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TOXIC EFFECT OF MERCURY ON THE FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT: The toxic effect of Mercury on freshwater fish *Oreochromis mossambicus* was studied. The LC_{50} values of mercury in *O. mossambicus* for 24, 48, 72 and 96 hours were 0.73, 0.69, 0.63 and 0.58 ppm respectively. Tilapia fish was silvery white in body in the control group throughout the experiment. The body colour changed from original silvery white to dark colour in heavy metal treated fish. The fish maintained in freshwater behaved normal as usual. But when the fish was exposed to mercury, erratic swimming, abnormal posture, dis-balance, sluggishness, imbalance in posture, increase in surface activity, opercular movement, gradual loss of equilibrium and spreading of excess of mucus all over the surface of the body were observed.

KEYWORDS: *Oreochromis mossambicus,* Acute Toxicity, Mercury, LC_{50}, Behaviour.

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1. INTRODUCTION

Pollution is the negative feedback of the environment which affects living organisms. The aquatic environment too is not spared from the adverse effects of pollution. In recent times interest has been focused on rivers and estuaries as these are considered major sources of pollutants of coastal seas and oceans. Human beings have been responsible for marine pollution, as they have introduced directly or indirectly, harmful waste substances into the marine environment. Estuaries and rivers have not been spared with the result the deleterious effects have paved the way for health hazards to human beings [1]. Industrialization, intensification of agriculture and rapid growth of human population have led to the increased discharge of pollutants which are harmful to the above biotopes.

Four principal categories of pollutants which jeopardize the marine environmental resources are viz., radionucleotides, petroleum hydrocarbons, pesticides and heavy metals. Among these, heavy metals are most dangerous because of their stability in the biological system. Metallic contamination in a stream may constitute a danger to public health if the water is to be used subsequently for drinking and other purposes. Increasing concern has been voiced in recent years on the effects of the different heavy metals on the freshwater ecosystems by surface run of either from rain or other sources. Rapid industrialization and urbanization have led to the utilization of heavy metals including mercury on a larger scale. These metals ultimately enter the aquatic ecosystems directly as effluents or indirectly by precipitation, thereby causing deleterious effects to aquatic life at an alarming level. Trace metals in general have a strong attraction to biological organisms and the slow acclimation of these chemicals in the biological systems, have led to their accumulation in the body tissues, resulting in the stimulation, irritation and inhibition of a variety of body functions. At present increased food production to meet the energy requirements of the ever increasing population, is a major problem of our country. To meet these requirements man has employed modern techniques both in agricultural and industrial production. But at the same time the population explosion possess a great threat to human society. The organisms inhabiting the marine, estuarine and fresh water environments are exposed to lethal levels of heavy metal pollutants due to industrialization and modern agricultural practices. Some of the heavy metals like iron, copper, manganese, magnesium and zinc are essential for the metabolism of organisms at optimal concentrations [2], but if the concentrations of these metals increase in the environment they may interfere with the metabolic activity in the organisms. The nonessential elements viz., mercury, cadmium, silver and lead are toxic to aquatic organisms even at very low concentrations. Mercury is used in large scale in industries, agriculture, military applications, medicines and dentistry and is considered most toxic amongst the heavy metals. The mercury released in the environment may enter the food chain by rapid diffusion and tight binding to protein, principally as methyl mercury. Mercury in any chemical form has the capability to denature proteins, inactivate enzymes and cause severe disruption in the physiological processes of any tissue with which it may come in contact in sufficient concentration [3,4,5,6]. Mercurials are well recognized neurotoxin and capable of inducing neuronal necrosis [7]. As the disruption of physiological processes or neurological effects being late manifestations of pollution, the need has continuously been felt to identify some non-invasive types of sensitive bio-indicators from early warming and monitoring the presence of pollutants in the environment. The heavy metal contaminations result in epidemic diseases such as “Minamata” [7], “Itai-Itai” [8] etc. Among the non-essential metals, arsenic, mercury, cadmium, lead and silver, poses serious threat [9]. They have many sources to reach the coastal system [10]. The human destructive influence on the aquatic environment is in the form of sublethal pollution which results in chronic stress conditions that have negative effect on aquatic life. The main source of freshwater pollution can be attributed to the discharge of untreated waste, the dumping of industrial effluent and the run-off from the agricultural fields. Mercury pollution in aquatic ecosystems has received a great deal of attention since the discovery of mercury as the cause of Minimata disease in Japan in the 1950s [11]. The fate of mercury in the environment depends on the chemical form of mercury released and the environmental conditions. The elemental mercury, the inorganic mercury and the methyl-mercury are the three most important forms of mercury in natural aquatic environments. Most mercury is released into the environment as inorganic mercury, which is primarily bound to particulates and organic substances and might not be available for direct uptake by aquatic organisms [12]. Toxicity is influenced by the form of mercury, environmental media, environmental conditions, sensitivity or tolerance of the organism, and its life history stage. The inorganic mercury is less acutely toxic to aquatic organisms than methyl-mercury, but the range in sensitivity among individual species for either compound is large. The undesirable effects of heavy metals and other toxic elements has long been recognized for creating havoc in the aquatic and terrestrial environments. Acute poisoning of aquatic environment leading to ravages of lives occurs from either the occasional industrial and haulage accidents or during routine dumping and unconscious leaching practices. Long term exposure to heavy metals and toxic elements has cataclysmic implications to our environment. It may prove to be hazardous and chronic to the animals living in water or associate with it either directly or in a roundabout way. Indeed, the presence of certain metals like iron, copper, cobalt, calcium and zinc at certain level in their exploitable forms is essential for life as a source of minerals. The mere presence of metals does not constitute a major threat to fisheries and other aquatic animals' health. But certain metals for instance, mercury, cadmium, silver, lead and arsenic have no nutritive value and are critically idiom as environmental contaminants. With this in view an attempt has been made in the present study to investigate the toxicity impact of heavy metal mercury on *O. mossambicus* since very little information is available in this important edible freshwater fish.

2. MATERIALS AND METHODS

The freshwater healthy fish, *O. mossambicus* of the weight (8±1g) and length (7±0.5 cm) were selected for the experiment and were collected from Ponds in and around Adirampattinam. Fish were screened for any pathogenic infections. Glass aquaria was washed with 1% $KMnO_4$ to avoid fungal contamination and then sun dried. Healthy fishes were then transferred to glass aquaria (35×20×20 cm) containing dechlorinated tap water (Temperature – 28 ± 2°C**;** total hardness – 518 ± 23 mg/l; DO - 5.6 ± 0.2 mg/l; salinity - 1.2 ± 0.13 ppt and pH - 7.8 ± 0.04). Fish were acclimated to laboratory conditions for 10 to 15 days prior to experimentation. They were regularly fed with commercial food *ad libitum* and the medium (tap water) was changed daily to remove faeces and food remnants.

Metal for toxicity studies (Mercury)

Toxicity studies were conducted to obtain reliable data regarding the effects of the toxicant on the test species. Static bioassay tests were conducted as per standards set by the American Public Health Association [13]. The toxicant sample used possessed the following characteristics.

Molecular weight	:	271.52
Colour	:	White powder poison
Specific gravity	:	5.440^{25}
Melting point	:	276°C
Boiling point	:	302°C
Solubility cold	:	Freely soluble
Hot	:	61.3^{100}

Acute toxicity test

Toxicity tests were conducted in accordance with standard methods [14]. Stock solution of mercury with a concentration of 1 g per litre (equivalent to 1 ppt) was prepared in distilled water and different dilutions were prepared by adding required amount of distilled water. Based on the progressive bisection of intervals on a logarithmic scale, log concentrations were fixed after conducting the range finding test. The fish were starved for 24 hours prior to their use in the experiments as recommended by storage to avoid any interference in the toxicity of the heavy metal mercury by excretory products. After the addition of the toxicant into the test tank with 10 litres of water having twenty fish, mortality was recorded after 24, 48, 72 and 96 hours. Five replicates were maintained simultaneously. Percent mortality was calculated and the values were transferred into probit scale. Probit analysis was carried out as suggested by Finney [15]. Regression lines of probit against logarithmic transformations of concentrations were made. Confidential limits (upper and lower) of the regression line with chi-square test were calculated by a computerized programme for Finney's [15] probit analysis.

3. RESULTS AND DISCUSSION

Mercury caused 50% mortality of *O. mossambicus* (96 hours) at 0.58 ppm. The LC_{50} values obtained at 24, 48, 72 and 96 hours exposures and the 95% confidence limits for the heavy metal revealed that mercury showed higher toxicity. The LC_{50} values of mercury for 24, 48, 72 and 96 hours were 0.73, 0.69, 0.63 and 0.58 ppm respectively (Table 1; Fig.1-4).

Table 1: Percent mortality of *O. mossambicus* exposed to different concentrations of mercury for different periods

Hours of Exposure	LC_{50}	L.C.L	U.C.L	Regression Equation	Calculated χ^2 value	Table χ^2 value
24	0.7386002	0.7635602	0.6942667	Y= 7.310755 + 12.68144 X	10.15498	9.49
48	0.6964271	0.7227551	0.6309157	Y= 6.698146 + 10.4917 X	15.11307	11.07
72	0.6382911	0.6960618	0.6461523	Y= 6.387431 + 7.846049 X	0.2337875	11.07
96	0.5835661	0.6302218	0.5952265	Y= 5.837121 + 7.421019 X	0.7186737	12.59

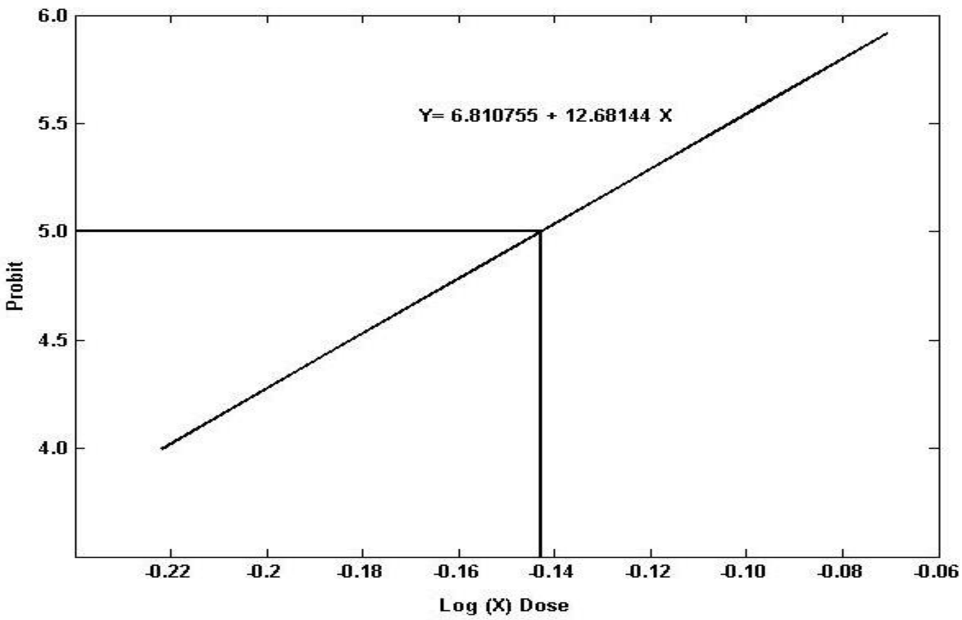

Fig.1. Regression line (based on probit analysis) of log concentration of mercury Vs Percent mortality of *O. mossambicus* 24 Hours

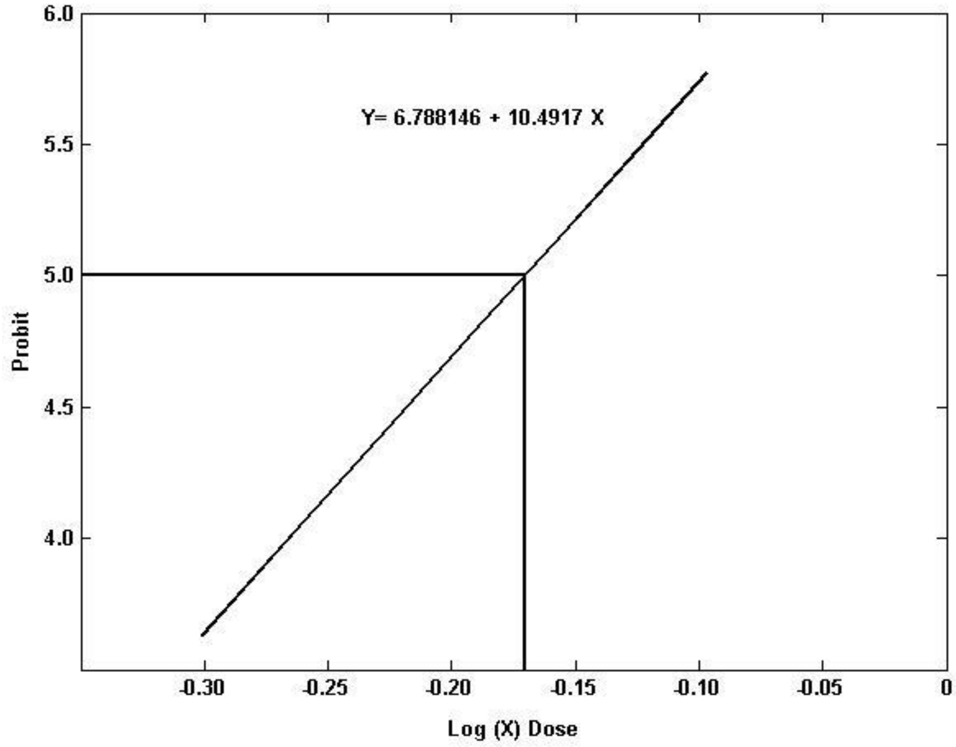

Fig.2. Regression line (based on probit analysis) of log concentration of mercury Vs Percent mortality of *O. mossambicus* 48 Hours

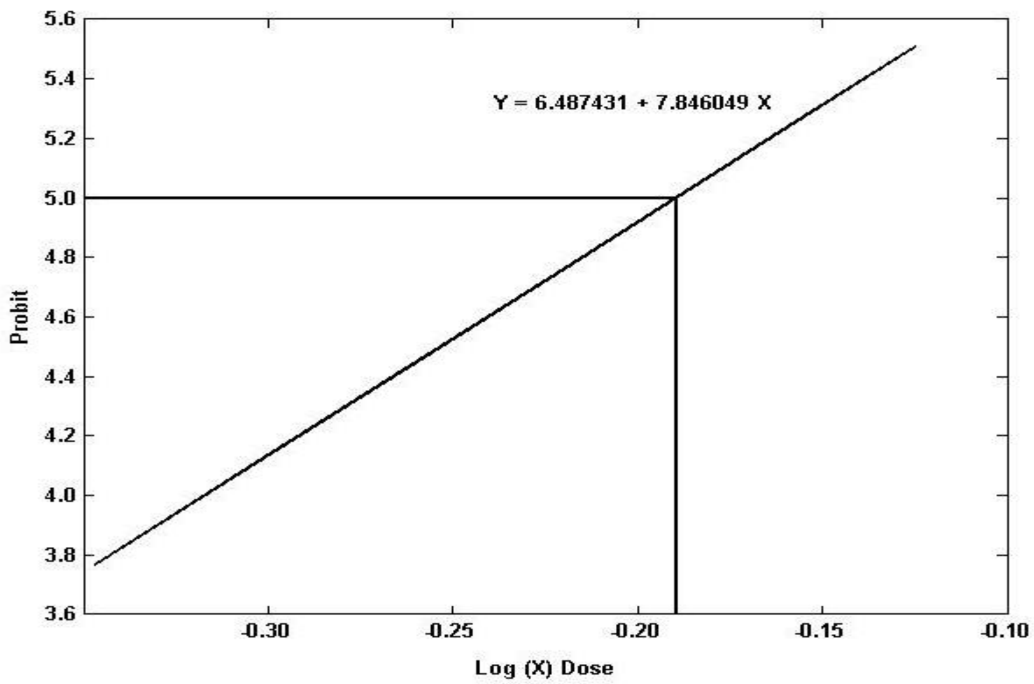

Fig.3. Regression line (based on probit analysis) of log concentration of mercury Vs Percent mortality of *O. mossambicus* 72 Hours

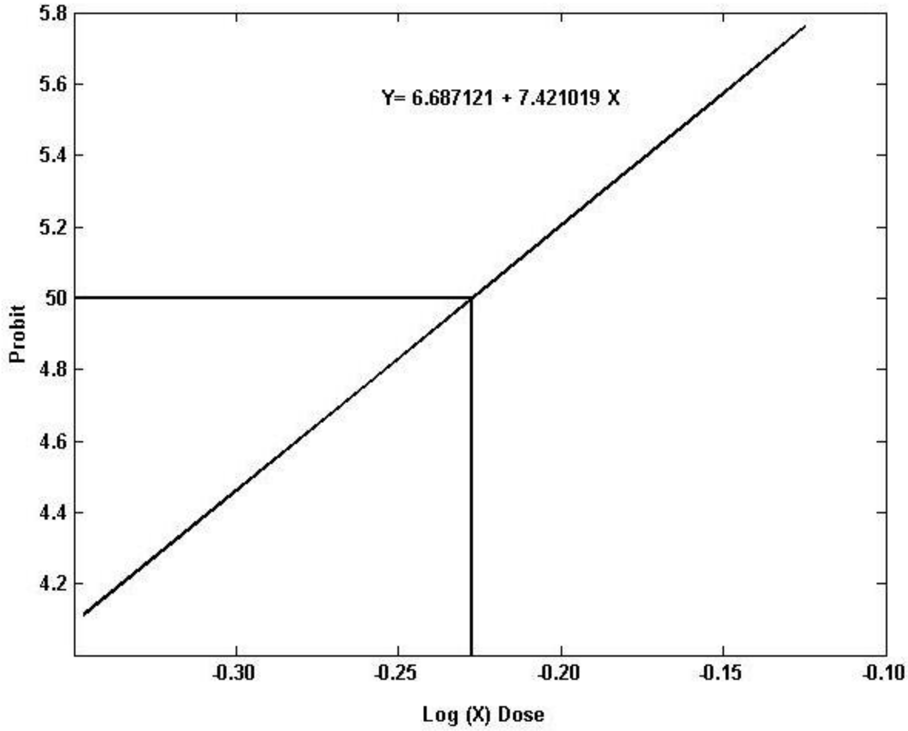

Fig.4. Regression line (based on probit analysis) of log concentration of mercury Vs Percent mortality of *O. mossambicus* 96 Hours

O. mossambicus was silvery white in body in the control group throughout the experiment. The body colour changed from original silvery white to dark colour in heavy metal treated fish. The fish maintained in freshwater behaved normal as usual. But when the fish was exposed to mercury, erratic swimming, abnormal posture, dis-balance, sluggishness, imbalance in posture, increase in surface activity, opercular movement, gradual loss of equilibrium and spreading of excess of mucus all over the surface of the body were observed. A survey of LC_{50} values of different heavy metals to the fish for different periods of exposure reveals the occurrence of a wide differences between duration of exposure and types of fishes [16,17,18,19,20,21,22,23,24,25]. Changes in body colour have been reported in *Anabas testudineus* after exposure to monocrotophos [23], and *Cyprinus carpio* to ammonia stress [26]. The behavioural changes are considered directly related to complex physiological responses and have often been used as a sensitive indicator of stress [27]. Tilapia fish exposed to sub lethal concentrations of mercury settled immediately at the bottom of the aquarium. The shoal was disturbed in the first day itself. Fish occupied larger area than to that of control group. They were spread and found swimming independently. Irregular, erratic and darting movements with imbalanced swimming activity and attempt to jump out of the toxic medium were observed. Similar behaviour patterns were observed in fish, trout and *L. rohita* exposed to fenvelrate [28]. Increased opercular movements, loss of equilibrium, erratic swimming and jerky movement and mucous secretion all over the body were observed in *Heteropneustes fossilis* after exposure to rogor and endosulphan pesticides [29]. Erratic swimming, imbalance in posture, increased surfacing activity with gradual decrease in opercular movement, loss in equilibrium, excess of mucus all over the body surface followed by sluggishness and death of *A. testudineus* after exposure to monocrotophos was reported by [23]. Surfacing phenomenon was also seen on second day. On third day swimming behaviour was in cork-screw pattern rotating along horizontal axis. Fish on fourth day showed signs of tiredness and lost positive rheotaxis characterized by weakness and apathy. Fish frequently came to water surface. Similar trend has observed in *O. mossambicus* exposed to endosulfan [30]. Finally fish turned upside down and dead. Fish in sublethal concentrations were found under stress but that was not fatal. The settlement of the fish to the bottom of the tank on addition of endosulfan reveals the avoidance behaviour of fish as observed in trout and tilapia by Murthy [28] and Devi Swetharanyam [30]. Shivakumar *et al.* [31] also observed the avoidance behaviour in *Ctenopharyngodon idellus* on exposure to endosulfan. Irregular and erratic swimming indicates loss of equilibrium [30]. This must be due to the damage caused at the centre associated with the maintenance of equilibrium in the brain [32]. Many workers have observed erratic swimming, equilibrium loss and surfacing phenomenon in the fish following pesticide exposure. Surfacing phenomenon shown by the fish might be to gulp maximum possible air to ease the tension. Rao and Rao [32] also observed this phenomenon in the fish, *Channa punctatus* exposed to two different pesticides viz., carbaryl and phenthoate. In relation to this they also reported that the surfacing phenomenon was due to hypoxic condition of the fish. Increased opercular movements were seen in the fish, *O. mossambicus* exposed to mercury, which was in accordance to the report put forth by Amitakiran and Jha [33] in *Clarias batrachus* exposed to herbicide, herboclin. The rapid opercular movements may be due to accumulation of mucous over gill due to the toxicant [21,34]. Similar findings were observed by Prasanth *et al.* [35], when freshwater fish *C. mrigala* exposed to cypermenthrin. The fish *O. mossambicus* exhibited irregular, erratic darting movements with imbalanced swimming activity. Occasionally the fish tried to jump out of the toxic medium, which shows the avoidance behaviour of the fish to the toxicant. Similar behavioural patterns were observed in *L. rohita* exposed to endosulfan [31]. The change of body colour, behavioural changes such as irregular swimming movements, loss of equilibrium, restlessness and excess secretion of mucous suggest that *O. mossambicus* has undergone chemical stress when exposed to heavy metals and the present study could be taken as an indicator of heavy metals pollution. The changes in the swimming behaviour and the opercular movements were more obvious in fishes subjected to prolonged exposure period at the acute concentration level while it was not so pronounced at the sub-lethal concentration. The impaired equilibrium observed in the present study concurs with the results obtained in the fresh water fishes. *Barbus aurilus* and *Lepidocephalecthyes quntea* exposed to lead, mercury, copper and zinc [36]. It was also noticed that the swimming activity of the mercuric chloride exposed fish significantly decreased. The increased opercular movements are inversely proportional to the decreased swimming activity. Increased opercular movements have been observed in *Tilapia mossambica* exposed to mercuric chloride [37]. Similar observations were made in several fishes viz., Stickle backs [38], Minnows [39] and *Salmo gairdneri* [40]. Preliminary range finding and screening tests have been made for establishing the water quality and also to serve as a baseline study for 96 hr sub-lethal and median-lethal exposure in toxicological investigations. Limited data are available on the relative toxicity of mercury compounds to aquatic organisms. Jackim *et al.,* [41] and Klavnig *et al.,* [42] reported acute toxicity levels during 96 hr. of exposure. The LC_{50} values for mercuric chloride toxicity in *Fundulus heteroclitus* were reported as 230 and 201 µg/l respectively. Portmann [43] obtained a 48 hr. LC_{50} value as 3.3 ppm of mercury for the fish *Pleuronectes flesus*. Saxena *et al.* [44] reported LC_{50} values at 24 hr with mercuric chloride for *Danio malbaricus* and *Puntius ticto* 2 mg/l and 0.30 mg/l respectively. Dhanekar *et al,* [45] obtained LC_{50} values at 96 hr mercuric chloride exposure for *Puntius sophore*, *Labistes reticulates*, *Sarotherodon mossambicus*, *Channa punctatus* and *Heteropneustes fossilis* as 0.15 mg/l, 0.25 mg/l, 0.50 mg/l, 1.0 mg/l and 1.0 mg/l respectively. Since the publication of the standard bioassay procedures [46,47,48,49], there have been a multitude of tests developed by researchers for evaluating or measuring toxicity using various organisms living in different environments and representing different levels in the food chain [14]. It is well known that toxicity will depend upon (a) the chemical form of the metal (b) the presence of other metals (c) the physiological status of the organisms and (d) the environmental, physico-chemical parameters like temperature, dissolved oxygen and the pH of the water. The toxicity of the metal is also dependent upon the residence time of the metal concerned. Generally most metals have long residence timers and hence, exert their toxic effects over long periods.

4. CONCLUSION

The LC_{50} can be used as a relative measure to study the impact of the heavy metal concentration on test fishes at different intervals. This toxicity test on the effect of mercury on *O. mossambicus* offers a rapid method for assessing the heavy metal impact on this fish. This type of preliminary investigations can be useful for deriving the safe level of heavy metal concentration (especially mercury) that can be released into the aquatic environments.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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