cultured in fish farms in Nigeria and of great economic interest, it is also the most common fresh water fish widely consumed in Nigeria [9], it can therefore be a good model to study responses of fresh water fishes to various environmental pollutants especially heavy metals.

This study aims to determine the acute toxicity and the rate of bioaccumulation of zinc and lead in gills and flesh of juvenile of *C. gariepinus*.

II. Materials And Method

2.1 Test Animals; Description, Source and Acclimatization

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 colour and white on the ventral surface. The head is dorso-ventrally flattened, with skin usually smooth in the young and coarsely granulated in adult.

C.gariepinus juveniles and post juveniles were used in these studies. Live juveniles (4-6 weeks old) and post juveniles (6-8 weeks old) were purchased from local fish farms in Lagos, Nigeria and transported in polythene bags half filled with pond water to holding tanks (length: 45.00cm, height: 34.00cm, bottom diameter: 25.00cm and top diameter: 35.00cm) in the laboratory.

The juveniles and post juveniles were kept in the plastic holding tanks, containing dechlorinated water for a period of seven days to acclimatize to laboratory conditions (28 ± 2^0C, R.H 70±2%) before they were used in the bioassays. The juveniles were fed with fish food (Coppens,) at 3% of body weight twice daily, and the water was changed once every 48hours, aerating it continuously with Bzagdon air pump (model-double type 1200).

2.2 Test Compounds and Preparation

The test compounds used were Zinc as $ZnCl_2.4H_2O$ (molecular weight 136.28g, purity 98%) and Lead as $Pb(NO_3)_2$ (molecular weight 331.21g, purity 99.5%). Both metals were of analytical grade and manufactured by J.T. Baker, a division of Mallinekrodt Baker Inc. Stock solutions of both metals were prepared by taking computed amount (1g) and made up to the desired volume (1 liter) using distilled water to achieve a stock of 1g L^{-1} for each metal. The stock solutions were serially diluted to obtain solutions with desired concentrations selected after range finding experiments.

2.3 General Bioassay Techniques

2.3.1 Bioassay Containers

Circular plastic bowls (volume: 6.00 liters, bottom diameter: 22.00cm and top diameter: 33.00cm) were used as bioassay container.

2.3.2 Selection of Animal for Bioassay

Active juveniles of similar age and size (age: 4-6 weeks old, mean snout to tail length: 8.00-11.50cm, mean weight: 4.80-11.00g) and active post juveniles of similar age and size (age: 6-8 weeks old, mean snout to tail length: 15.00-22.00cm, mean weight: 31.00-55.00g) were taken from holding tanks and randomly assigned to experimental containers.

2.3.3 Quantal Response

Juveniles and post juveniles were taken to be dead if no body movements including the operculum were observed, even when prodded with a blunt glass rod.

2.4 Bioassays

2.4.1 Relative Acute Toxicity of Test Heavy Metals Acting Singly against Juveniles of Clarias gariepinus

Four active juveniles of similar age and size were taken from plastic holding tanks, using a sieve and randomly assigned to bioassay containers already with test media or untreated control. Each treatment was replicated twice, giving a total of 8 juveniles exposed per treatment. Mortality was assessed once every 24hrs for a period of 4 days.

Zn^{2+} against juveniles at; 0.07, 0.11, 0.18, 0.29, 0.40 mMol L^{-1} and untreated control.

Pb^{3+} against juveniles at; 0.06, 0.11, 0.15, 0.21, 0.30mMol L^{-1} and untreated control.

2.4.2 Bioaccumulation studies of Clarias gariepinus Exposed to Sublethal Concentrations of Zinc and Lead acting singly in Semi Static Bioassays.

8 active post juveniles of similar age and size were exposed to sub lethal concentration and untreated control in 2 replicates (4 post juveniles per replicate). These series of bioassays went on for 28 days and the semi static bioassay procedure was adopted in order to avoid drastic changes in concentration of test media via evaporation and excessive reduction in dissolved oxygen level. In the semi static procedure, each test media was changed into a fresh solution of exactly the same concentration of heavy metal salt or untreated control respectively once every four days, transferring the same exposed test animals into the freshly prepared test media over the 28 day period of the experiment. The physico chemical parameters of the test media were measured at set up of experiment and before test media was changed to evaluate change in the respective parameters, especially dissolved oxygen.

At pre-determined time intervals; 7, 14 and 28 days, one live C. gariepinus per replicate,(two per treatment and two from untreated control) ,were randomly selected , dissected and the liver, gills and flesh taken and preserved at 4^0C till further analysis.

C. gariepinus were exposed to sublethal concentrations of test heavy metals in separate experiments as follows:

a. $ZnCl_2.4H_2O$ was tested at:

0.01mMol/l (0.1 of $96hrLC_{50}$)
0.001mMol/l (0.01 of $96hrLC_{50}$)
0.0001mMol/l (0.001 of $96hrLC_{50}$)

b. $Pb(NO_3)_2$ was tested at:

0.02mMol/l (0.1 of $96hrLC_{50}$)
0.002mMol/l (0.01 of $96hrLC_{50}$)
0.0002mMol/l (0.001 of $96hrLC_{50}$

2.4.3 Bioaccumulation Studies of Gill and Flesh of Clarias gariepinus exposed to Sublethal Concentrations of Test Heavy Metals ($ZnCl_2$ and $Pb(NO_3)_2$)

A known weight of gill and flesh was digested separately using nitric acid (10ml), the samples were then heated until all the brown fumes disappeared. The samples were then cooled, and distilled water was added to make up to 50ml in a standard volumetric flask. The solution was filtered and the filtrate was analyzed using the Atomic Absorption Spectrophotometer.

2.5 Statistics

The dose-response data of the acute toxicity test of the test heavy metals against the test animals was analyzed using SPSS (Statistical Package for Social Sciences) model 16.0. Indices of measuring toxicity (LC_5, LC_{50}, LC_{95}) and their 95% confidence limits were employed as follows;

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
LC_5- lethal concentration that will kill 5% of the exposed population of test animals

III. Results

The acute toxicity of $Pb(NO_3)_2$ against juveniles of Clarias gariepinus was found to be less toxic against the fish species with a 96hr LC_{50} value of 0.16mMol L^{-1} as compared to $ZnCl_2$ with a 96hr LC_{50} value of 0.11mMol L^{-1} (Fig 1, 2 and 3).

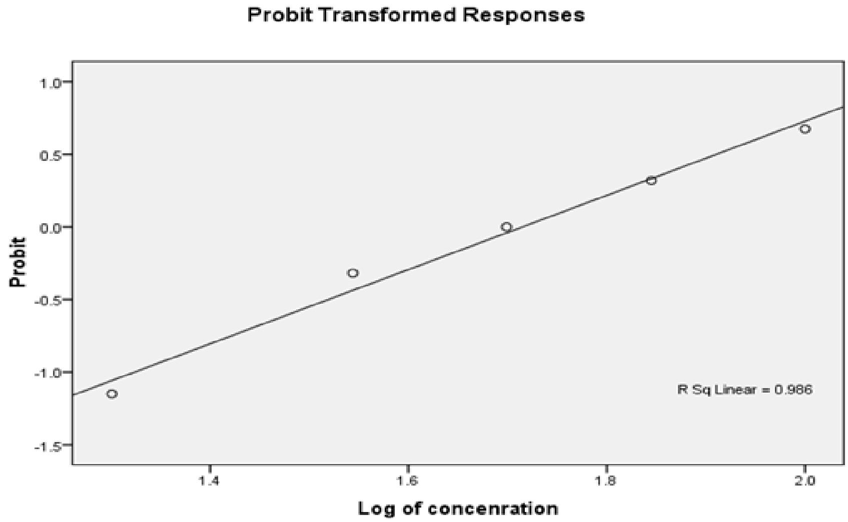

Figure 1: Probit Log-Dose of relative acute toxicity of $Pb(NO_3)_2$ (Based on 96hr dose Mortality data) against Clarias gariepinus.

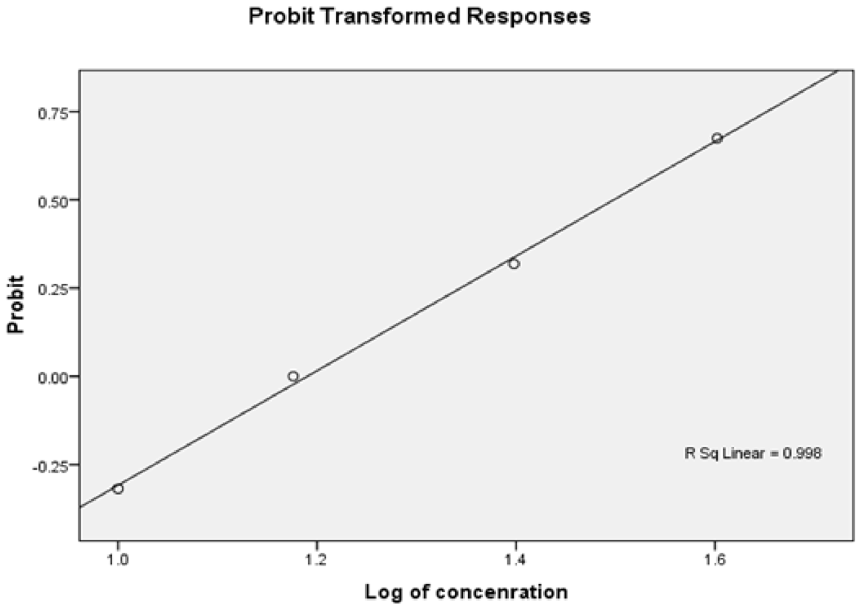

Figure 2: Probit Log-Dose of relative acute toxicity of $ZnCl_2$ (Based on 96hr dose Mortality data) against Clarias gariepinus.

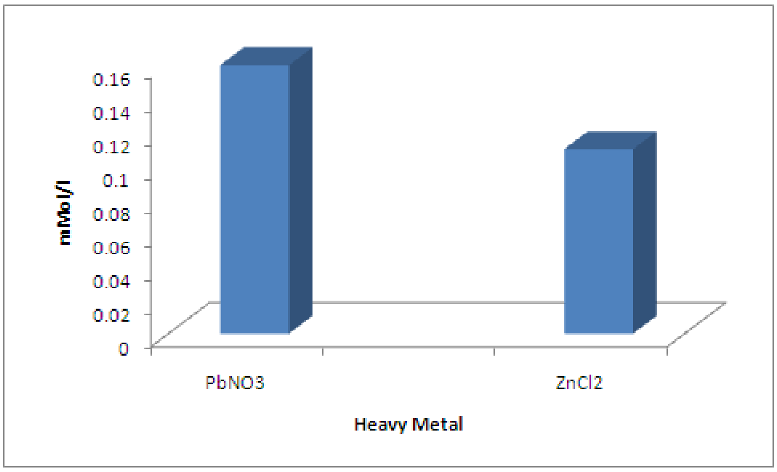

Figure 3: 96hrLC_{50} values of $Pb(NO_3)_2$ and $ZnCl_2$ against Clarias gariepinus juveniles

There were variations in the bioaccumulation of $Pb(NO_3)_2$ in the gills and flesh of Clarias gariepinus. $Pb(NO_3)_2$ was not bio-accumulated in the test organism after the first 7 days of the experiment in all concentrations and control. However, at 14 days, bioaccumulation occurred in the gills and flesh of test organism in all treated groups, with the highest concentration in test organism exposed 0.002mMol L^{-1} (1/100 96hr LC_{50} value). At the conclusion of the experiment (after 28 days), high concentrations of $Pb(NO_3)_2$ was recorded in the gill and flesh (75.39mg kg^{-1} and18.62mg kg^{-1} respectively) of test organism exposed to 0.02mMol L^{-1} (1/10 96hr LC_{50} value) but none in test organisms in the other treated groups including those exposed to 0.002mMol L^{-1} (1/100 96hr LC_{50} value) where representative samples recorded the highest values at 14 days (Table 1).

Bioaccumulation of $ZnCl_2$ in the gills and flesh of Clarias gariepinus followed a defined pattern, as bioaccumulation was recorded at each sampling stage throughout the experimental period in all concentrations and untreated control. Bioaccumulation occurred in the gills and flesh but with lower concentrations recorded in the gills. There was also a general decrease in concentration in both organs over the experimental period with the lowest concentrations recorded after 28 days. The highest concentrations of $ZnCl_2$ was recorded in gills and flesh of organisms exposed to 0.011mMol L^{-1} (1/10 96hr LC_{50} value) at 7 and 28 days and in those exposed to 0.001mMol L^{-1} (1/100 96hr LC_{50} value) at 14 days (Table 2).

Table 1: Concentrations (mg kg^{-1}) of $Pb(NO_3)_2$ in Gill and Flesh of Clarias gariepinus

	7 DAYS		14 DAYS		28 DAYS	
CONCENTRATION	GILL	FLESH	GILL	FLESH	GILL	FLESH
CONTROL	ND	ND	0.22	0.16	2.67	1.72
5.15mg L^{-1} (1\10	ND	ND	0.12	0.05	75.39	18.62
0.52mg L^{-1} (1\100)	ND	ND	1.06	1.20	ND	ND
0.052mg L^{-1}(1\1000)	ND	ND	0.13	0.07	ND	ND

ND: Not Detected.

Table 2: Concentrations (mg kg^{-1}) of $ZnCl_2$ in Gill and Flesh of Clarias gariepinus

	7 DAYS		14 DAYS		28 DAYS	
CONCENTRATION	GILL	FLESH	GILL	FLESH	GILL	FLESH
CONTROL	75.96	31.80	15.21	7.66	0.26	0.13
1.53mg L^{-1} (1\10)	86.89	67.77	12.57	1.41	0.65	0.12
0.15mg L^{-1}(1\100)	62.12	42.35	18.22	6.24	0.23	0.91
0.015mgL^{-1}(1\1000)	68.69	36.00	21.21	7.48	0.32	0.13

IV. Disscussion And Conclusion

Acute toxicity results showed that lead nitrate ($Pb(NO_3)_2$, a non essential heavy metal was less toxic when compared to zinc chloride ($ZnCl_{2)}$, an essential heavy metal, against juveniles of Clarias gariepinus. This is in agreement with the findings of [10] who recorded 96hr LC_{50} values of 83.10mg L^{-1} and 370.77mg L^{-1} for $ZnCO_3$ and $Pb(NO_3)_2$ respectively against Tympanotonus fuscatus (periwinkle). [6] also recorded a 96hr LC_{50} value of 72.92mg L^{-1} for $Pb(NO_3)_2$ against C. gariepinus while [11] obtained 96hr LC_{50} value of 7.5mg L^{-1} for $ZnCl_2$ against catfish (Heteropneustes fossilis) .These previous investigations have shown that some essential heavy metals can be more toxic to aquatic organisms than their non essential counterparts when present in high enough concentrations.

Pattern of bioaccumulation results obtained from this study, showed that zinc ($ZnCl_2$) was bio-accumulated at a faster rate than lead ($Pb(NO_3)_2$) in the gills and flesh (muscle) of the juvenile fishes. [12] carried out a study on heavy metal accumulated in tissues of edible fish species in upper stretch of Gangetic West Bengal and reported that bioaccumulation trends in the tissues of the fishes was Zinc> Chromium> Copper> Cadmium> Lead. The slow rate of absorption of Lead by the juvenile fishes can be attributed to limited bio-availabilty of Lead in the exposure media. Bio-availability of $Pb(NO_3)_2$ has been reported [13] to be affected by water hardness being more bio-available in soft water (10 mg L^{-1}) and less bio-available in hard water (340 mg L^{-1}). The water hardness of exposure media used in this study ranged between 40-130mg L^{-1}.

Higher concentrations of both heavy metals were detected in the gills than in flesh of the juvenile fishes, across all concentrations during the exposure period. Earlier, [14] who studied heavy metals concentrations in certain tissues of five commercially important fishes from El-Mex Bay, Alexandria, Egypt, observed that lower concentrations of all heavy metals (including lead and zinc) were found in the muscles as compared to gills and went on to explain that this observed trend was due to the physiological roles of the various tissues, where target tissues of heavy metals are the metabolically active ones. The gills are highly metabolic active tissues when compared to the flesh. This is due to the major role they play in gaseous exchange in aquatic organisms, which also makes them a target site for active uptake of any pollutant that may be present in the water. Concentrations of $ZnCl_2$ in the gills and flesh of the juveniles reduced with increased exposure period, with highest concentrations recorded at 7 days and lowest at 28 days. Similar trend was reported by [8] from their study of bioaccumulation pattern of zinc in freshwater fish Channa puncatus after chronic exposure. They reported lower concentration of zinc in the gill at the end of the exposure period (45^{th} day) as compared to levels found at 30 days, and attributed this trend to induction of regulatory process and biochemical defense mechanisms. Zinc is an essential element which can be regulated by fish over wide range concentrations [15].

Essential heavy metals are commonly detected in water bodies and as shown by this study, can be more toxic than the non essential counterparts; hence more attention should be given to essential heavy metals when setting effluent limitation standards for industries and facilities generating toxic wastes.

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