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Acute Toxicity of Mercury Chloride on Fingerlings of *Clarias gariepinus*

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ABSTRACT

In this study, 90 fingerlings of Clarias gariepinus were divided into six treatments of five fish each. The different groups were exposed to the different concentrations of 0mg/L, 2mg/L, 4mg/L, 6mg/L, 8mg/L and 10mg/L for a period of 96 hours. The experiment was triplicated. The results revealed that all the fish of treatments exposed to 0mg/L of $Hgcl_2$ (control) survived whereas all the fish of treatments exposed to 8mg/L and 10mg/L died. The determination of 96 hours LC_{50} was carried out by computing the mortality result in probit program of SPSS. The median lethal concentration was 0.55mg/L with lower and upper confidence limits of 3.188mg/L and 6.96mg/L respectively. Also in this study, the histopathological alteration in the muscle tissue of Clarias gariepinus caused by the mercury chloride were observed. After 96 hours of introducing different concentration of mercury chloride of the determination of 96 hours LC_{50}. The control (0mg/L) shows the fish skeletal muscle tissue with evenly sized muscle bundles displaying typical but less obvious cross striation as well as thick intermuscular connective tissue while the highest concentration (10mg/L) compared to 0mg (control) shows there is severe variability in muscle bundle size, accentuation of cross striations and severe reduction of muscle density with prominent myonecrosis as well as moderate thinning of intermuscular connective tissue.

Keywords: *Toxicity, Clarias gariepinus, Mercury Chloride, Histopathology*

INTRODUCTION

In modern times, one of the main threats to the health of ecosystem is the exposure to a myriad of toxic substances and compounds such as mercury, cadmium, lead, copper, arsenic, air pollutants, pesticides, plastics, cigarette smoke, diesel fumes and nano-particles found in products like perfumes and sunscreens. (Guedenon *et al*., 2012). Because of their high toxicity conferred by their persistent nature in the environment, heavy metals come to the forefront of dangerous substances causing serious health hazard in ecosystems and organisms (Bhattacharya *et al*., 2011; Adamu *et al*., 2011; Babu *et al*., 2011). Metal concentrations in aquatic organisms appear to be of several magnitudes higher than concentrations present in the ecosystem. (Law, 2000). This is attributed to bioaccumulation, whereby metal ions are taken up from the environment by the organism and accumulated in various organs and tissues. Metals also become increasingly concentrated at higher trophic levels, possibly due to food-chain magnification (Nwakanma and Hart, 2013). Since fish are animals particularly affected by these pollutants they are widely used to evaluate the health of aquatic ecosystems (Olaifa *et al*., 2014). Some particular heavy metals, such as mercury (Hg), are especially of a deep concern due to their high toxicity. Mercury occurs naturally as a mineral and is widely distributed throughout the environment as a result of natural and human activities. Inorganic mercury is the most common form of the metal release by industries in the environment (Sunderland and Chimura, 2000). Heavy metals are produced from a variety of natural and anthropogenic sources (Baurais *et al*., 2015).

In aquatic environment, heavy metal pollution results from direct atmosphere deposition, industrial waste products also through waste water treatment plants. (Demirak *et al*., 2006; Maier *et al*., 2014; Dhanakumar *et al*., 2015; Garcia *et al*., 2015; Wanger and Boman, 2003). Fishes are considered to be of most significant advantage in describing the natural characteristics of aquatic systems and in assessing changes to habits. (Lamas *et al*., 2007). Fish are used as bio-indicators to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in aquatic system (Farkas *et al*., 2002). In addition, fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic and acute disease. (AL-Yousuf *et al*., 2000). Fish have the ability to uptake and concentrate metal directly from the surrounding water or indirectly from other organisms such as small fish, invertebrates and aquatic vegetation and the effects become apparent when concentration in such tissues attain a threshold level (Maier *et al*., 2014). However, this accumulation depends upon their intake, storage and elimination from the body (Abdallah and Morsy, 2013). Studies from the field and laboratory works showed that accumulation of heavy metals in a tissue is mainly dependent on water concentrations of metals and exposure period, although some other environmental factors such as water temperature, oxygen concentration, pH, Hardness, salinity, alkalinity and dissolve organic carbon, may affect and play significant roles in metal's accumulation and toxicity to fish (Abbas and Ali, 2002; Adil *et al*., 2013; Berlin *et al*., 2007; Dahunsi *et al*., 2011). The aim of this study is to determine the effect of mercury chloride on the survival rate of *Clarias gariepinus* fingerlings; the histopathological effect of muscle tissue of *Clarias gariepinus* fingerlings exposed to mercury chloride over 96 hours period and to determine the acute concentration of the mercury chloride (96hrs LC_{50}) on *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

The experiment was carried out at Michael Okpara University of Agriculture, Umudike in the Department of Environmental Management and Toxicology (EMT) Laboratory. 100 *Clarias gariepinus* fingerlings of the same breed was purchased from a reputable fish farm in Abia State. The fingerlings was transported in an oxygen bag to the department of EMT teaching and research laboratory in a 20 litres plastic gallon cut open half way on top. The fish was kept in plastic tank which was half filled with dechlorinated water. They were acclimatized to laboratory conditions over one week. During acclimatization, the fingerlings was fed thrice daily (morning, afternoon, evening) and the water was changed every twenty four (24) hours to prevent the accumulation of waste metabolite and particles. The experiment was laid out in a completely randomized design (CRD) with six (6) treatments. The treatments were 0mg/L (control treatment), 2mg/L, 4mg/L, 6mg/L, 8mg/L and 10mg/L of mercury chloride ($Hgcl_2$). Five fingerlings were kept in a transparent plastic bucket in 15 litres of water measuring to be replicated three (3) times across each giving a total of fifteen (15) samples. The following water quality was determined during the experiment and they include: temperature, dissolved oxygen and pH using recommended standards, (AOAC, 2000). For the histopathological analysis the samples were put into separate sample bottles with label, and fixed in 10% formal saline solution. Thereafter, the samples were dehydrated through ascending grades of alcohol viz: 70% (1hour); 95% twice (1hour each); absolute alcohol twice (1hour 30min each); and another absolute alcohol for 2 hours; equal volumes of absolute alcohol and xylene, i.e. 50/50 (overnight); then cleared in two changes of xylene for one hour each. Paraffin wax infiltration was carried out with two changes of paraffin wax at one hour each, in an electric hot air oven at 60^0C. The samples were then embedded in paraffin wax, trimmed and mounted in a wooden chuck. Each sample was sectioned on the microtome at 5μm and floated on the floatation bath. The floated sections were picked out with clean albumenized microscopic slides; the slides were stained using Haematoxylin and Eosin (H & E) protocol, and cover-slipped with a Depex mountant. The slides were later viewed with a binocular Olympus microscope.

RESULTS AND DISCUSSION

The physico-chemical parameters of the water measured were: Temperature, dissolved oxygen and pH. The mean Temperature of the water during the study was at 26.7^0c; however pH and

Dissolved oxygen mean values of 6.6 and 5.4 respectively were maintained throughout the duration of the study. The values of physic-chemical parameters of test water observed during the experiment are presented in Table 1.

Table 1: Mean value of the physic-chemical parameters of the water used for the experiment

	Toxicants Concentration					
Parameters	**Control (0mg)**	**2mg**	**4mg**	**6mg**	**8mg**	**10mg**
Temperature ^{0}C	26.7	26.8	26.7	26.4	26.7	26.6
Dissolved oxygen (DO)	5.4	5.3	5.5	5.6	5.2	6.4
pH	6.7	6.8	6.6	6.5	6.7	6.5

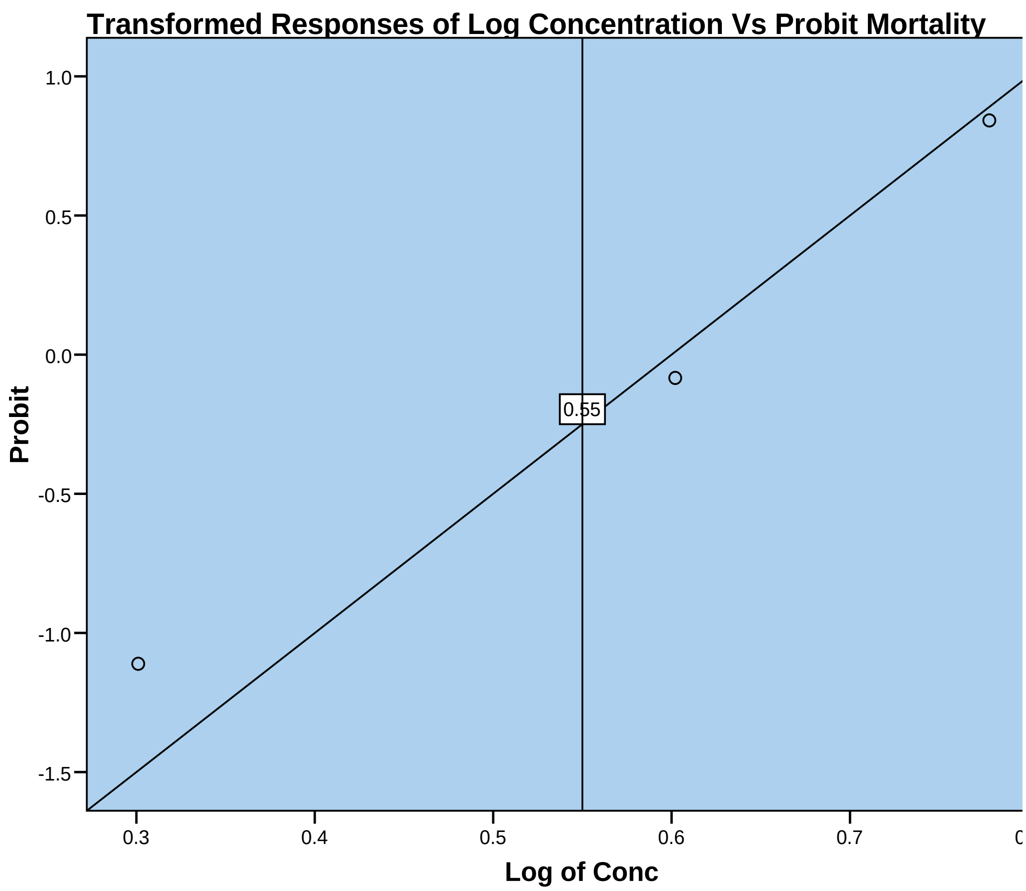

Figure 1: Lethal Concentration (LC50 of *Clarias gariepinus)* exposed to mercury chloride

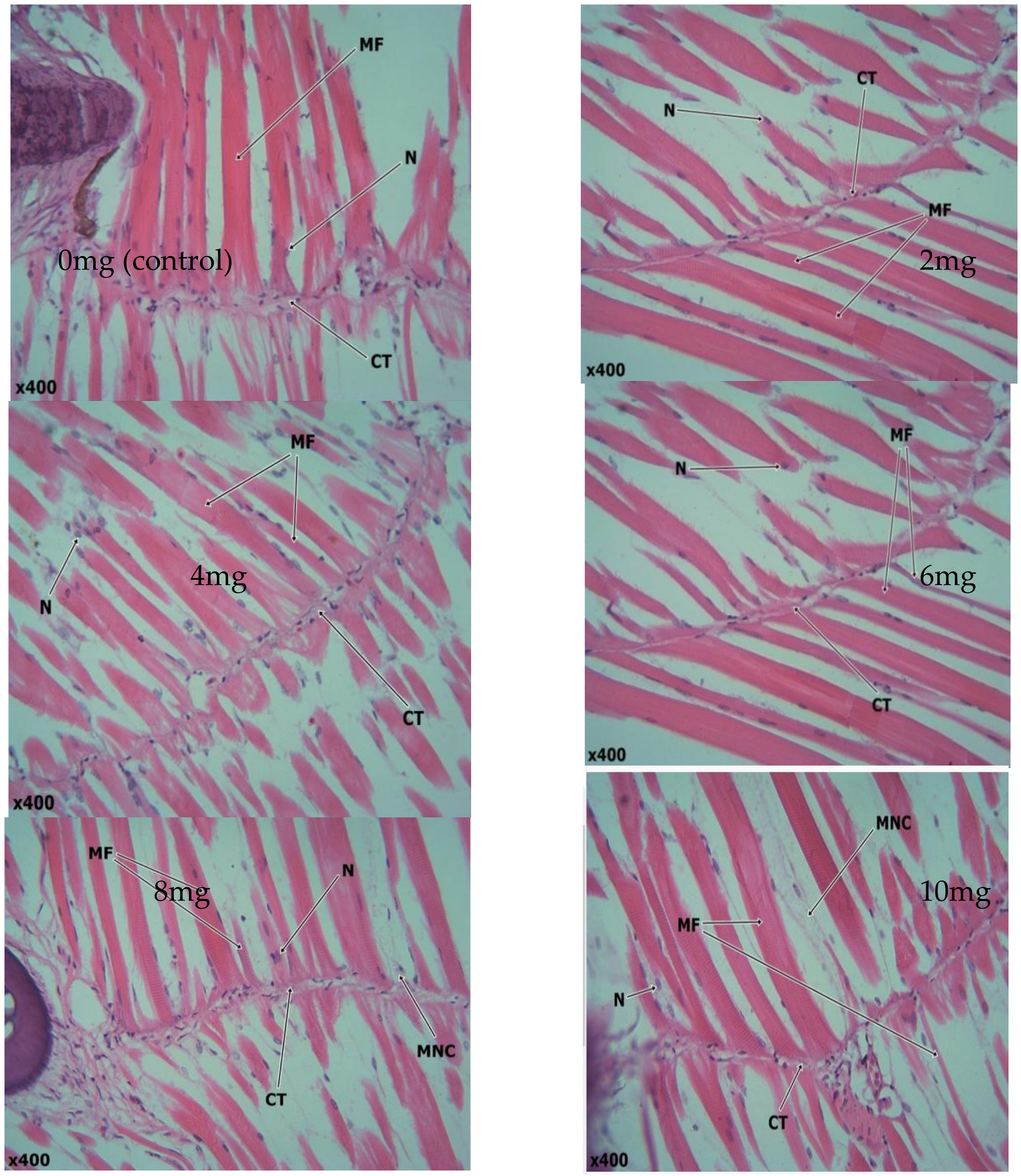

Plate 1: Photo micrographs of the muscle of *Clarias gariepinus* exposed to different Mercury Chloride Concentration

Keywords for Fish Muscle. MF= Muscle Fibre, N=Nucleus, CT=Connective Tissue, MNC= Myonecrosis

Table 2: Explanations to Plate 1: Photo micrographs of the muscle of *Clarias gariepinus* exposed to different Mercury Chloride Concentration

0mg	2mg	4mg	6mg	8mg	10mg
Photomicrographs show fish skeletal muscle tissue with evenly sized muscle bundles displaying typical but less obvious cross striations as well as thick intermuscular connective tissue.	Compared to 0mg there is mild variability in muscle bundle size, accentuation of cross striations and mild thinning of intermuscular connective tissue.	Compared to 0mg there is moderate variability in muscle bundle size, accentuation of cross striations and mild thinning of intermuscular connective tissue.	Compared to 0mg there is moderate to severe variability in muscle bundle size, accentuation of cross striations and reduction of muscle density as well as mild thinning of intermuscular connective tissue.	Compared to 0mg there is severe variability in muscle bundle size, accentuation of cross striations and severe reduction of muscle density with focal myonecrosis as well as moderate thinning of intermuscular connective tissue.	Compared to 0mg there is severe variability in muscle bundle size, accentuation of cross striations and severe reduction of muscle density with prominent myonecrosis as well as moderate thinning of intermuscular connective tissue.

The results of mortality of C*larias gariepinus* after 96 hours of exposure to mercury chloride are shown in table 3. It was observed that the mortality recorded in this investigation increased with the rise in concentration. The first death was noticed twenty minutes after the introduction of toxicant in the plastic with the highest concentration in mercury chloride (10mg/L). Olaifa *et al.,* 2004 reported the first death, three hours after introduction of toxicant in the exposure of *clarias gariepinus* to lethal and sub-lethal concentration of copper Datta and Raviras, 2002; Fafioye *et al*., 2014 and Okomoda *et al*., 2010 recorded the first death 36 hours after the exposure to acute toxicity treatments of *Clarias gariepinus* with synthetic pyrethroid. Guedenon *et al*., 2011 recorded the first death after thirty hours while treating *Clarias gariepinus* with 120mg/L of cadmium sulphate. The duration of resistance of *Clarias gariepinus* in the present study appeared to be lowest compared to those studies mentioned. Although *Clarias gariepinus* has proved to be very resistant to various toxicants, at has shown very little resistance to mercury. In the plastics with 8mg/L and 10mg/L concentrations of mercury chloride all the fish died. However, no death was recorded in the control plastic (0mg/L) devoid of mercury chloride. This observation confirmed that the mortality registered could entirely be attributed to the chronic effects of mercury chloride. The estimation of the lethal concentration values (LC_{50}) was carried out using the probit analysis. The result is shown in figure 1. From the graph of figure, the 96 hours LC_{50} value was determine to be 0.55mg/L with lower and upper confidence limits of 3.188mg/L and 6.961mg/L respectively at 95%. The 0.55mg/L concentration was at the point where mortality was increased and achieved. Ishikawa *et al*., 2007 recorded 0.22mg/L in an acute mercury toxicity treatment to O*reochromis nitoticus*.the median lethal concentration in this study was the highest recorded compared to those reports in the investigations mentioned. The chemical product being the same, the difference in the results could be attributable to the variety in species used. Here, *Clarias gariepinus* proved to be more resistant to mercury chloride than the various species involved on the studies already mentioned. However, the LC_{50} found in the present study was by far lower than those reported with *Clarias gariepinus* by Aguba and Ofojekwa, 2002; Ezike and Ufodike, 2008; Lawson *et al*., 2011 and Guedenon *et al*., 2011 which are respectively 204.17mg/L, for Datara innoxia, (334mg/L) for petrol, 129mg/L for lidane (Gamma Hexachloro-cyclo hexane) and 46, 11mg/L for cadmium sulphate. The difference might be due not only to the various substances and compounds used in the experiments but also the distinct environmental conditions.

Behavioural changes were observed in the catfish in the poisonous solutions. Those symptoms were hyperactivity and attempts to jump out due to skin irritation, restlessness, loss of balance, gulping for air due to respiratory rate impairment, darkening of the body, sudden and quick movement, rolling movement, back stroke, all these ending on death. The reaction to the toxic coat was more noticeable in the media containing the highest two concentrations of mercury chloride. The observations accord with those remarked by Ezike and Ufodike, 2008 and Guedenon *et al.,* 2011 during acute toxicity. This study of exposing *Clarias gariepinus* fingerlings to mercury chloride highlighted the high toxicity of mercury by the mortality recorded in the poisonous fish. Histopathological study offers a definitive toxicological effect of toxicants on the aquatic organisms. From the observation made in this study, a particular attention should be given to the use of product containing mercury. This study also showed the necessity to regulate the discharge of mercury in effluents from domestic and industrial sources into aquatic systems. There is a need for further research on this subject so as to establish standards for tropical fish such as *Clarias gariepinus* and other fish meant for human consumption throughout Africa in general and particularly in Nigeria.

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