Online Journal of Animal and Feed Research
Volume 1, Issue 4: 139-143 (2011) ISSN 2228-7701

TOXICOLOGICAL STUDY OF SINGLE ACTION OF ZINC ON TILAPIA SPECIES (*Oreochromis Niloticus*)

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ORIGINAL ARTICLE

ABSTRACT: *Lethal effects of zinc sulphate ($ZnSO_4.H_2O$), a widespread environmental pollutant was evaluated following exposure at different concentrations of the toxicant to Tilapia species, Oreochromis niloticus based on toxicity index of 96 hrs LC_{50} values. The obtained results were analyzed by the Finney's Probit Analysis LC_{50} Method and 96 hrs LC_{50} value for Oreochromis niloticus was found to be 72.431 mg/l. The work further documented the lower and upper confidence limits for the LC_{50} to be 77.288 mg/l and 67.682 mg/l, respectively. The research showed zinc to be lethal to the test organism and recommends proactive control measures to be put in place to avert possible disaster of zinc poisoning.*

Keywords: *Lethality, heavy metal, single action, pisces, 96 hrs LC_{50}.*

INTRODUCTION

Essential heavy metals are generally considered to be less toxic than non-essential metals (Batley, 1993). Metal such as zinc exhibit aquatic toxicity when present above recommended standard in that they can contaminate surface and groundwater bodies, soil, plant, aquatic life, and man, through bioaccumulation.

There are a numerous studies carried out on the toxicity of zinc sulphate. Malik et al. (1998) studied the effect of zinc toxicity on biochemical composition of muscle and liver of murrel (*Channa punctatus*). Selected specimens of murrel were exposed to a sub-lethal zinc concentration. They had reported that the zinc exposure produced marked changes in the chemical composition of liver and muscle tissues. The metabolism of the fish decreased with the time of exposure and there was a decline in the calorific value of lipid, protein, and glycogen in muscle and liver.

Uviovo and Beatty (1979) investigated effects of chronic exposure to zinc on reproduction in the guppy (*Poecilia reticulata*). They concluded that zinc had no effect on brood time, but the total number of young produced and brood size fell with increasing zinc concentrations (0.36 - 1.7 g Zn/l); dead young with abnormalities were not included in brood size and live young were significantly smaller in the presence of zinc. According to the results, this change in size of young at birth provides a very sensitive parameter of zinc toxicity.

Khunyakari et al. (2001) investigated toxicity of nickel, copper, and zinc in *Poecilia reticulata.* Heavy metal exposure caused increased mucus like secretion over gills, excessive excretion, anorexia and increased fin movement. Copper was found to be the most toxic followed by zinc and nickel.

Pierson (1981) studied effects of chronic zinc exposure on the growth, sexual maturity, reproduction and bioaccumulation of the guppy, *Poecilia reticulata*. The zinc burden increased from 56 to 70 days during the onset of pregnancy. The experiments show that uptake of zinc during logarithmic growth and pregnancy indicates that females actively transfer zinc to the embryo. When guppies exposed to 0.607 mg/l zinc for 134 days, the wet weight of females was reduced by 40%.

It is generally accepted that metal accumulation in tissues of aquatic animals is dependent upon the exposure concentration and period, as well as some factors, such as salinity, temperature, interacting agents and metabolic activities of tissue concerned (Shukla et al., 2007). The mobility as well as the toxicity of zinc to aquatic species is enhanced by the physicochemical characteristics of inhabiting medium, such as temperature, hardness, and pH. Karakoc and Dincer (2003) reported highest accumulation of zinc in kidney tissue of *Oreochromis niloticus* at 15 ^{0}C and 30 ^{0}C for different concentrations, which is followed by gills and liver. In all tissues, zinc accumulation increases with increasing temperature.

To cite this paper: Ezeonyejiaku, C.D. and Obiakor, M.O. 2011. Toxicological study of single action of zinc on Tilapia species (*Oreochromis niloticus*). *Online J. Anim. Feed Res.*, 1(4): 139-143.
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Monitoring pollution limits of heavy metals is important in the aquatic ecosystems, so that approximate measures of the potential hazards can be attained. These measures should give an estimation of the type of effects that could be expected after exposure to heavy metals. Thus, the aim of the research was to assess the mortality response of *Oreochromis niloticus* following different concentrations of zinc exposures using the toxicity scale of 96 hr LC_{50} value.

MATERIALS AND METHODS

Adult *Oreochromis niloticus* were obtained from a commercial hatchery and brought to the laboratory within 30 min in plastic bags with sufficient air. The plastic bags were placed into the maintenance aquarium for 30 - 35 min 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 liter 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 DANA feed food at least once 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.

Zinc sulphate ($ZnSO_4.H_2O$) in tap water was used in the static bioassays. The test organisms were subjected to different concentrations (50, 60, 70, 80, 100 and 120 mg/l) of the zinc sulphate ($ZnSO_4.H_2O$). 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.

The experiment was carried out in a series and 140 *Oreochromis niloticus* species were used. This species was selected for static bioassays because it can be easily cultured and raised under laboratory conditions through a complete life cycle, and it is one of the standard test species used for laboratory toxicity studies.

Water quality parameters (temperature, dissolved oxygen (DO), $CaCO_3$ hardness and pH) using in the aquaria were periodically determined before the bioassay tests (Table 1). The water temperature was kept 27 ± 2.00 ^{0}C. Also the experimental medium was aerated in order to keep the amount of oxygen not less than 6 mg/l.

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

Concentration (mg/l)	Temperature (^{0}C)	pH	Hardness (mg/l)	Dissolved Oxygen (mg/l)
50	27.3	7.50	225	6.8
60	28.2	7.65	225	6.0
70	28.1	7.70	215	6.7
80	27.2	7.70	220	6.0
100	25.9	7.63	225	6.2
120	26.8	7.70	235	6.5
Control group	27.7	7.60	230	6.3

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 experiments were carried out with static acute experimental method. In this method the experimental solution and the samples (i.e. fish) are put in a suitable experimental cell (i.e. aquarium) and kept like that for a certain period. Since the decreased amount of oxygen and increased metabolic waste become a problem in long term experiments, the duration of such experiments are usually kept at 96 hours or less. The bioassay system was as described in standardized methods (OECD, 1993; APHA/AWWA/WPCP, 1971).

Assessment of mortality response on zinc exposures of the test species, *Oreochromis niloticus* was determined by the use of Finney's Probit Analysis LC_{50} Determination Method (Finney, 1971). 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 96th hour.

RESULTS

The toxicity data revealed 96 hour LC_{50} value for *Oreochromis niloticus* exposed to different zinc concentrations as 72.431. Theoretical Spontaneous Response Rate was zero (for control experiment). Around 95% lower and upper

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confidence limits for the LC_{50} values were 77.288 mg/l and 67.682 mg/l, respectively. These toxic effects increased, as the dose was increased (Table 2 and 4 and Figure 1).

The parameter estimates and the obtained results for the acute 96 hrs toxicity estimated lethal concentration values and their confidence limits are shown in Table 3 and 4, respectively. Figure 1 displays the Probit line graph of acute toxicity of zinc on *Oreochromis niloticus* with Table 5 showing the 96 hrs LC_{50} value for the Tilapia species.

Table 2 - The relationship between the zinc concentration and the mortality rate of *Oreochromis niloticus* for the 96-hour exposure

Conc(Mg/l)	Test Animals	24 hrs	48 hrs	72 hrs	96 hrs
50	20	0	2	2	3
60	20	3	4	5	7
70	20	2	7	7	9
80	20	5	7	10	11
100	20	10	12	13	16
120	20	13	13	15	19
Control	20	0	0	0	0

Table 3 - Parameter Estimates for the Probit Analysis

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PROBIT[a]	Concentration	6.390	0.800	7.987	0.000	4.822	7.958
	Intercept	-11.884	1.499	-7.930	0.000	-13.383	-10.386

[a] PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.); Theoretical Spontaneous Response Rate = 0.000

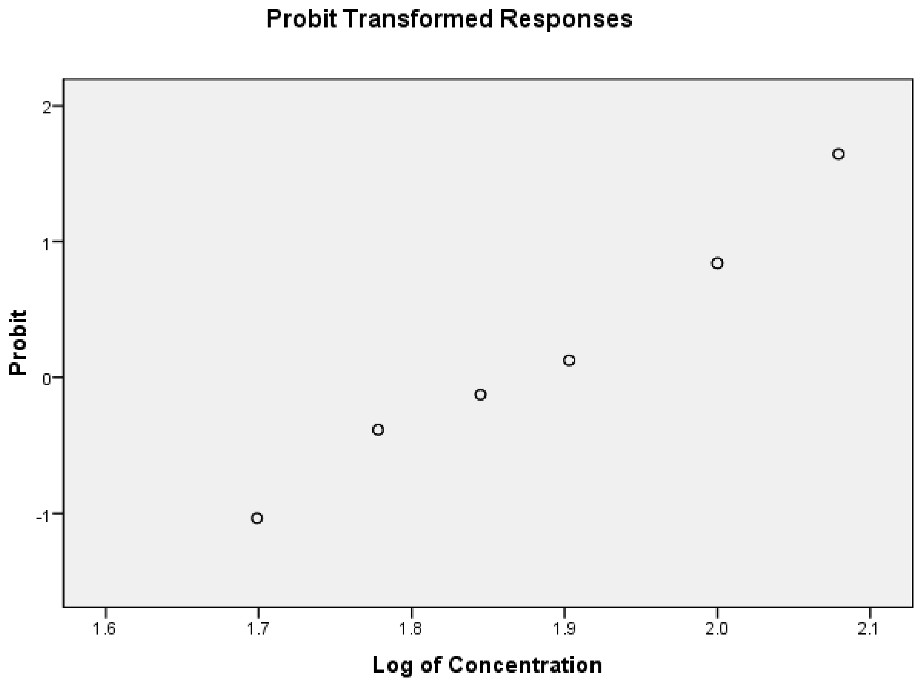

Fig. 1 - Probit line graph of acute toxicity of Zinc on Tilapia (*Oreochromis niloticus*)

DISCUSSION

Gül et al. (2009) found the 96 hrs LC_{50} value for Guppies (*Poecilia reticulate* P., 1859) to be 30.826 mg/l. similarly, Williams and Holdway (2000) examined that the effects of pulse-exposed cadmium and zinc on embryo hatchability larval development and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatis*). The LC_{50} values of zinc were found to be 0.51, 0.56, and 1.57 mg/l for 24-h, 3-4-day, and 9-10- day-old larval rainbow fish.

Herrera et al. (1995), reported the 96 hrs LC_{50} value of $ZnCl_2$ on *Chanos chanos* as 25 mg/l. Finlayson and Verrue (1982), investigated toxicities of copper, zinc and cadmium mixture to juvenile *chinook salmon*. They found that median lethal concentrations during 4 days (96-hour LC_{50} values) were most variable for zinc (39 to 122 mg/l).

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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	31.321	23.448	37.467	1.496	1.370	1.574
	0.02	34.554	26.677	40.590	1.539	1.426	1.608
	0.03	36.777	28.948	42.711	1.566	1.462	1.631
	0.04	38.542	30.780	44.384	1.586	1.488	1.647
	0.05 (LC_5)	**40.040**	**32.352**	**45.797**	**1.602**	**1.510**	**1.661**
	0.06	41.361	33.752	47.038	1.617	1.528	1.672
	0.07	42.556	35.027	48.156	1.629	1.544	1.683
	0.08	43.654	36.207	49.182	1.640	1.559	1.692
	0.09	44.678	37.314	50.137	1.650	1.572	1.700
	0.10	45.641	38.360	51.034	1.659	1.584	1.708
	0.15	49.856	42.991	54.955	1.698	1.633	1.740
	0.20	53.482	47.026	58.336	1.728	1.672	1.766
	0.25	56.802	50.740	61.459	1.754	1.705	1.789
	0.30	59.959	54.268	64.473	1.778	1.735	1.809
	0.35	63.041	57.687	67.480	1.800	1.761	1.829
	0.40	66.111	61.041	70.564	1.820	1.786	1.849
	0.45	69.224	64.365	73.805	1.840	1.809	1.868
	0.5 (LC_{50})	**72.431**	**67.682**	**77.288**	**1.860**	**1.830**	**1.888**
	0.90	114.946	103.570	134.957	2.060	2.015	2.130
	0.91	117.425	105.435	138.728	2.070	2.023	2.142
	0.92	120.178	107.493	142.952	2.080	2.031	2.155
	0.93	123.280	109.795	147.755	2.091	2.041	2.170
	0.94	126.840	112.417	153.321	2.103	2.051	2.186
	0.95 (LC_{95})	**131.025**	**115.474**	**159.938**	**2.117**	**2.062**	**2.204**

[a] Logarithm base = 10

Table 5 – 96 hrs acute toxicity of Zinc on *Oreochromis niloticus*

Test Animals	96 hrs LC_{50} (mg/l)	96 hrs LC_5 (mg/l)	96 hrs LC_{95} (mg/l)	S.E
Oreochromis niloticus	72.431 (77.288-67.682)	40.040 (45.797-32.352)	131.025 (159.938-115.474)	1.499

DISCUSSION

Gül et al. (2009) found the 96 hrs LC_{50} value for Guppies (*Poecilia reticulate* P., 1859) to be 30.826 mg/l. similarly, Williams and Holdway (2000) examined that the effects of pulse-exposed cadmium and zinc on embryo hatchability larval development and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatis*). The LC_{50} values of zinc were found to be 0.51, 0.56, and 1.57 mg/l for 24-h, 3-4-day, and 9-10- day-old larval rainbow fish.

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Khangarot et al. (1981) examined toxicity of interaction zinc-nickel, copper-nickel and zinc-nickel-copper to a freshwater teleost, *Lebistes reticulatus*. The 48-hour median lethal concentrations (LC_{50}) for individual salts were 75 mg/l for Zn^{2+}, 37 mg/l for Ni^{2+} and 2.5 mg/l for Cu^{2+}. Their experiment showed that in the Zn^{2+} – Ni^{2+} mixture, when Ni^{2+} was more in proportion, the toxicity was more than additive. The results indicate that heavy metallic mixtures would pose a greater toxicological danger to fish than the respective individual metals.

Zinc lethality has been widely described and its mixture with certain metals further documented to be synergistic. Joint action toxicity testing with metals involving zinc should be further carried out on different sensitive aquatic organisms to measure deviation from its single component toxicity, since these heavy metals are rarely/ or do not occur in isolation within the environment of influence.

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