EFFECT OF ZINC ON THE DISTRIBUTION OF MAJOR CATIONS AND ANIONS IN THE MUSCLE OF THE FRESH WATER CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822) COLLECTED FROM EBONYI RIVER, SOUTH EAST NIGERIA.

OTI, E.E., [1] UDEH, G.N., [1] EKWU, A.O. [2] OKEYE, J.[1]

1. Department of Fisheries and AquacultureEbonyi State University, AbakalikiEmail- Otiegwuemmanuel@yahoo.com or graceinfinito@gmail.com

2. Department of Fisheries and AquacultureUniversity of Uyo, Akwa Ibom State.

ABSTRACT

The effect of zinc exposure evaluated during a four day exposure periods at 13.88ppm, 26.75ppm, 53.5ppm, 107ppm and 214ppm to *Clarias gariepinus* sub-adults showed the 96h LC_{50} as 26.62ppm. The threshold value was 20.30ppm. The muscle of the exposed fish analyzed showed a significant decrease in all the major cations and anions (Na^+, CL^-, Ca^{2+}, K^+, Mg^{2+}) at ($p<0.05$). The research revealed that there was no total inhibition of uptake of the cations and anions and also that uptake of these metals, that is cations and anions (Na^+, CL^-, Ca^{2+}, K^+, Mg^{2+}) increased rapidly during the 24hr and dropped at 48hours and 72hours and gradually increased at the end of 96hours showing that it was time dependent. During exposure period affected fish stood in upright position with their snouts above the water surface, gasping for air. Observation of the exposed fish showed that they were peeling of skin, increase in opercula movement, erratic swimming, quietness and finally death. This research therefore recommends that zinc should be applied appropriately at 26.30ppm to avoid death of fish and man that are the chief consumer and other aquatic organism.

Key words: Zinc, Muscle, Cations, Anions, *Clarias gariepinus*

INTRODUCTION

Zinc is omnipresent in the environment and an essential trace element in air, water and all living organism. Zinc 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 product, 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 (Schroeder's *et al.*, 1967). It occurs widely in nature as sulphide carbonate and hydrated silicate ores, frequently accompanied by other metals, mainly iron and cadmium. Zinc at high concentration has adverse effects in fish such as affecting the development, growth and survival of fish, induces changes in ventilation and heart physiology (Hughes and Tort, 1985). Zinc accumulates in fish gills and induced depressive effect on tissue respiration leading to death by hypoxia (Burto *et al.*, 1972). Zinc have also been said to adversely affect hatchability, survival, hematological parameters of fish (Flos *et al.*, 1987). Oti and Avoaja (2005), observed a decreased in haematocrite, hemoglobin corpuscular and mean corpuscular volume and increased blood cell count while white blood cell count were decreased in *Clarias gariepinus* exposed to zinc. The decrease in the red blood cell count of the species is attributed to swelling of the erythrocytes. Low concentrations of zinc are therefore toxics to both the juveniles and adults of various fish species. Zinc is a common pollutant of the environment and so its effect on aquatic ecosystem cannot be under estimated because of the long term effect. It is the objective of this study to determine the acute concentration on 96h LC_{50} of zinc on *Clarias gariepinus*, study the effect of zinc on the uptake of some major cations and anions (Na^+, CL^-, K^-, Mg^{2+} Ca^{2-}) in the muscle of the exposed fish and also 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 in Ebonyi State and used for the experiment. The fish were acclimatized in laboratory conditions of 24.3 ± 0.6^0C for 14days and fed twice daily at 5% of their body weight with artificially formulated diet. This was carried out in concrete fish pond containing dechlorinated water at fish farm complex, department of Fisheries and Aquaculture. Feeding was stopped a day before the commencement of the experiment.

Exploratory test were carried out to determine the appropriate concentration for testing the chemical. 50 litres capacity plastic bowl was used for the experiment containing 40 litres of dechlorinated 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 authodelvery systems controlled by adjustable regulation. The experiment lasted for 4days (96 hours) and a total fifty fish were used. The experimental fish were not fed throughout the experimental periods of the acute toxicity. Within these four days of acute experiment, the fish were monitored to know their response to different concentration of the toxicant. A single fish was taken from each bowl containing different concentrations of the toxicant including the control which has no toxicant for trace metal analysis. Their muscles were extracted (cuts) and analyzed to determine the effect of toxicant (zinc) on the uptake of Na^+, CL^-, K^+, Ca^{2+}, and Mg^{2+} in the muscle of exposed fish.

Physico-chemical parameters such as Temperature, pH, Dissolved Oxygen, Frees carbon (IV) oxide and total alkalinity were measured twice (before and after the introduction of the toxicant). Temperatures were measured using thermometer, pH using meter JENWAY Model No 4076, Dissolved Oxygen by winkler method (APHA, AWWA and WPCF, 1989), CO_2 and alkalinity by titration using phenolphthalein.

Determination of cations and anions

The cations and anions were determined by wet acid digestion method. 0.5g of the sample (muscle) was weighted into a conical flask and 5ml H_2SO_4 selenium salicylic acid solution added and allowed to stand overnight. This was heated at 30^0C for 2 hours and 5ml of perchloric acid (conc.) added and heated the vigorously until sample digested. It was made up in 50ml flask and used for the determination of the elements. Magnesium was determined by Edtaver-sanate complerometric titration method, potassium and sodium by the flame photometry method.

Statistical Analysis of Data

The confidence limit to 96hr LC_{50} was calculated by Litchfield and wilcoxon simplified method for evaluating dose effect experiment (Litchfield and Wilcoxon, 1949). The significant difference was calculated based on the standard error of the difference (Finney, 1971).

RESULTS AND DISCUSSION

During the experiment, the values of the physicochemical parameters observed are shown in Table 1. This is similar to earlier reports by Okpi (2003). Statistical analysis showed that dissolved oxygen, Co_2, total alkalinity, temperature and pH did not vary significantly at ($P<0.05$) from the control. Their values were also within the suggested tolerance limit for fresh water fish.The 96h LC_{50} obtained in this study was 26.62ppm and thresh value was 20.30ppm. These result were not in agreement with what was reported by Okpi (2003) which may be as a result of the size, age and species of fish used for the experiment or the source of water, experimental techniques and conditions. 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). During the exposure period, the test fish exhibited some behavioural pattern which includes initial increase in opercula movement, standing upright with snout above the water surface gasping for air, curvature of body, peeling of skin, loss of balance, erratic swimming, quietness and finally death. This behavioural response was similar with earlier report by Oti (2003).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 A.T.P. which may reduce the energy available for respiration. This depletion could have also resulted in exhaustion and consequently, death. It may also have interfered with the Osmoregulatory activity of the test organism. There was no total inhibition in the uptake of the cations and anions (Na^+, K^+, CL^-, Ca^{2+}, and Mg^{2+}) studied table 2. The uptake of many trace element concentrations in the tissues of aquatic organism at the permeable surface generally considered to be a passive process not requiring the expenditure of energy. But this contrast markedly with that pertaining to the major ions of alkali metals (e.g. Sodium, potassium, calcium) which are taken up through active transport pump. The key to this difference lies in the different chemistries of the two groups of metals (Neiber and Richardson, 1980).Trace metal uptake of potassium, sodium and chloride ions did not vary significantly between the concentration at ($p<0.5$) while the uptake of magnesium and calcium ions varied significantly at ($p<0.5$). 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 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 24hrs and decrease at the end of 96hrs may be as a result of the fish trying to adjust to different concentrations. The high uptake in the control (0.00ppm) tank may be because it was devoid of the toxicant. The above result also showed that the uptake of these cations and anions (Na^-, CL^-, K^+, Ca^{2+}, and Mg^{2+}) is time dependent. Uptake of cations and onions varied significantly between the concentrations and the control at $p<0.05$. Observation made during the experiment shows high uptake of the cations and onions in the control tank while decrease in uptake in the different concentrations.

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 (^{0}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(Mgl^{-1})	6.20±0.5	5.88±0.5	5.53±0.5	5.10±0.5	6.43±0.5
$C0_2$ (Mgl^{-1})	1.45±0.1	1.53±0.1	1.52±0.1	0.80±0.1	0.54±0.1
Total alkalinity (Mgl^{-1})	8.2±0.2	8.2±0.2	8.0±0.2	5.0±0.2	11.0±0.2

Table 2: Uptake of anions and cations in the muscle of experimental fish in each of the toxicant concentrations.

Con (ppm)	Duration in hours																			
	24hours					48 hours					72 hours					96hrs				
	Mg	Ca	K	Na	Cl	Mg	Ca	K	Na	Cl	Mg	Ca	K	Na	Cl	mg	CA	K	Na	Cl
13.88	620	2610	15.4	18.4	0.12	620	2601	15.0	18.37	0.12	625	2604	15.0	18.4	0.12	617	2603	15.0	18.2	0.12
26.75	1500	4000	22.0	19.4	0.13	1504	3770	23.1	19.12	0.11	1492	3350	22.0	19.3	0.12	1907	3721	19.2	19.0	0.10
53.5	1611	5000	23.1	22.1	0.13	1506	4811	25.0	23.00	0.13	1502	3812	24.0	24.1	0.12	1500	3712	21.0	19.60	0.11
107	1701	5806	35.0	22.6	0.13	1728	5000	35.4	21.40	0.12	1700	4000	35.0	20.0	0.11	1702	3900	22.0	19.50	0.10
214	1890	6005	35.1	24.0	0.14	1722	5006	34.8	21.00	0.12	1688	4000	33.0	20.0	0.12	1688	3920	25.0	19.10	0.10

CONCLUSION

In this study, it was identified that zinc is harmful to both target and non target organism when applied above the threshold level (i.e. 26-36ppm). It also affects the uptake of Na^+, CL^-, K^+, Ca^{2+}, and Mg^{2+}. These cations and onions function in acid base balance, osmoregulation, nerve transmission and born formation etc. This toxicant (zinc sulphate) did not inhibit the uptake of these cations and anions. The fluctuation in their uptake might also affect the formation of the cations and onions in the body of fish. Increase in mortality ratio increases as the concentration of the toxicant increases as evident in T_5 (214ppm), T_4 (107ppm), and T_3 (53.5ppm) in the present study. Although there was no total inhibition of uptake of the cations and anions studied, the results revealed that the uptake of these cations and anions increased rapidly during the 24hrs period and dropped at 48hrs and 72hrs and gradually increased at the end of 96hrs showing that it was time dependent.It can be concluded that zinc sulphate when applied above threshold level (26.30ppm) is poisonous to both target and non target organism. Therefore, it should not be applied in the environment above the threshold in the level.

REFERENCES

APHA, AWWA and APCP, American Public Health Association, American Water Works Association and Water Pollution Control Federation (1989). Standard method for the examination of water and waste water 17 edition APHA Washington, D.C. USA 1391pp.

Avoaja, D.H. and Oti, E.E. (1997). Effect of sublethal concentration of pesticides on the growth and survival of the ginger lings of the African catfish "Heteroclarias" hybrid Nigeria. *Journal of Biotechnology 8 (1):* 40-45pp.

Burto, D.T., Jones A-H.D., Cairns, I. (1972). Acute zinc toxicity to rainbow trout (Salmon gainder) conformation of the Hypothesis that death is related to tissue hypoxia. *Journal fish Research Bd. Carrads, 29:*1463-1466.

David, J.H.P., Philips, S.R. (1993). Bio-monitoring of trace aquatic contaminants. Chapman and Hall, London, Elsevier Science Publishers Ltd 79-132pp.

Flos, R., Tort, L. and Balasch, I. (1987). Effect of zinc sulphate on haematogical parameters in dogfish, scyhiorhinus canicula and influence of MS 222. *Marine Environmental Research 21:* 289-298.

Gerlarch (1981). Industrial effluent in Marine pollution . Springer-Verlag New York, 37-40ppm.

Hughes, G.M. and Tort, L. (1985). Cardia-respiratory responses of rainbow trout during recovery from zinc treatment. *Environment Pollution Service 37(A)*:22-226.

Litchfield, J.I. and Wilcoxon (1949). A simplified method of evaluating dose effect experiments. *Journal pharmacol experimental/hour 96:* 99-173pp.

Mchonald, P. Edwards. R.A., Green halgh, J.F.D., Morgah, C.A. (1995). Animal nutrition Longman group Ltd U.K. 97-108pp.

Nieber, E., Richardson, D.H. (1980). Replacement of Non-descript term Heavy metals by a biological and chemically significant classification of metal ions. *Environmental pollution series 3 (1):* 3-26.

Oti, E.E. (2003). Acute toxicity of gammalin 20 to *chrysichthys Nigroditatus* (Lacepede). *Science Forum Journal Pure and Applied Sciences 6(2)*:219-225pp.

Oti, E.E. and Avoaja, D.A. (2005). Haematological assessment of freshwater catfishes, *Clarias gariepinus* (Burchell, 1822) exposed to sublethal concentration of zinc. *Pakistan journal of zoology 37 (3).* 101-105pp.

Okpi, K. (2003). Acute toxicity of lambols cyhalotrin pyrethroid (Karate 2.5 Ec) to the freshwater catfish. *Heterobranchus longifilis* (cuvier and valencennes). A. B. Sc. Thesis submitted to the Department of fisheries. Michael Okpara University of Agriculture Umudike Nigeria 1.46pp.

Omoregie, E. and Ufodike, E. B. C. (1991). Histopathology of *Oreochromis niloticus* exposed to actelli 25 E.C. *Journal of Aquatic Science 6:* 13-17.Schroeder, H. A. P., Tiapton, I. H. and Balassa, J.J (1967). Essential trace metals in man: relation to environmental cadmium. *Journal Chronic Disease Volume 20:*179-219.