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Lethal Influence of Zinc Exposure to *Clarias gariepinus* (Burchell, 1822, Pisces, Clariidae)

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Abstract: Acute toxicity of zinc ($ZnSO_4.7H2O$) on *Clarias gariepinus* (Burchell, 1822) was investigated using toxicity scale of 96hrLC_{50} values and the quantal response determined by the statistical Probit Analysis Method. Fish species displayed different mortality responses to the varying concentrations of zinc studied (50, 60, 70, 80, 100 and 120mg/l) 96-hour LC_{50} value for *Clarias gariepinus* was found to be 78.178mg/l. The lower and upper confidence limits for the LC_{50} were 86.313mg/l and 71.035mg/l, respectively. While asserting the fact that zinc is an important constituent in municipal wastes discharged into freshwater and marine, there is clear indication from our results of the necessity to control the use of this metal. Further toxicity testing approaches on fish will be very useful in assessing possible ecological risk of heavy metals.

Key word: LC_{50} % Toxicity % Zinc % Bioassay % Pisces % Catfish

INTRODUCTION

Heavy metals are important environmental pollutants. Metal contamination of the environment results both from natural sources and industrial activities. Metals in soil and water may enter the food cycle with an additional contribution from air [1].

Various physical parameters such as temperature, pH, water hardness, salinity and organic matter can influence the toxicity of metals in solution [2-4]. Also, the lack of natural elimination process for metals aggravates the situation [5]. As a result, metals shift from one compartment within the aquatic environment to another including the biota often with detrimental effects, through sufficient bioaccumulation. Food chain transfer also increases toxicological risk in humans [6, 7]. Bioconcentration or bioaccumulation of heavy metals over time in aquatic ecosystems has been reported by Alabaster and Lloyd [8], Otitoloju [9], Friberg *et al.* [10], Fischer [11] and Groundwork [12] Similar trend has been reported in Anambra River by Igwilo *et al*. [13]) working with water and bank sediments and Obodo [14] in fish (*Clarias sp*.). According to Merian [15], heavy metal pollution is one of the five major types of toxic pollutants commonly present in surface and ground waters. The environmental pollutants tend to accumulate in organisms and become persistent because of their chemical stability or poor biodegradability and that they are readily soluble and therefore environmentally mobile, forming one of the major contributors to the pollution of natural aquatic ecosystems [16, 17].

There are a number of studies carried out on the toxicity of zinc sulphate [1]. Zinc is an essential mineral in cellular metabolism. It is a cofactor for the activity and folding of proteins [18]. Because of the pleiotopic effects of zinc on every aspect of cell physiology, zinc deficiency or excessive rise in its cellular concentration, can have catastrophic consequences and are linked to major patho-physiologies [18]. However, its prolonged and excessive intake may lead to toxic effect such as carcinogenesis, mutagenesis and teratogenesis as a result of its bioaccumulation [19, 20].

Although the toxicity of zinc to several fish species has been documented, the toxicity of this metal is not well known for all aquatic organisms [21]. Alsop *et al*. [22] examined chronic waterborne zinc exposure and the consequences of zinc acclimation on the gill/zinc interactions of rainbow trout in hard and soft water. They found that all zinc-exposed trout had been acclimated to the metal, as seen by an increase of LC_{50} by 2.2 to 3.9 times over that of control fish.

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Bieniarz *et al*. [23] examined reproduction of fish in conditions disadvantageously altered with the salts of zinc and copper. They report that elevated Zn levels in water affected the reproduction of fish.

Obiakor *et al.* [24] evaluated the genotoxicity of copper, zinc and their binary mixture on *Synodontis clarias* and *Tilapia nilotica* using micronucleus test in fish genome. The authors documented elevated micronuclei frequency following exposure to highest and lowest concentration of zinc on the fish species at 96-hrLC_{50}. The authors went further to report that the studied concentration to could not result in mortality but possessed genotoxic effect (EC_{50}).

The toxic characteristics of zinc depend largely on the physicochemical characteristics of water. The principle factors effecting toxicity are the hardness and the pH of water. Decreasing hardness and the increasing pH increases the lethality of dissolved zinc [25-27]. It has also been established that there are marked differences in zinc sensitivity between various species. For instance the order Perciformes was found to be the most resistant and Clupeiformes the most sensitive [28]. Salmonoid fishes were found to be highly susceptible to zinc intoxication in soft water and the 96-hr LC_{50} value for the rainbow trout was found to be 0.066 mg/l [27]. However, this value goes up to 2.5 to 4.71mg/l in very hard water [29]. Non-salmonoid fish on the other hand are known to be less sensitive than salmonoids. 96-h LC_{50} for common carp in soft water was found to be 3.12 mg/l [30]. Seven-day LC_{50} values of fish species such as *Gobio gobio, Abramis brama, Rutilus rutilus, Cyprius carpio, Scardinus erytrophtalmus* and *Perca fluviatilis* were found to range between 8 to 17 mg/l [31].

This study was therefore, designed to investigate the toxicity of zinc on the available local and aquacultural test fish species, *Clarias gariepinus* (Burchell, 1822) by determination of 96-hrLC_{50} values at different concentrations of the toxicants.

MATERIALS AND METHODS

Live adult *Clarias gariepinus* were obtained from a commercial hatchery and brought to the laboratory within 30 minutes in plastic bags with sufficient air, irrespective of gender. The plastic bags were placed into the maintenance aquarium for 30-35 minutes for acclimatization. Then the bags were cut open and the fish were allowed to swim into the aquarium water. Test chambers were glass aquaria of about 40-litre capacity.

The aquaria were aerated with a central system for a period of 48 hours and the fish were exposed to 15 days conditioning period at room temperature. The fish were fed with commercial feed food twice a day during this period. Acclimated fish were not fed 24-h before the start of the tests. Care was taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started.

Chemically pure salt of zinc sulphate ($ZnSo_4.7H_2O$) dissolved in distilled water was used as toxicant. The test organisms were subjected to different concentrations (50, 60, 70, 80, 100 and 120mg/l) of the zinc sulphate ($ZnSO_4$.7H2O). For the acute bioassay tests, 20 fish were used per concentration. The containers were not aerated at the dosing time. The amount of zinc sulphate to be added in each aquarium was calculated after the volume of each aquarium was accurately determined.

There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the zinc sulphate; keeping all other conditions constant. The mortality rate in the control group did not exceed 10% and 90% of the fish looked healthy throughout the experiment.

Water quality parameters (temperature, dissolved oxygen (DO), $CaCO_3$ hardness and pH) using in the aquaria were periodically determined before the bioassay tests. The water temperature was kept 27 ± 2.0°C. In addition, the experimental medium was aerated in order to keep the amount of oxygen not less than 6mg/l.

All experiments were carried out for a period of 96 hours. The number of dead fish were counted every 24 hours and removed from the aquaria as soon as possible. The mortality rate was determined at the end of the 96th hour. No food was given to the fish during the experiments.

The bioassay system was as described in standardized methods [32, 33]. Assessment of quantal response (mortality) following toxicity of zinc on the test species, *Clarias gariepinus* (Burchell, 1822) was determined by the use of Finney's Probit Analysis LC_{50} Determination Method [34]. The mortality response of the fish species was taken to be when the animals sank down to the bottom of the containers and became motionless, rate determined at the end of the 96^{th} hour.

RESULTS

The selected physicochemical characteristic variables maintained in the aquaria for the toxicity testing of the fish species are shown in Table 1, while Table 2 gives the relationship between the different exposure

Table 1: The temperature, pH, hardness and oxygen demand (DO) of the aquaria in zinc toxicity tests

Concentration (mg/l)	Temperature (°C)	pH	Hardness (mg/l)	Dissolved Oxygen (mg/l)
50	27.2	7.55	215	6.0
60	26.5	7.80	200	6.0
70	27.1	7.70	215	6.5
80	28.2	7.54	230	6.3
100	25.7	7.8	225	6.5
120	27.1	7.85	235	6.2
Control group	27.0	7.70	220	6.4

Table 2: The relationship between the zinc concentration and the mortality rate of *Clarias gariepinus* (Burchell, 1822) for the 96-hour exposure

Conc (mg/l)	Test Animals	24hr	48hr	72hr	96hr
50	20	0	0	1	1
60	20	1	2	3	5
70	20	4	5	6	8
80	20	7	7	10	13
100	20	8	11	14	14
120	20	10	13	14	17
Control	20	0	0	0	0

Table 3: Parameter Estimates for the Probit Analysis

						95% Confidence Interval	
	Parameter	Estimate	Std. Error	Z	Sig.	Lower Bound	Upper Bound
PROBIT[a]	Concentration	6.317	1.107	5.706	.000	4.147	8.487
	Intercept	-11.959	2.094	-5.711	.000	-14.053	-9.865

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

Theoretical Spontaneous Response Rate = 0.000

Table 4: Estimated lethal concentration values and confidence limits

		95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)a		
	Probability	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	0.01	33.483	21.314	42.057	1.525	1.329	1.624
	0.02	36.981	24.752	45.368	1.568	1.394	1.657
	0.03	39.387	27.208	47.615	1.595	1.435	1.678
	0.04	41.300	29.210	49.386	1.616	1.466	1.694
	0.05 (LC_5)	42.925	30.943	50.882	1.633	1.491	1.707
	0.06	44.357	32.495	52.197	1.647	1.512	1.718
	0.07	45.653	33.916	53.383	1.659	1.530	1.727
	0.08	46.845	35.239	54.472	1.671	1.547	1.736
	0.09	47.956	36.483	55.486	1.681	1.562	1.744
	0.1	49.002	37.665	56.441	1.690	1.576	1.752
	0.15	53.582	42.936	60.631	1.729	1.633	1.783
	0.2	57.525	47.573	64.282	1.760	1.677	1.808
	0.25	61.138	51.860	67.703	1.786	1.715	1.831
	0.3	64.576	55.931	71.066	1.810	1.748	1.852
	0.35	67.934	59.855	74.497	1.832	1.777	1.872
	0.4	71.282	63.667	78.108	1.853	1.804	1.893
	0.45	74.678	67.388	82.009	1.873	1.829	1.914
	0.5 (LC_{50})	78.178	71.035	86.313	1.893	1.851	1.936
	0.9	124.724	107.747	164.120	2.096	2.032	2.215
	0.91	127.445	109.592	169.449	2.105	2.040	2.229
	0.92	130.468	111.624	175.447	2.116	2.048	2.244
	0.93	133.875	113.893	182.302	2.127	2.056	2.261
	0.95(LC_{95})	142.384	119.473	199.851	2.153	2.077	2.301

a. Logarithm base = 10

Table 5: 96hrs acute toxicity of Zinc on *Clarias gariepinus* (Burchell, 1822)

Test Animals	96hrs LC_{50} (mg/l)	96hr LC_5 (mg/l)	96hrs LC_{95} (mg/l)	S.E
Clarias gariepinus	78.178 (86.313-71.035)	42.925 (50.882 -30.943)	142.384 (199.851-119.473)	2.094

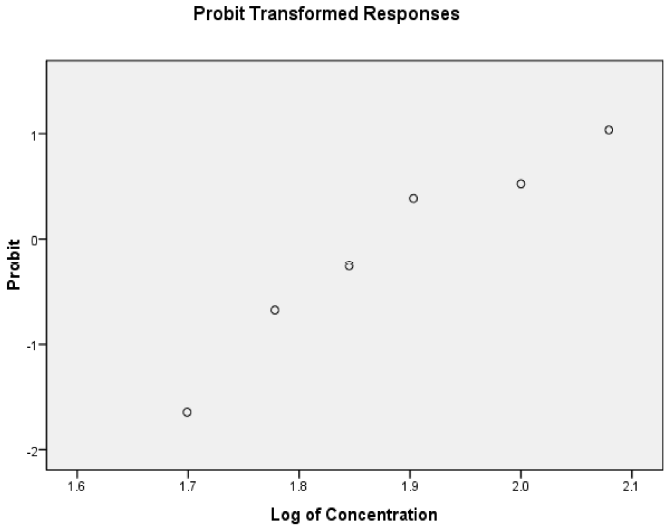

Fig. 1: Probit line graph of acute toxicity of Zinc on Catfish (*Clarias gariepinus*, Burchell, 1822)

concentrations and the mortality response rate of *Clarias gariepinus* (Burchell, 1822). The Probit parametric estimates and the obtained results for the acute static 96-hour toxicity estimated lethal concentration values and their confidence limits are shown in Tables 3 and 4, respectively. Figure 1 displays the Probit line graph of acute toxicity of zinc on *Clarias gariepinus* with Table 5 showing the 96-hrLC_{50} value for the fish species.

DISCUSSION

Finney's Probit Analysis gave 96- hour LC_{50} value for *Clarias gariepinus* (Burchell, 1822) exposed to different zinc concentrations as 78.178 mg/l. Control mortality was zero. 95% lower and upper confidence limits for the LC_{50} values were 86.313 and 71.035 mg/l, respectively. These toxic effects increased, as the dose was increased (Table 2 and 4 and Figure 1).

The observed differential toxicity of heavy metals can be attributed to several factors such as the type of heavy metal tested, solubility of the compound, predominant ions in test solution, physico –chemical characteristics of the test solution and the mechanism(s) of action of the different metals. All of these factors determine the availability, including the penetrability of the metals into the test animals and hence, their toxicity. Khangarot, while working on the toxicity testing of *Poecilia reticulata* in hard water (260 mgCaCO3 / l) [35] found the 96-h LC_{50} value as 55 mg/l [35]. This result is slightly lower to our observations in *Clarias gariepinus* (78.178mg/l- Table 4 and 5). The difference could be attributed to variations in the hardness, pH value and the temperature of water (Table 1), which had earlier been documented [25-27]. Consequently, the observations could be explained by the species of fish employed in the work, which supports the work of Otitoloju [9] and Obiakor *et al*. [24]. The authors reported differential toxicities following exposures of different species of fish to varying concentrations of heavy metals.

However, age of the fish could play significant role in response to a given contaminant or toxicant in a given medium [24]. Spehar [36], investigated cadmium and zinc toxicity to flagfish, *Jardanelle floridae*. The 96-hr LC_5 values for cadmium and zinc detected by the author to juvenile flagfish were 2.5 mg/l and 1.5 mg/l respectively, which were lower than the value (Zn) recorded in our toxicity data (Table 4 and 5) for similar 96-hrLC_5.

Gomez *et al*. [37], studied zinc toxicity in the fish *Cnesteron decemmaculatus* in the Parana River and Rio de La Plata Estuary. They found 24-h LC_{50} 93.2 mg/l for *Cnesteron decemmaculatus* and contaminant load in the natural waters tested was similar at both sites, with Zn concentration 40 and 44 mg/l, respectively.

CONCLUSION AND RECOMMENDATION

Lethal effects of zinc have been widely reported for different aquatic organisms and exposure routes. Since this metal is an important constituent in municipal wastes discharged into freshwater and marine, there is need to regulate and control the use of this heavy metal (zinc) to avert possible ecological damage. Further work with toxicity testing methods directly on fish both in laboratory and in its corresponding natural setting will be very useful in assessing possible ecological risk of heavy metals.

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