

Original article

## TOXIC STRESS OF LEAD ON *CLARIAS GARIEPINUS* (AFRICAN CATFISH) FINGERLINGS

OLAIFA, F .E<sup>1\*</sup>, OLAIFA, A.K<sup>\*\*</sup> AND LEWIS O.O\*.

\*Department of Wildlife and Fisheries Management, University of Ibadan,

\*\* Department of Veterinary Surgery and Reproduction, University of Ibadan,

The toxic stress of lead on fish was tested employing a 96-hour bioassay test. The experimental fish used were *Clarias gariepinus* fingerlings. Lead in the form of lead chloride was used to prepare the stock solution. The concentrations of lead used for the experiment were 0, 1.8, 3.2, and 5.6, and 10.0mg/l. The lethal concentration (LC<sub>50</sub>) was estimated using the logarithmic method and was found to be 0.6, 0.58 and 0.62mg/l for replicates 1, 2 and 3 respectively.

Keywords: Lead, toxic stress, Catfish, *Clarias gariepinus*, fingerlings

<sup>1</sup>Author for correspondence

### INTRODUCTION

Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety (Ward and Parrish, 1982).

Lead is a potentially toxic chemical that may be directly ingested by man or indirectly through aquatic animals like fish and shellfish. The effects of lead on man include mental retardation, learning dysfunction, and loss of coordination (Goodman and Gilman, 1992). Though the effect of lead toxicity is well elucidated in man (Klaassen, 1992), there is paucity of information on its effects on fish, which are eaten by man.

This work is therefore aimed at assessing the toxic stress of lead on fish using a static bioassay technique (Reish and Oshida, 1987). The fish *Clarias gariepinus* is a hardy fish and highly valued in Nigeria. It would therefore be of interest to study the quantity of lead that can be accumulated within its tissues; which would then give an indication of how much lead is indirectly consumed by man.

### MATERIALS AND METHODS

A 96 –hour short-term static bioassay (Reish and Oshida, 1987) was conducted using the fingerlings of *Clarias gariepinus* as test organisms. This was done in order to study the toxicity of lead on fish, and indicate allowable levels or concentrations of lead for very short exposures.

#### **Collection and acclimatization of fish:**

The fish used for the experiment were purchased from a fish farm in Ibadan. The choice of *Clarias gariepinus* was informed by its ability to withstand stress and its high commercial value in Nigeria. The average weight and length of fish used for the experiment were 6g and 5cm respectively. The fish were held in 20-litre capacity plastic bowls containing dechlorinated water. The fish were allowed to acclimatize for about one and a half months. The period of acclimatization was extended beyond two weeks to ascertain the condition of the fish. The fish were inspected for disease conditions and general fitness. Water was changed every other day. Fifteen fingerlings were kept per bowl. There were four different treatment groups and each had three replicates. The system was aerated using an

aerator and the fish were fed four times daily on a 40-percent crude protein diet during the period of acclimatization. Feeding was discontinued while aeration continued during the 96-hour test period.

#### **The Determination of the physico-chemical parameters of the water:**

The physico-chemical parameters of the water used were examined after exposure to air to lose chlorine. These parameters included temperature, dissolved oxygen (D.O.) and the hydrogen ion concentration (pH). The temperature was measured with mercury-in-glass thermometer. The dissolved oxygen of the water was measured with a Griffin oxygen meter (model 40). The D.O. was calculated as shown below:

$$D.O = \frac{S \times P}{100} \text{ (mg/l)}$$

Where

*S* = Conversion of percentage saturation to parts per million (ppm).

*P* = Reading on the oxygen meter.

The hydrogen ion concentration (pH) was measured using a pH paper (Seele-Hannover pH paper). The pH paper had a range of 1 to 14 with 7 as the neutral point.

#### **Preparation of the stock and test solutions of lead:**

The test chemical used for the experiment was anhydrous lead chloride. The chloride form of the metal was chosen because of its lower toxicity than the other forms of lead (Odiete, 1999). After a range – finding test, the concentrations prepared for the experiment were 1.8, 3.2, 5.6, and 10.0 mg/g of lead respectively. A stock solution of 1000mg/l (1g/l) of the lead was prepared by adding 1.0 g of lead to 1litre of distilled water. The amount of lead chloride which contained 1.0 g of lead was determined from the molecular and atomic weights as:

$$\frac{\text{Molecular weight of lead chloride}}{\text{Atomic weight of lead (Pb)}}$$

The different concentrations required were calculated as follows:

$$\frac{\text{Wt of lead required}}{\text{Atomic weight of lead}} \times \text{molecular wt of lead}$$

#### **Preservation of dead fish samples:**

Dead fish were identified by an absolute lack of movement. They were removed as soon as this was noticed, wrapped in pre-cleaned plastic bags and frozen. The toxicity of the test chemical was determined using the logarithmic method of analysis (Litchfield and Wilcoxon, 1949).

#### **Digestion of samples:**

The digestion of samples was carried out by placing the samples in open beakers on a hot plate. (FAO/SIDA, 1983). 10.0g of thawed fish sample were placed in a beaker and 15mls of freshly prepared 1:1 nitric acid – hydrogen peroxide added. The beaker was covered with a watch glass and left for the reaction to subside. The beaker and its content were heated on a hot plate with the temperature not exceeding 160°C. The boiling was done for about two hours till the content of the beaker reduced to about 2 to 5mls. The content of the beaker was transferred to a 25ml volumetric flask and diluted to volume with distilled water. The digest was transferred to plastic bottles and later analyzed using the Atomic absorption spectrophotometer (AAS). The actual concentration of the lead in fish tissue was determined as:

$$\begin{aligned} \text{Actual concentration of lead in sample} \\ = \text{PPMR} \times \text{Dilution Factor} \end{aligned}$$

Where PPMR represented the AAS reading

$$\text{Dilution factor} = \frac{\text{Volume of digest used}}{\text{Wt of sample digested}}$$

## **RESULTS**

#### **Physico-chemical parameters**

The results of the physico-chemical parameters measured are given in table 1.

**The concentration of the toxicant used:**

The toxicant used was anhydrous lead chloride and standard concentrations were prepared according to the method of Reish and Oshida (1987). The concentrations of the toxicant prepared per 8 litres of water are given in table 2.

**Table 1:** The physico-chemical characteristics of the water used:

Parameter	Value
Temperature	26 °C
Dissolved oxygen	7.4mg/l
Hydrogen ion concentration	7.0

**Table 2:** Concentrations of the toxicant in 8 litres of water.

Required concentration of lead /liter (mg/l)	Required concentration of lead chloride (mg/l)	Lead chloride in 8 liters of water (mg)
1.8	2.416	19.33
3.2	4.297	34.37
5.6	7.519	60.15
10.0	13.427	107.41

**Table 3:** Concentration of lead in water (mg/l) Concentration of lead in muscle tissue(ppm)

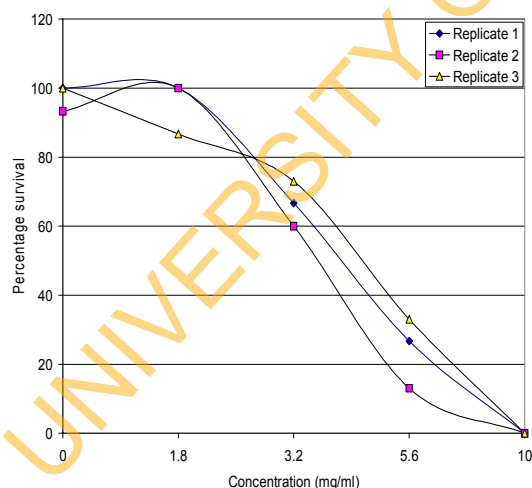
Treatment	Concentration of lead in water (mg/L)	AAS reading	Actual concentration of lead in muscle tissue (ppm)
A	0.0	0.38	0.95
B	1.8	0.57	1.425
C	3.2	0.62	1.55
D	5.6	0.68	1.7
E	10.0	0.95	2.38

The estimation of the lethal concentration values (LC 50) was carried out using the Logarithmic method (Litchfield and Wilcoxon, 1949). The results were subjected to an analysis of variance and a follow-up procedure using the Least Significant Difference (LSD). The 96-hr LC<sub>50</sub> values were determined from the graphs to be 0.6, 0.58 and 0.62 mg/l for replicates 1, 2 and 3 respectively. There were significant differences among treatment concentrations used.

**DISCUSSION**

Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals. Bioassay is necessary to determine the concentration of a toxicant, which may be allowed in receiving waters without adverse effects on the living resources (Standing Committee of Analysts, 1981; Ward and Parrish, 1982; Reish and Oshida, 1987)

The effects of lead on fish observed during this experiment showed that caution should be exercised in allowing lead into the aquatic environment. These effects included loss of balance, skin bleaching and weakness. A thick layer of mucus on the skin covered the dead fish and there were air bubbles on the water. There was reduced activity evidenced by vertical positioning and less mobility of the fish in solutions with less



**Fig. 1:** The fish survival in 96 hours:

**Tissue Analysis:**

The result of the tissue concentration of lead in the fish is given in table 3.

concentration of lead (3.2 and 5.6mg/l), while no survivors were recorded for the highest concentration (10.0mg/l).

The percentage and number of survivors decreased with increasing concentrations of lead in the water. Also, the accumulation of lead in fish tissue increased with increasing lead concentration in water. There were significant differences in the survival and tissue concentrations among the treatment groups. The result of this study agreed with that of (James *et al.*, 1996) who observed that fish exposed to sub lethal levels of lead produced dose – dependent significant increases in the concentration of lead in the liver and muscle of *Oreochromis mossambicus*. The concentrations of lead in these tissues declined on transfer of fish to lead –free water. The recovery of fish was faster for those placed in lower concentrations of lead than those that had been placed in higher concentrations.

The effects of heavy metals such as lead on the environment is usually highlighted and addressed in respect to their effects on man. In some countries like Sweden, Japan, Switzerland and Germany, there are maximum permissible levels of metals allowed in fish (about 1mg/kg body weight). Italy allows 0.7mg/kg body weight while the standard for the United States and Canada allow 0.5mg/kg body weight (Gerlach, 1981). There is a need for more work to set maximum permissible levels of metals for fish meant for human consumption in Nigeria.

#### REFERENCES:

**FAO/SIDA (1983).** Manual of methods in aquatic environmental research, part

9. Analyses of metals and organochlorines in fish. *FAO Fisheries / Technical paper* 212.

**Gerlach, S.A. (1981).** Marine Pollution, Diagnosis and therapy. SpringerVerlag, Berlin. Pp218.

**Goodman, L.S. and Gilman, A. (1992).** The Pharmacological Basis of Therapeutics. (Editors: Gilman, A.G., Rall, T.W., Nies, A.S., and Taylor, P). 8th edition. Mc Graw-Hill International Edition. Pp 1592-1614.

**James, R., Sampath, K. and Alagrathinam, S., (1996).** Effects of lead on respiratory enzyme activity, glycogen and blood sugar levels of the teleost *Oreochromis mossambicus* (Peters) during accumulation and depuration. *Asian Fish. Sc.* Vol .9 No 2, pp87-100.

**Klaassen, C.D. (1992).** Heavy metals and heavy metal antagonists. In: Goodman and Gilman's Pharmacological Basis of Therapeutics. (Editors: Gilman A.G., Rall, T.W., Nies, A.S. and Taylor, P) 8<sup>th</sup> edition. Mc Graw – Hill.

**Litchfield, T. Jr and Wilcoxon, F.A. (1949).** A simplified method for evaluating dose –effect experiments. *Journal of Pharmacological Experimental Theory*, 96,99-113.

**Odiete, W.O. (1999).** Environmental Physiology of Animals and pollution. Pp1-261.

**Reish, D.L, and Oshida, O.S. (1987).** Manual of Methods in aquatic environment research. Part 10. Short-term static bioassays. *FAO Fisheries Technical Paper* No 247. Rome. 62pages.

**Standing Committee Of Analysts (1981).** Acute Toxicity testing with aquatic organisms 1981. Methods for the Examination of Waters and Associated Materials. Her majesty's Stationery Office, London.

**Ward G.S. and Parrish, P.R. (1982).** Manual of Methods in Aquatic environmental research. Part 6. Toxicity Tests. *FAO Fisheries Technical Paper*, No 185 FRI/T185.

Received: February 2002

Accepted in final form: March 2003