EFFECT OF ZINC ON THE DISTRIBUTION OF MAJOR CATIONS AND ANIONS IN *Clarias gariepinus* FROM EBONYI RIVER, SOUTHEAST NIGERIA

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ABSTRACT

A 96 hour bioassay was conducted to evaluate the effect of zinc on the distribution of major cations and anions in the muscle of the catfish Clarias gariepinus sub-adults at 13.88 ppm, 26.75 ppm, 53.5 ppm, 107 ppm and 214 ppm. The 96 h LC_{50} obtained in this study was 26.62 ppm and threshold value of 20.30 ppm. Statistical analysis of physicochemical parameters showed that dissolved oxygen, carbon (IV) oxide, total alkalinity, temperature and pH did not vary significantly at ($P < 0.05$) from the control. Mortality increased with increased concentration of zinc sulphate. Quantal response included initial increase in opercula movement, erratic swimming, gasping for air, curvature of body, loss of balance, quietness and finally death. The increase in opercula movement was due to possible inhibition action of the toxicant on respiration as well as possible depletion of A.T.P. which may reduce the energy available for respiration. This depletion could have resulted in exhaustion and ultimately, death. All cations and anions (Mg^{2+}, Ca^{2+} Na^{+}, K^{+} and Cl^{-}) showed highest uptake at 214 ppm toxicant (Zn) concentration while the lowest muscular uptake was observed at 13.88 ppm Zn concentration during the 96 hr exposure period, with a threshold concentration of 20.30 ppm. There was however, no total inhibition of ionic uptake (both cations and anions) during the study period.

Keywords: harmful, osmoregulation, physicochemical, trace element, uptake

INTRODUCTION

Zinc occurs in the environment as an essential trace element in air, soil, water and all living organisms. It is found in natural water supplies, but the content may be increased if the water flows through galvanized, copper or plastic pipes. Sea foods, meat whole grains, dairy products, nuts and legumes are high in zinc content. Geographic variation in zinc tissue level may be due to the reduction of zinc by the refining of grains (Reed *et al*., 1980). It occurs widely in nature as sulphide carbonate and hydrated silicate ores, frequently accompanied by other metals, mainly iron and cadmium (Leena *et al.*, 2012).

Zinc is used to galvanize brass and some alloys and other compounds commonly used in manufacturing industries (Alabaster and Lloyd, 1982). Consequently, it can be an important pollutant from both mining and other industrial processes. Allochthonous introduction of zinc into the aquatic environment is from natural sources such as weathering of rocks and from anthropogenic sources through industrial and domestic effluents (Nriagu, 1989), with the resultant uptake in tissues of aquatic organisms. Bioaccumulation of Zn among other metals has been reported in fin fishes (Ekwu, 2010), while Mohamed (2008) reported Histopathological Alterations in tissues by the metal, having adverse effects on the hepatic distribution of other trace metals in *Oreochromis niloticus* and *Lates niloticus*. This report was corroborated by Bashir *et al*., (2013) for fishes in the coastal waters of Kapar and Mersing, Malaysia. Eneji *et al*. (2011) reported that Zn is among the highest bioaccumulated metals in tissues of organisms with bioaccumulation factors of 229 and 226 for *Tilapia zilli* and *Clarias gariepinus*, respectively.

High concentrations of zinc especially in high alkalinity waters have been reported to produce adverse effects on growth and survival in fishes (Reed *et al.*, 1980). A similar observation was reported by Tuurala and Soivio (1982). Zinc amasses in fish gills (Crespo *et al.*, 1979) and prompts depressing effect on respiration resulting in death by hypoxia (Burton *et al*., 1972). Zinc contamination also makes changes in air circulation and heart structure and function (Hughes and Tort, 1985). Zinc has also been reported to adversely affect hatchability, survival and hematological parameters of fish (Flos *et al.,* 1987). Oti and Avoaja (2005), noted that zinc at higher concentration in catfish (*Clarias gariepinus)* and heterodoxies ("Hybrid'') subjected to sub-lethal concentrations: 2.5 and $4.0mg^{-1}$ respectively for six weeks showed decreased haematocrit, hemoglobin corpuscular and mean corpuscular volume while white blood cell counts were decreased. Decrease in heamatocrit levels of both species following zinc exposure was attributed to haemo-dilution. Furthermore, these authors opined that a decrease in red blood cell count of *Clarias* species was due to cell shrinkage caused by osmotic alteration of the blood cells exposed to the metal.

Zinc is a common pollutant of the environment and so its effect on the aquatic ecosystem cannot be under estimated because of its long term effect (Leena *et al.*, 2012). This study was embarked upon to determine the effect of high concentration of Zinc on the uptake of some major cations and anions (Na^+, Cl^-, K^+, Mg^{2+} Ca^{2+} Cl^-) in the muscle of *Clarias gariepinus* and also determine the threshold at which this metal can be safe to aquatic organisms in the environment.

MATERIALS AND METHODS

Sub-adults of *Clarias gariepinus* with mean weight and length 6.1g and 8.5cm respectively were collected from Ebonyi River, South East Nigeria and used for the research. The fish were acclimatized in the laboratory at 24.3^{0}C for 14days and fed twice daily at 5% of their body weight with artificially formulated diet. The tank set-up comprised of 15 concrete fish tanks containing de-chlorinated water at the fish farm complex, Department of Fisheries and Aquaculture, Ebonyi State University. Feeding was terminated 24 hours before the commencement of the assay.

A range finding test was carried out using different logarithmic concentrations of the toxicant and the lowest concentration (0.001 at log^{3}i.e. 1gm of toxicant in 1000ml of distilled water) was chosen. 50 litre capacity plastic bowls were used for the experiment containing 30 litres of de-chlorinated water and 10 fish per replicate. The following concentrations were prepared:

13.88, 26.75, 53.5, 107 and 214ppm respectively and designated as T_1, T_2, T_3, T_4, and T_5 with three replicates respectively. These concentrations were introduced into the test bowls after acclimation by means of gravimetric toxicant auto- delivery systems controlled by adjustable regulation. The assay lasted for 4days (96 hours). A total of one hundred and eighty fish (180) were used. Experimental fish were not fed throughout the experimental period of the acute toxicity.

Physico-chemical parameters such as temperature, pH, dissolved oxygen (DO), free carbon (IV) oxide (CO_2) and total alkalinity were measured twice (before and after the introduction of the toxicant). Temperature was measured using thermometer, pH was measured using a pH meter, while DO, CO_2 and Alkalinity were measured titrimetrically according to APHA (1989).

The Cations and Anions (Na^+, Cl^-, K^+, Ca^{2+}, and Mg^{2+}) were determined by wet acid digestion method of APHA (1989) 0.5g of the sample (muscle) was weighed into a conical flask and 5ml H_2SO_4 selenium salicylic acid solution added and allowed to stand overnight. This was heated at 30^{0}C for 2 hours and 5ml of Perchloric acid (conc.) added and heated until the sample was digested. It was thereafter made up in a 50ml flask and used for the determination of the elements. Magnesium was determined by the Edtaver-sanate complerometric titration method, while Potassium and Sodium were determined by the flame photometry method.

The 96hr LC_{50} was calculated by the Litchfield and Wilcoxon simplified method for evaluating dose effect experiment (Litchfield and Wilcoxon, 1949) and the test of significance was calculated at 95% confidence limit (Finney, 1971).

RESULTS

The values of the physicochemical parameters observed in this study are shown in Table 1. There was no significant variation ($P<0.05$) in the water quality parameters between the treatment tanks and the controls. The 96h LC_{50} obtained in this study was 26.62ppm and thresh value was 20.30ppm. Mortality increased with increased concentration of zinc sulphate. Quantal response included initial increase in opercular movement, vertical positioning of the fish with snout slightly above the water surface, curvature of the body, peeling of the skin, loss of balance, erratic swimming, quietness and finally, death.

The results obtained for the ionic uptake in the muscle of *Clarias gariepinus* fingerlings are shown in fig 1,2,3,4 and 5 below. There was significant variation ($P > 0.05$) in ionic uptake between the treatments and the controls, whereas there was no significant difference ($P < 0.05$) among the treatments. Furthermore, there was no significant variation in the uptake of K^+, Na^+, and Cl^- ions between the treatments whereas a significant variation ($P>0.05$) was observed in the uptake of Mg^{2+} and Ca^{2+} ions.

The uptake of the ions increased rapidly during the 24h period, varied at 48h and 72h and gradually dropped at the end of the 96h (Figs 1-5). There was however, no total inhibition of both cation and anion uptake.

Table 1: Mean water quality result for the period of the experiment and standard error of the mean

	Toxicant concentration (ppm)				
Parameter	**13.88**	**26.75**	**53.5**	**107**	**214**
Temperature (°C)	24.3 ± 0.6	24.2 ± 0.6	23.8 ± 0.6	24.3 ± 0.6	24.9 ± 0.6
pH	7.10 ± 0.4	6.95 ± 0.4	7.03 ± 0.4	7.02 ± 0.4	7.15 ± 0.4
Dissolved oxygen(mg l^{-1})	6.20 ± 0.5	5.88 ± 0.5	5.53 ± 0.5	5.10 ± 0.5	6.43 ± 0.5
CO_2 (mg l^{-1})	1.45 ± 0.1	1.53 ± 0.1	1.52 ± 0.1	0.80 ± 0.1	0.54 ± 0.1
Total alkalinity (mg l^{-1})	8.2 ± 0.2	8.2 ± 0.2	8.0 ± 0.2	5.0 ± 0.2	11.0 ± 0.2

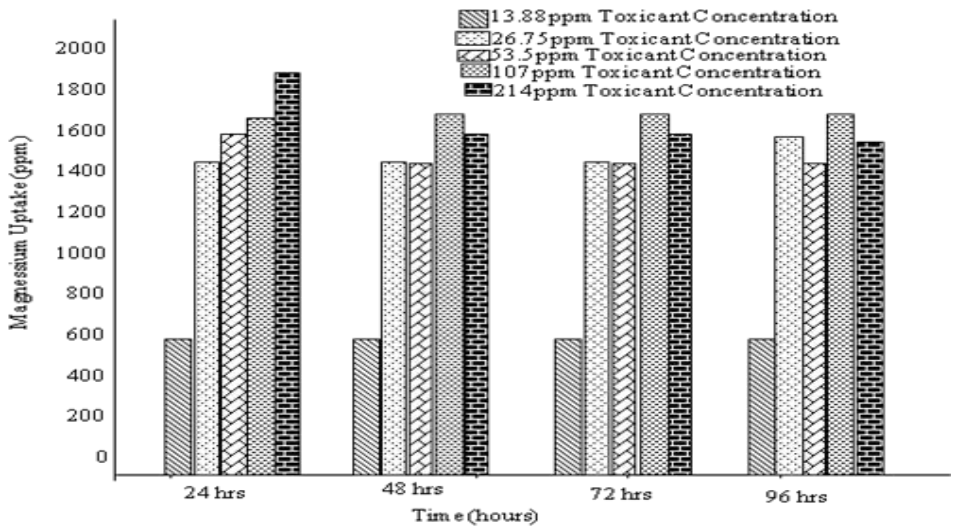

Fig 1: Uptake of Magnessium in the muscle of *Clarias gariepinus exposed* to different concentrations of Zinc

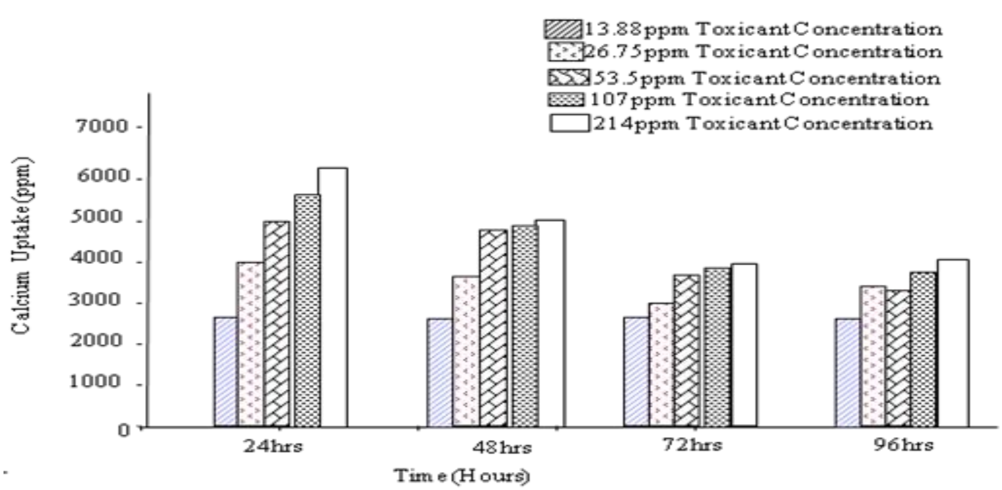

Fig 2: Uptake of Calcium in the muscle of *Clarias gariepinus exposed* to different concentrations of Zinc

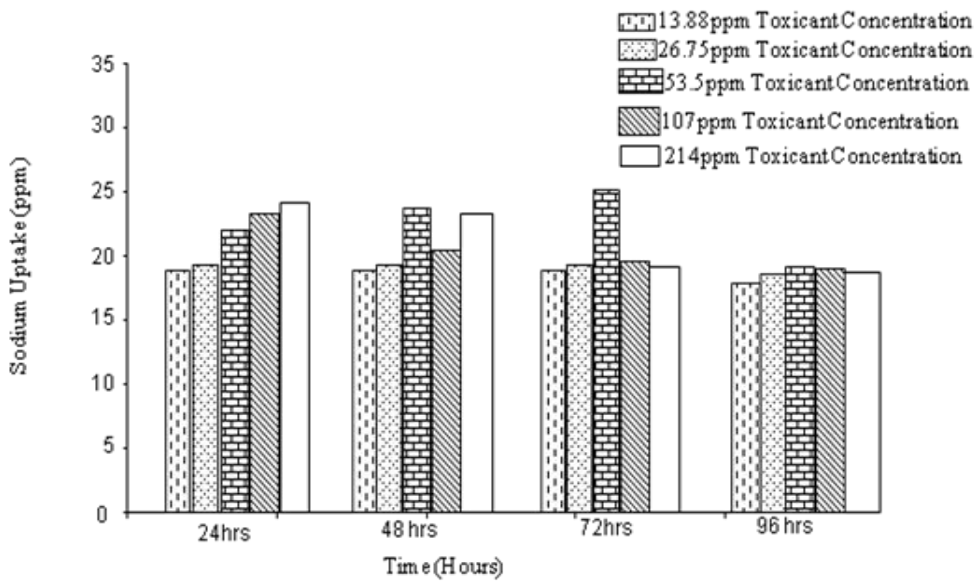

Fig 3: Uptake of Sodium in the muscle of *Clarias gariepinus exposed* to different concentrations of Zinc

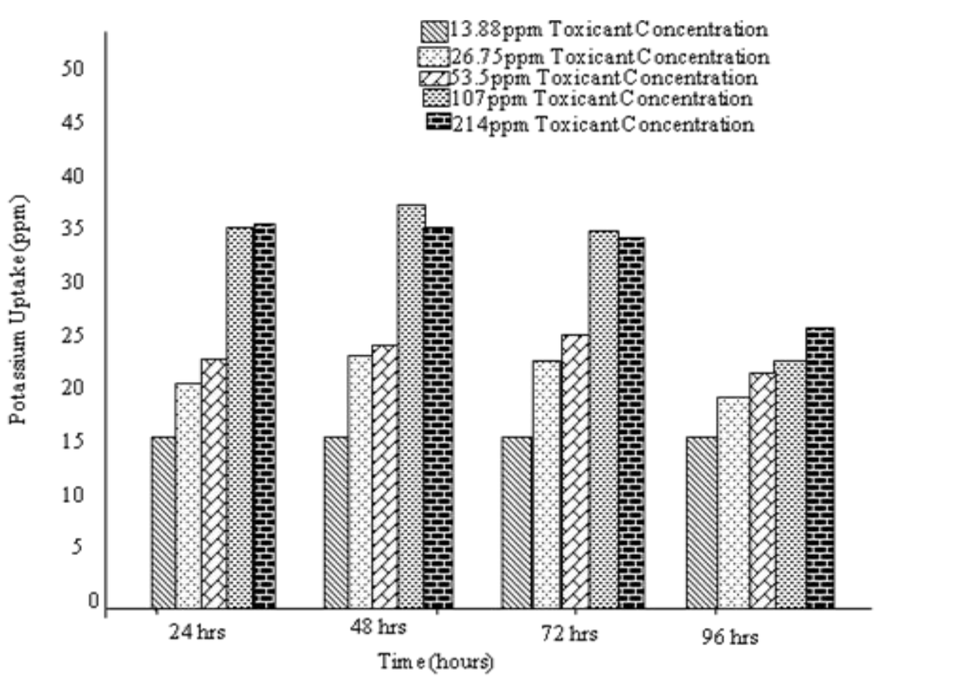

Fig 4: Uptake of Potassium in the muscle of *Clarias gariepinus exposed* to different concentrations of Zinc

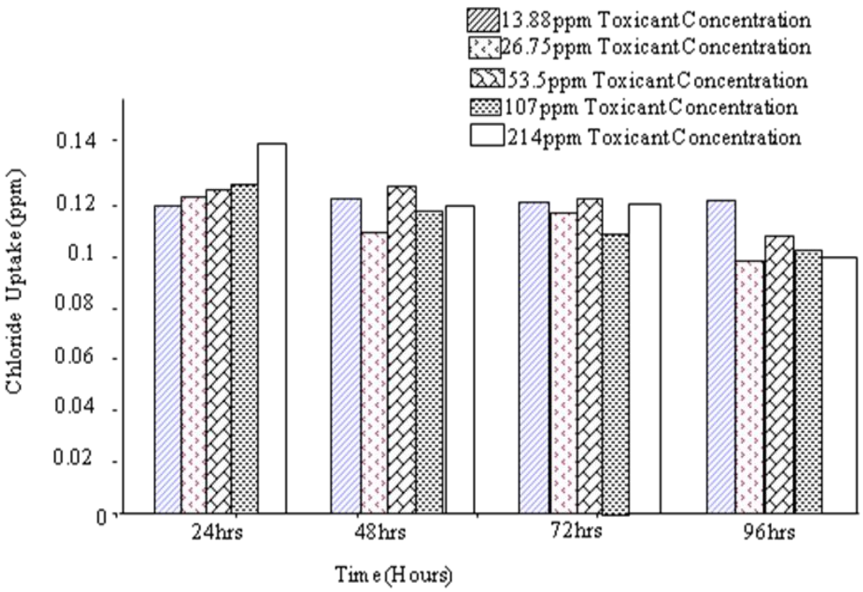

Fig 5: Uptake of Chloride in the muscle of *Clarias gariepinus exposed* to different concentrations of Zinc

DISCUSSION

The observed values of the physicochemical parameters in this study are in consonance with other reports for tropical waters (Offem *et al.*, 2011; Prakash and Dagaonkar, 2011). The 96h LC_{50} obtained in this study (26.62ppm) and threshold value (20.30ppm) were within the range reported by other workers (Oribhabor *et al*., 2012; Ekwu *et al.*, 2012) but however, not in agreement with the report of Okpi (2003). This could be attributed to the size and age of fish used for the assay. Mortality increased with increased concentration of zinc sulphate. This observation is in agreement with earlier reports by Omoregie and Ufodike (1991), Avoaja and Oti (1997) and Okpi (2003). The observed behavioral responses of the test organisms corroborated earlier reports by Oti (2003) and Ekwu *et al.*, (2012).

Initial increase in opercula ventilation which later decreased may be as a result of the possible inhibition action of the toxicant on respiration as well as possible depletion of ATP, which may have reduced the energy available for respiration. This depletion could have also resulted in exhaustion and consequently, death. Hughes and Tort (1985) reported severe effect of zinc on the cardio-respiratory function in Rainbow trout. It may also have interfered with the Osmoregulatory activity of the test organisms, as opined by Omoregie and Ufodike (1991).

There was no total inhibition of ionic uptake. This could be explained on the basis of the fact that uptake of many trace element concentrations in the tissues of aquatic organisms at the permeable surface is generally considered to be a passive process not requiring the expenditure of energy (Bashir *et al.*, 2013). However, uptake of major ions of alkali metals (e.g. Sodium, potassium, calcium) is through active transport pump, which requires energy (Neiber and Richardson, 1980).

Trace metal uptake of potassium, sodium and chloride ions did not vary significantly between the concentrations at ($P<0.5$) while the uptake of magnesium and calcium ions varied significantly at ($P<0.05$). The relationship between metals uptake and loss dictates the particular metal accumulation strategy of an organism (David and Rainbow, 1993). This brings about the variation in uptake of cations and anions. The significant difference at ($P>0.05$) in the uptake of potassium and chloride ions may be as a result of acid - base and osmoregulation functions in the test animals. Sodium and potassium are associated with chloride in acid - base balance and osmoregulation (Mchonald *et al.*, 1995).

Increase in the uptake of these cations and anions at 24 hrs and decrease at the end of 96 hrs may be as a result of the fish trying to adjust to different concentrations. The observed high uptake in the control (0.00 ppm) tank may be because this culture tank was devoid of the toxicant. The above result also showed that the uptake of these cations and onions (Na^+, Cl^-, K^+, Ca^{2+}, and Mg^{2+}) was time dependent.

CONCLUSION

Zinc is harmful to both target and non target organisms when present above the threshold concentrations (i.e. 20.30 ppm). It affects the uptake of Na^+, Cl^-, K^+, Ca^{2+}, and Mg^{2+}. These cations and anions function in acid base balance, osmoregulation, nerve transmission and bone formation etc. Increase in mortality ratio increases as the concentration of the toxicant increases as evident in T_5 (214 ppm), T_4 (107 ppm), and T_3 (53.5 ppm) in the present study. Although there was no total inhibition of uptake of the cations and anions studied, the results revealed that the influence of the toxicant (Zinc) on ionic uptake and consequent effects on the physiological functions of *Clarias gariepinus* is time dependent.

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