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Critical Toxicity Bioassays of Mercuric Chloride on the freshwater fish *Clarius gariepinus* (Burchell, 1822)

Chandanshive Navnath Eknath
Zoology Department, Fergusson College, Pune, SPP University Pune, Maharashtra State, Pin-411004, India.

ABSTRACT

The critical toxicity of Mercuric chloride to ***Clarius gariepinus (Burchell, 1822)*** *was examined with static bioassay's. The 96 h LC50 of mercuric chloride was determined by the graphical method of Krouwer and Monti (1995). The value of 96 h LC50 for mercuric chloride is found to be 1.8 mg/litre. During experiments the exposed fishes displayed several behavioural changes before death such as anxiety, rapid swimming, and loss of balance, secretion of enormous amount of mucus, respiratory distress and haemorrhaging of gill filaments amongst others. Fishes were remained in a vertical position for hours together with the anterior side or terminal mouth up near the surface of water, trying to gulp the air and the tail in a downward direction. Opercula ventilation rate as well as visual examination of dead fish indicates lethal effects of the Mercuric chloride on the fish.*

Introduction

The aquatic ecologies play a significant role in the lifecycle of aquatic organisms due to its physico-chemical properties and formation of food value. However, the major aquatic bodies of world are being heavily polluted due to the activities like urbanization, industrialization, agricultural and other activities. These activities has exploited the water resources and disturbed the ecological balance between living and non-living components. The heavy metals like Mercury, Cadmium and Nickel etc. are being discharged into ecosystem through industrial processes, sewage disposal, soil leaching and rainfall. These metals are highly toxic even at low concentration and affect the existence of fishes and other aquatic organisms. The biological effect of heavy metals in the aquatic environment is adverse mainly due to the complex nature.

Mercury has been used in many manufacturing industries such as plastics, chlorine, caustic soda and caustic potash, and agricultural fungicides. These industries are responsible for releases of mercury and other its compounds into the water. Mercury occurs in two forms i.e. inorganic and organic forms. Inorganic mercury is the most common form of the metal released in the environment by industries, causing more severe effect on aquatic organisms than that of the organic form of mercury [1].

Due to bio-magnifying tendency of mercury in aquatic environment and it became toxic to fish and wildlife. Mercury is become a wide-ranging pollutant of substantial aquatic environment. However, mercury is also occurs naturally in the environment, but its concentrations have increased due to its use in the industrial processes [2]. The methyl mercury (MeHg) is inorganic form of mercury and is the highly toxic that quickly accumulates in the tissues of organisms [3]; [4]. In sediments of marine and freshwater anaerobic sulfate-reducing bacteria synthesizes Methyl mercury, and aquatic animals are subjected to contamination by methyl mercury [5]. The lethal effects of Mercury exposure are associated with its nervous system. Mercury is also concerned with immune suppression, endocrine disruption, physical malforma.

The median lethal concentration (LC50 for 96 hrs) of cadmium in *L. calcarifer* was determined to be 6.08 ppm and for mercury 1.03 ppm. When fishes *L. calcarifer* were subjected to acute toxicity of mercury and cadmium in and reported that the fish was more susceptible to mercury, followed by that cadmium. This study indicates that mercury was more toxic (1.03 ppm) metal than that of cadmium (6.08 ppm) [6]. However, [7] have recorded LC50 value of mercury in the fish *Cyprinus carpio* was 0.5 ppm. In the case of *Rasbora daniconius*, 0.80 ppm was reported as LC50 value for mercury [8].

Material method

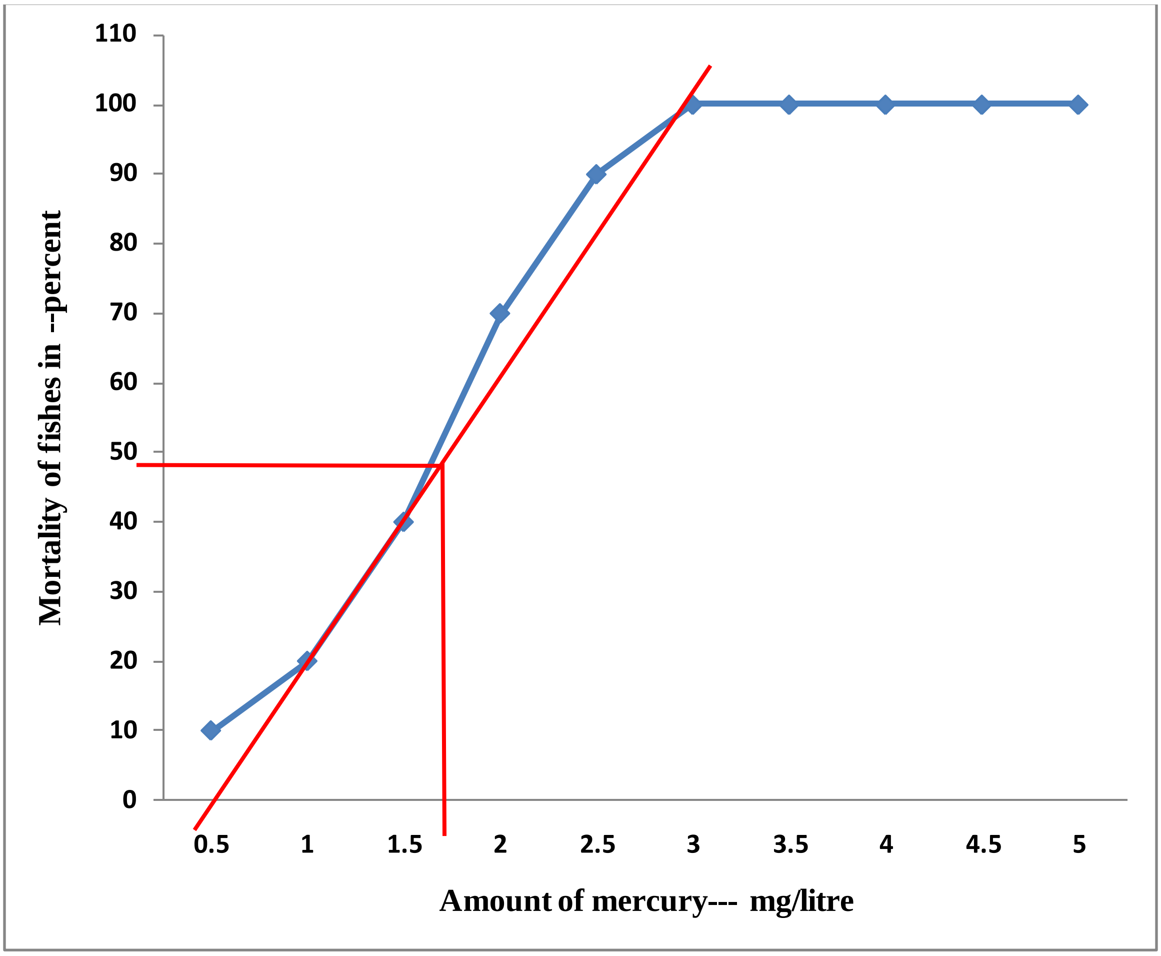
The freshwater fish *Clarius gariepinus* (Burchell, 1822) were separated from *Clarius batrachus* and collected fisherman near Kumbharwada were used for bioassay studies. The fishes were brought into the laboratory for the acclimation. The fish *Clarius gariepinus* (Burchell, 1822) were selected irrespective of sex for experiments. The size or length ranged from 30 cm to 34.5 cm and weight ranging from 251gm to 459gm. Fishes were acclimated in glass tank in the laboratory for seven days as per the method in APHA [9]. They were divided into different groups each containing ten fishes for the experiment. Mercuric chloride weighed accurately as per requirement and dissolved in water before the transfer of fishes into the aquarium. Dead fish number were also recorded simultaneously and removed immediately.

Static renewal bioassay tests were conducted in order to evaluate the acute toxicity of Mercuric chloride; de-chlorinated tap water was used for acclimation of fish as well as experiment and control. The moderate size fishes were selected for experiment and a set of another 10 fishes were exposed to the concentrations of 0.5, 1.0, 1.5, 2.2.5, 3.0,3.5, 4.0,4.5 and 5 ppm of mercuric chloride to determine LC50 values after 96 hours. Simultaneously a set of control fishes was maintained. Water temperature was 28.5 ± 1.5ºC; average pH was 6.5, Dissolved oxygen ranged from 8.5 to 9.5mg/lit. Mortality of Fishes was recorded at regular time intervals of 24, 48, 72 and 96h, during the period of experiment, dead fishes were removed immediately from the experimental tanks. Fish behaviour was also observed during exposure of different concentrations of Mercuric chloride. The 96 h LC50 tests were conducted to measure susceptibility and survival potential of fishes to $HgCl_2$. The 96 h LC50 of mercuric chloride was determined following the graphical method of Krouwer and Monti [10].

Observation table of mortality of fishes against mercuric chloride

Sub-group	No. of fishes exposed	Conc. of HgCl2 --mg/lit	Mortality of fishes noted after time intervals of									% mortality within 96 hrs
			30 min	60 min	2 hrs	6 hrs	12 hrs	24 hrs	48 hrs	72 hrs	96 hrs	
1	10	4.0mg/lit	5	5	---	---	----	---	---	----	----	100
2	10	3.5mg/lit	5	5	---	----	----	----	----	----	----	100
3	10	3.0mg/lit	---	2	1	2	1	4	1	---	----	100
4	10	2.5mg/lit	----	1	---	1	2	2	3	1	----	90
5	10	2.0mg/lit	----	----	----	----	1	1	1	1	3	70
6	10	1.5mg/lit	----	1	----	----	----	----	1	1	1	40
7	10	1.0mg/lit	---	1	----	---	----	---	----	---	1	20
8	10	0.5mg/lit	----	----	----	----	----	----	1	----	----	10
9	10	**Control**	----	----	----	----	----	----	----	----	----	Nil

Graphical presentation of LC50 value in *Clarius gariepinus* (Burchell, 1822) with Mercuric chloride



Result and Discussion

Lethal Concentration LC_{50} Value.

In the present investigation 96hrs LC_{50} value of Mercuric chloride in *Clarius gariepinus* (Burchell, 1822) is 1.8 mg/litre. Similar 96hrs LC_{50} values observations were reported by many workers. The 96hrs LC_{50} value was investigated for Mercury in the fish *Channa punctatus* as 1.21 ppm [11]. Similarly, in *Cirrhinus mrigala* the LC_{50} value for mercury was 1.11 ppm reported [12]. In the fish *Tinca tinca* the LC50 value was 1.0 ppm for mercury noted. [13]. Our results are in agreement with all present studies. However, the LC50 value was observed of 2.0 ppm mercury in the fish *Boleophthalmus dussmieri* [14]. The LC50 value of $NaAsO_2$ and $HgCl_2$ for 96 hours of exposure were 18.211 ppm (95% confidence limit, 5.962 to 53.724) and 0.606 ppm (95% confidence limit, 0.228 to 1.293), respectively was noted [15]. Methyl mercury was the compound that showed the higher toxicity for SAF-1 (EC50 0.01mM) compared to mercury chloride [16].

The studies on the acute toxicity of mercury, zinc and cadmium in rainbow trout and reported that the fish was more susceptible to mercury, followed by that of zinc and cadmium [17]. [18] They believed that the stress response is expressed in the form of physiological changes. These changes might be identical for stressors and could be as varied as anesthesia, flight, forced swimming, disease treatments, handling, scale loss, or rapid temperature change. A study showed that stressor increases the permeability of the surface epithelia, including the gills to water and ions and thus induces hydro mineral disturbances. Acute toxicity studies were the very first step in determining the water quality requirements of fish. These studies obviously reveal the toxicant concentrations (LC50) that cause fish mortality even at short exposure [19].

Behaviour of Fish

In control tank, experimental organisms exhibited normal behaviour and swimming patterns, there was no mortality. The fishes exposed to different concentrations of metals mercuric chloride showed abnormal behaviours. In the beginning of exposure, fishes were attentive, stopped swimming and remained static. Later on, they were trying to avoid the toxic medium with fast swimming and jumping. The opercular activity was faster, surfacing and gulping of air. In tanks with higher concentrations of **2.5, 3.0, 3.5** and **4** ppm of Mercury, the fishes swam unpredictably with irregular movements and show more excitability. They secreted ample amounts of mucus continuously, Fish lost balance, became tired, unconsciousness and lethargic. Lastly, they remained in a vertical position for hours together with the anterior side or terminal mouth up near the surface of water, trying to gulp the air and the tail in a downward direction. After some time their bellies turned upward and the fish died. The haemorrhaging and oedema was seen in and around the mouth and below buccal cavity. Some fishes died with open mouth. In dead fishes the colour of body became dispigmented**.** The belly of dead fish's became enlarge and pale yellow in colour. The bleeding was seen from operculum and gill filaments, at the base of pectoral, anal and pelvic fins. Body was slimy due to mucus secretion from epithelium of gills and skin.

A similar observation has been reported by [6] **in** *L. calcarifer* when exposed to the Cadmium and mercury. *L. calcarifer* exposed to different concentrations of heavy metals both cadmium and mercury showed abnormal behaviour. In the begining of exposure, for both the metals fishes were alert, stopped swimming and remained static position in response to the sudden changes in the surrounding environment. After some time, they tried to avoid the toxic water with fast swimming and jumping. Faster opercular activity was observed as surfacing and gulping of air. In tanks with higher concentrations of 10 and 12 ppm of cadmium, 1.4 and 1.6 ppm of mercury, the fishes swam erratically with jerky movements and hyper excitability. Fins became hard and stretched, due to stretching of body muscles. They secreted copious amounts of mucus continuously, body pigmentation decreased with blackening noticed on the body surface. Ultimately, fish lost balance, became exhausted, lost consciousness, and became lethargic. Lastly, they remained in a vertical position for a few minutes with the anterior side or terminal mouth up near the surface of water, trying to gulp the air and the tail in a downward direction. Soon they settled in the bottom of the tank and after some time their bellies turned upward and the fish died.

In the present study *Clarius gariepinus* (Burchell, 1822) exhibited characteristic avoidance behaviour by rapid and erratic swimming with jerky movements and hyper-excitability such type of changes were also observed in *Channa punctata* after cadmium toxification [20].

Similar observations were made earlier by [21] in the fish *Chanos chanos* and [22] in the fish *Therapon jarbua*. Different actions occurred in the present study was in agreement with [23]. who has also reported similar abnormal behaviour in *C. punctatus* exposed to mercury. Likewise, in the fish *Ambassis commersoni* exposed to cadmium abnormal behaviour and altered movements are observed [24]. It is reported that heavy secretion of mucus over the body of the fish and dispigmentation are attributed to dysfunction of the endocrine or pituitary gland under toxic stress, causing changes in the number and area of mucus glands and chromatophores [25]. The preceding behavioural abnormalities of the fish and subsequent death imply that the toxic effect is mediated through the disturbed nervous or cellular enzyme system affecting the respiratory function and nervous system, which involves control of almost all vital activities, as many earlier studies have demonstrated that heavy metals have been shown to affect Na2+ -K2+ -ATPase in the brain [26]. Acute toxicity studies were the very first step in determining the water quality requirements of fish. These studies obviously reveal the toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. Therefore, studies demonstrating the sensitivity of xenotoxic effects of heavy metals in aquatic organisms, particularly in fish are needed. Thus, it can be concluded from the present study that the fish *Clarius gariepinus* (Burchell, 1822) was highly sensitive to mercury and the mortality rate is dose dependent.

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