Acute Toxicity of Mercury (HgCl$_2$) to African Catfish, *Clarias gariepinus*

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Abstract

In order to assess the acute toxicity of mercury on Clarias gariepinus, 108 fish of mean weight 51.27 g ± 2.01 and mean length 20. 2 cm ± 0.72 were divided into six groups of six fish each. The different groups were exposed to the different concentrations of 0 mg/L, 0.3 mg/L, 0.5 mg/L, 0.8 mg/L, 1 mg/L et 1.50 mg/L for a period of 96 hours. The experiment was triplicated. The results revealed that all the fish of groups exposed to 0 mg/L of HgCl$_2$ (control) survived whereas all the fish of groups exposed to 1 mg/L and 1.5 mg/L died. The determination of 96 hours LC50 was carried out by computing the mortality results in Probit program of SPSS (version 17.0). The median lethal concentration was 0.60 mg/L with lower and upper confidence limits of 0.135 mg/L and 3.519 mg/L respectively at 95%.

Introduction

In modern times, one of the main threats to the health of ecosystems 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.

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[1-3]. Their introduction into aquatic environment is caused by direct or indirect agricultural and industrial discharges.

Heavy metals contamination could be detrimental to ecological balance of the recipient environment and to a diversity of aquatic organisms[4,5]. Since fish are animals particularly affected by these pollutants they are widely used to evaluate the health of aquatic ecosystems[6].

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 released by industries in the environment [7]. Once in the aquatic ecosystem, part of the inorganic mercury can be microbiologically converted into methyl-mercury. The resulting organomercury compounds are rapidly absorbed by the gastrointestinal tract where 90% of ingested mercury is directly absorbed by fish from water through gills, skin and digestive tract[8]. Once contaminated by Hg, fish suffer pathological alterations, with consequent inhibition of metabolic processes, haematological changes, and decline in fertility and survival [9].

The aim of this study was to determine the acute toxicity of mercury chloride to Clarias gariepinus. C gariepinus was chosen as an experimental animal on the basis of important criteria. The organism is a representative species and widely used in aquaculture throughout Africa. Moreover it is largely tolerant of high concentration of heavy metals. In addition, *Clarias gariepinus* is a hardy fish and can survive difficult conditions.

Material and Methods

One hundred and fifty juveniles of *Clarias gariepinus* of the same brood were purchased from a reputable fish farm (Yanaplaza) in Lagos and transported in an oxygen bag to the aquaculture laboratory of the Faculty of Science of University of Lagos. Their mean weight and length were 32 g ±1.82 and 18.2cm ± 0.61 respectively. The fish were kept in plastic tanks (30x 30x60cm) which were half filled with dechlorinated water. They were acclimatized to laboratory conditions over six weeks. During acclimatization, the juveniles were fed with commercial fish feed known as Coppens at 4% of their body weight. Proximate composition of Coppens is shown in table 1.The Juveniles were fed thrice daily (morning, afternoon and evening) and the water was changed every twenty four (24) hours to prevent the accumulation of waste metabolite and food particles. They were kept at 12 hours of photoperiod. During the whole period of acclimatization, the mortality recorded was below 2%. At the end of acclimatization the new mean weight and length were 51.27g ± 2.01 and 20.2 cm ± 0.72 respectively. The juveniles were randomly divided into six groups of

eighteen fish and each group subdivided into a set of three subgroups consisting of six fish each. The eighteen subgroups were kept in eighteen different tanks in 15 liters of water. The six groups were respectively exposed to 0 mg/L, 0.3 mg/L, 0.5 mg/L, 0.8 mg/L, 1.5mg/L and 3 mg/L of mercury chloride (HgCl$_2$). In other words, there were five experimental groups and one control group, each group having three replicates.

In respect of the preparation of the stock and test solutions of mercury the test chemical used for the experiment was anhydrous mercury chloride. Mercuric Chloride powder (HgCl$_2$ =271.50; minimum assay Hg: 98%) was purchased from "General Purpose reagent BDH Chemicals Ltd Poolo England". The chloride form of the metal was chosen because of its lower toxicity compared to the other forms of mercury[10]. After a range finding test, the concentrations prepared for the experiment were 0 mg/L, 0.3 mg/L, 0.5 mg/L, 0.8 mg/L, 1.5mg/L and 3 mg/L of mercury chloride (HgCl$_2$) respectively. A stock solution of 100mg/L (0.1g/l) of the mercury was prepared by adding 100 mg of mercury to 1 liter of distilled water. The different volumes of the solution stock used are shown in table 2.

Twenty four hours (24) prior to the exposure to mercury chloride and during the whole period of exposure of 96 hours the feeding of the fish was stopped. During our investigation, methods for acute toxicity test recommended by UNEP were implemented[11]. A static renewal bioassay technique in which the test media were renewed at the same concentration once every twenty four (24) hours was adopted [12]. The bowls were covered with mosquito mesh–sized nets to prevent fish from jumping out or move from one bowl into another. The set up was monitored hourly to observe changes in fish behavior and remove dead fish. A fish was considered dead when observed to be totally immobile with no opercula movement seen when probed with a glass rod.

With respect to the water used in the experiment, its physico-chemical parameters were measured after exposure of the tap water to air in order to lose chlorine. These parameters included temperature, dissolved oxygen, and the potential of hydrogen (pH). They were measured using Horiba multi-parameter by submersing the sensor in the water at a depth of 10 cm from the water surface. All parameters displaced on the screen of the apparatus.

Table - 1
Proximate centesimal Composition of Coppens[13]

Moisture	Ash	Crude protein	Crude lipid	Crude fiber
8.2%	9.5%	45%	12%	1.5%

With regard to statistical analysis, all the mortality results were treated with the computer statistical package (SPSS, version 17.0) SPSS. Linear regression analysis of Probit program was carried out to determine the median lethal concentration (LC50). Also the student test was carried out to assess if the differences between experimental groups and control were significant. The difference is regarded as highly significant if *P* value is lower than 0.01, statistically significant if *P* value is lower than 0.05, and non significant if *P* value is higher than 0.05.

Results and discussion

Bioassay is a necessity to determine the concentration of a toxicant, which could be allowed in waters without adverse effects on the living organisms[14,15].

The physico-chemical parameters of the water measured for were temperature, dissolved oxygen and pH. The results are given in table 3.

Table – 2
Preparation of the Toxicant Concentrations Used

Required concentration of HgCl$_2$ (mg/L)	Volume of stock solution (mL) used to meet the required concentration	Total volume of stock solution (mL) to meet the required solution in 15L
A: 0.0	0.0	0.0
B: 0.3	3	45
C: 0.5	5	75
D: 0.8	8	120
E: 1.5	15	225
F: 3	30	450

Notice: Prior to addition of the calculated volume of the toxicant, the same amount of tap water was removed from the tank.

Table - 3
The physico-chemical characteristics of the water used

Parameters	Values
Temperature	27ºC
Dissolved oxygen	7.44 mg/L 6.5
pH	7.02 6.5–8.

The physico-chemical parameters recorded were within the permissive limits fixed by WHO which are 6.5 and 6.5- 8.0 respectively for dissolved oxygen and pH.

The results of mortality of *Clarias gariepinus* after 96 hours of exposure to mercury chloride are shown in figure 1. It was observed that the mortality recorded in this investigation increased with the rise in concentration. The first death was noticed thirty minutes after the introduction of toxicant in the bowl with the highest concentration in mercury chloride (3mg/L).

Olaifa et al.[6] reported the first death three hours after introduction of toxicant in the exposure of *Clarias gariepinus* to lethal and sub-lethal concentrations of copper. Datta and Kaviraj [16], Fafioye *et al.* [17] and Okomoda et al. [13] recorded the first death 36 hours after the exposure to acute toxicity treatments of Clarias *gariepinus* with Synthetic Pyrethroid

Deltamethrin, *Parkia biglobosa* and *Raphia vinifera* extracts and Formalin respectively. Guedenon et al.[18] remarked the first death after thirty hours while treating *Clarias gariepinus* with 120 mg/L of cadmium sulphate.

The duration of resistance of *Clarias gariepinus* in the present study appeared to be the lowest compared to those in the aforementioned studies. Although *Clarias gariepinus* has proved to be very resistant to various toxicants [6, 13, 16-18], it has shown very little resistance to mercury.

In the tanks with 1.5 mg/L and 3 mg/L concentrations of mercury all the fish died. However no death was recorded in the control tank devoid of mercury. This observation confirmed that the mortality registered could entirely be attributable to the chronic effects of mercury. The estimation of the lethal concentration values (LC 50) was carried out using the linear regression of Probit program. The result is shown in figure 2.
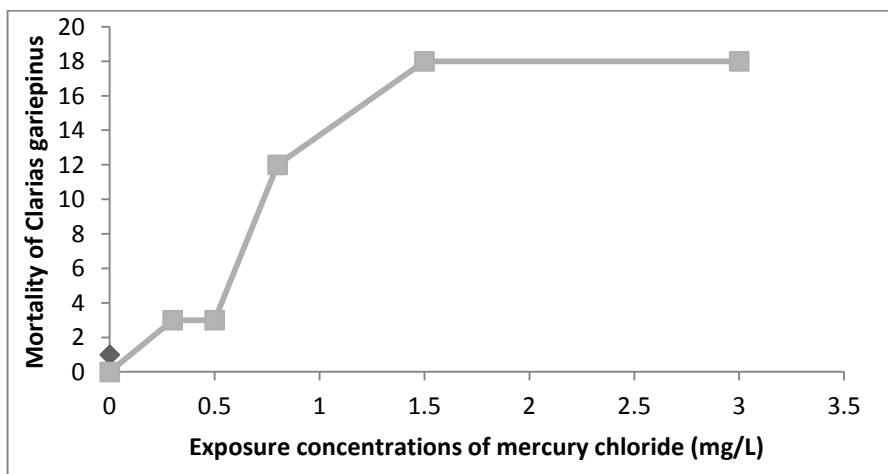

Figure - 1
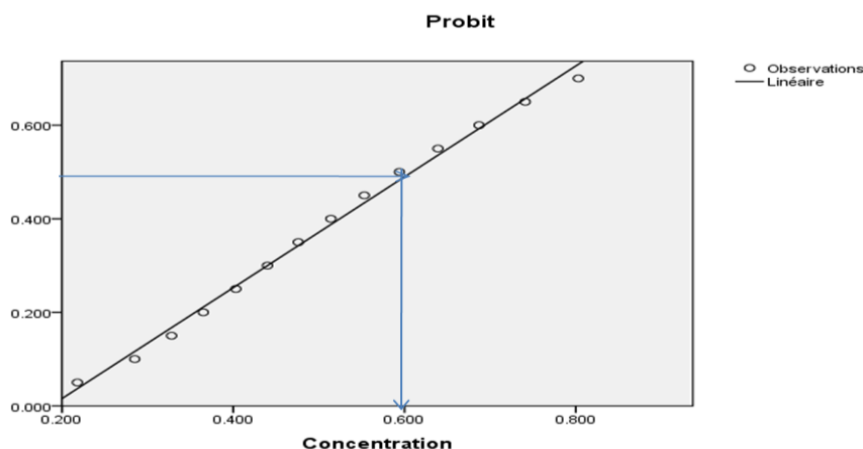**Graph depicting the evolution of mortality with the varying concentrations**

Figure - 2
Lethal concentration (LC50) of *Clarias gariepinus* exposed to mercury

From the graph of figure 2 the 96-hour LC50 value was determined to be 0.60 mg/L with lower and upper confidence limits of 0.135 mg/L and 3.519 mg/L respectively at 95%. The LC50 found in this investigation is similar to that of Hirt and Domitrovic[19] in the exposure of *Aequidens portalegrensis* to acute concentrations of mercury chloride. Slabbert and Venter [20] reported LC50 of 0.200 mg/L in the treatment of *Poecilia reticulate* with mercury chloride. Shyong and Chen [21] found LC50 values of 0.168 mg/L and 0.161 mg/L in the exposure of *Variocorhinus barbatulus and Zacco barbata* respectively.

Ishikawa *et al*. [22] recorded 0.22mg/L in an acute mercury toxicity treatment to Oreochromis *niloticus*. The median lethal concentration in our study was the highest recorded compared to those reported in the aforementioned investigations. 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 in the studies already mentioned.

However the LC50 found in the present study was by far lower than those reported with *Clarias gariepinus* by Ayuba and Ofojekwu [23], Ezike et Ufodike [24], Lawson *et al*. [25] and Guedenon *et al*. [18] which are respectively (204.17 mg/L) for *Datura innoxia*, (3,34mg/L) for petrol, (1,29mg/L) for Lindane (Gamma Hexachloro-Cyclohexane) 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.

Behavioral changes were observed in the catfish in the poisonous solutions. Those symptoms were hyper-activity and attempts to jump out due to skin irritation, restlessness, respiratory distress, loss of balance, gulping for air due to respiratory rate impairment, darkening of the body, sudden and quick movement, rolling movement, back stroke, excessive accumulation of mucus, all these ending in death. The reaction to the toxicant was more noticeable in the media containing the highest two concentrations of mercury chloride.

These observations accord with those remarked by Hirt and Domitrovic[19], Oti[26], Oshode *et al*,[27], Ezike et Ufodike [24] and Guedenon *et al*. [18] during acute toxicity tests. The accumulation of mucus on the body could be connected to the intensification of mucus secretion of mucous cells activated by the toxicants[28,29]. The ensuing death might be due to increased heart failure, hypertension, gastric hemorrhage, convulsion, paralysis, heart failure and suffocation[30].

Conclusion

The investigation of exposing *Clarias gariepinus* to mercury highlighted the high toxicity of mercury by the mortality recorded in the poisonous fish and hence a particular attention should be given to the use of products containing mercury. This study also demonstrated 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 *C.gariepinus* and other fish meant for human consumption in throughout Africa in general and particularly in Benin.

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