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Evaluation of toxicity of heavy metal iron on a fresh water fish *Labeorohita* and its behavioural impacts.

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ABSTRACT

Heavy metals have been considered to be the serious pollutants of the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic animals. In the present studied static renewal bioassay tests were performed to evaluate the 96-hr LC_{50} value of heavy metal iron on an Indian major carp *Labeorohita*. Various concentration of iron was prepared and a toxicity test was done in order to determine the 96hr-LC50 of Iron. The result of the present work was found out to be the 70.79 mg/l of iron (357 mg/l of $FeSO_4.5H_2O$). The present work also observed behavioural change of fish, which shows uncontrolled swimming, erratic movement, lying on the sides of test chamber, change in opercula movement etc and precipitation of iron hydroxide on all parts of body including gills.

Keywords: Heavy metal iron, *Labeorohita*, 96-hr $LC_{50,}$ Behavioural changes;

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INTRODUCTION

Water pollution is the major threat of urbanization, industrialization and present agricultural practices. It leads to deviation in physical, chemical and biochemical properties of water bodies. The aquatic environment has always been subjected to different types of pollutants [1]. Various workers [2-3] reported that rapid industrialization in India has resulted in substantial rise in the liquid wastes, which are usually being discharged into open land or nearby natural water, cause a number of problems to plant and animal [4]. The problems of environmental pollution and its detrimental effect on aquatic biota including fish, is receiving focus during the last few decades [5-6]. Industrial discharges containing toxic and hazardous substances, including heavy metals contribute tremendously to aquatic ecosystem [7-8]. Heavy metals are natural trace mechanism of the aquatic environment, but their levels have been increased due to domestic, industrial and agricultural activities. Among several metals, iron plays an important role in the biology of living organisms. It forms complexes with molecular oxygen in haemoglobin and myoglobin, thereby acting as common oxygen transport proteins in vertebrates. Excess iron is toxic and acts as a catalyst in the Fenton reaction. This generates free radicals, which are detrimental to health [9]. A possible mechanism for dissolved iron toxicity is disruption of sodium balance. Several pathological changes like a fusion of gill lamellae, separation of the outer epithelial layer, hypertrophy and necrosis of the lamellar epithelium have been observed in teleost exposed to excess iron [10].

Quantitative parameters as well as survival and mortality of fish are used to assess the acute toxic effects of diverse toxicants for the fish [11] and to evaluate the sensitivity of different fish species against metal's toxicity [12]. On the other hand, metals concentration in tissues reveals past exposure of fish through water or food that can act as bio-indicator of metallic pollution of the environment [13].

Fish is an indirect target to pollutants, pathogens and xenobiotic substances present in these water bodies. These substances cause stress, which in turn affects the metabolism and biochemical profiles of fish. Fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants. They are sensitive to contamination and the pollutants may damage some physiological and biochemical processes when they enter the organs of fishes [14]. Changes in the quality of water, interactions between individuals, high fish stocking density [15-16] may bring about a wide range of physiological changes in fish. The altered physiological adaptations are however variable and flexible in fish in response to water quality of a large variety of aquatic habitats. Being at a higher level of the food chain, they accumulate a significant amount of pollutants and this accumulation depends on the intake and elimination from the body [17].

The LC_{50} value of this essential metal and its impact on the behaviour of fish during 96-hr time periods was revealed in the present work Currently, there is little information available on toxicity of heavy metal iron on the fresh water fish Labeorohita; therefore this study was performed to gain more information in this field. Specifically, we evaluated lethal toxicity of essential heavy metal Iron in an edible fish *Labeorohita*.

MATERIAL AND METHOD

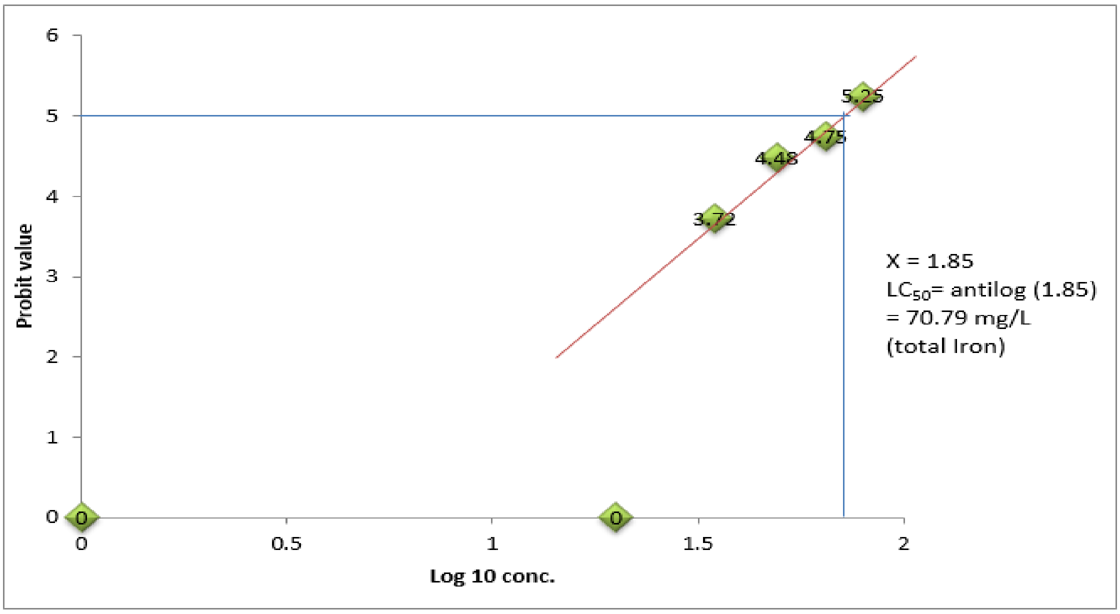
The fresh water fish species of *Labeorohita* was collected from the Narmada River at Hoshangabad. They weighed 32g±1g and their length was in the range 12cm±1.The fish were transported in polythene bags containing sufficient amount of oxygen, in order to reduce stress. Laboratory aquariums and equipments were cleaned and rinsed completely before filling with water. Potassium Permanganate was used as a disinfectant. Before fish was brought into laboratory, the fish were examined for any lesions or external infection. Then fish were treated with $KMnO_4$ so that fish were disinfected of external infection. The fish were acclimatized two weeks before the experiment. Acute toxicity tests, determining the toxicity of particular toxicant were done and a wide range was determined, with in which lay a concentration that caused 0.0% and 100.00% mortality. Pure compound of Iron [$Fe_2.7H_2O$] was dissolved in deionised water and its stock solution (1000 ppm) was prepared. To avoid stress to the fish, the desired metal concentration in each aquarium was attained within 7 h of the start of experiment. Metal toxicity concentration for each fish species was started from zero with an increment of 0.05 and 5 mg L^{-1} (as total concentration) for low and high concentrations, respectively. Ten fish of each age group and species were tested against various Fe concentrations for the determination of 96hr LC_{50}. During acute toxicity tests, the fish were subjected to 12 hr photoperiod and not fed during acute toxicity trials. The aquaria were examined after every 2 hr for the fish mortality. The physico-chemistry of the test media viz. water temperature, pH, total hardness, dissolved oxygen, carbon dioxide, sodium, potassium, calcium and magnesium were determined at 12 h intervals throughout the test period of each 96hr experiment by following the methods of APHA (1998). The LC_{50} concentration for 96h was calculated by Probit analysis method [18].

RESULTS

The results obtained regarding the acute toxicity (mortality) test of iron for the fish are presented in Table 1. The 96 hr-LC_{50} is the basic value in the acute toxicity test and it was 70.79 mg/L (353.95 mg/l of ferrous sulphate) for Iron against the test fish. During the experiment the precaution were taken as suggested in standard method (APHA et al., 1979). During the present bioassay experiment the physio-chemical parameter of test water was found within the range of (temperature 24.26-22.45^{0}C, pH 7.6-6.1, dissolved oxygen 6.30-5.50mg/l, carbon dioxide 3.50-4.50mg/l, alkalinity 95.40-85.62mg/l, hardness 110.65-105.69mg/l, chloride 25.69-24.36mg/l and salinity 45.40-44.65mg/l). The water of aquarium was changed after 24 hr of exposure with a required heavy metal iron concentration. The LC_{50} was calculated for 96hr by using graphical method by plotting a graph with log10 concentration on X-axis and Probit value (percent mortality) on Y-axis. The point at which the curve crossed or touch Probit of 5 represented 50% lethal concentrated value (Graph 1).

Table 1: Median lethal concentration (96-hrsLC_{50}) of *Labeorohita* exposed to different concentration of Iron (Ferrous sulphate)

Conc. mg/l	Log10 conc.	Total no. of fish	No. of fish death	% age of mortality	Probit value
Control	0	10	0	0	0
20 mg/l of Fe	1.30	10	0	0	0
35 mg/l of Fe	1.54	10	1	10%	3.72
50 mg/l of Fe	1.69	10	3	30%	4.48
65 mg/l of Fe	1.81	10	4	40%	4.75
80 mg/l of Fe	1.90	10	7	70%	5.25



Graph 1: Showing Probit lines for *Labeorohita* exposed to Iron ($FeSO_4$).

During the present experiments the behaviour of the fresh water fish *Labeorohita* was observed carefully. The ethological observation of *Labeorohita* at different exposure concentration and time duration for iron in the form of ferrous sulphate was noted. The fish were exposed to 20 to 80 mg/l of concentration of total iron for time period of 96 hrs. During the iron intoxication the fish displayed physiological malfunctions such as erratic swimming, hyperactivity, loss of equilibrium, increase in opercular movement and vertical movement of the fish. A layer of ferric hydroxide has been found all over the body of *Labeorohita* especially at the gills causes damage of gill tissues which directly affects the respiratory system of fish.

DISCUSSION

Bioassay tests are often employed at a very first step in determining the toxicity of any compound. Some scientists [19-20] has suggested the utility of bioassay method in determining the toxicities of industrial wastes to bivalve. In bio-assay determination, acute toxicity tests are considered to be most important, since these provide information about the relative lethality of heavy metal. Various scientists [21-22] have evaluated various types of toxicity tests to asses' effects of chemicals to aquatic life. Iron plays a key physiological role in all aspects of animal life. However, it causes deleterious effects on living organisms at supra-optimal concentrations [23]. Ferrous iron is considered to be the most toxic form of iron to aquatic fauna, in part; because it is the most readily bioavailable form under most circumstances. The LC_{50} recorded for present test fresh water fish *Labeorohita* in case of heavy metal total iron was 70.79 mg/l (354.81 mg/l of ferrous sulphate) in 96 hrs. Several authors have made studies for acute toxicity test. The effects of cadmium chloride and lead nitrate on the Indian major carp, *Labeorohita*, in order to determine survival range, LC_{50} values and safe concentration were studied. Cadmium was found to be more toxic than lead and inferred by their LC_{50} values. The safe concentration of cadmium chloride and lead nitrate were estimated at 5.89 and 6.81 mg/l respectively [24]. The 96-hr LC_{50} of lead has been reported as 19.00 mg/l for *Colisa fasciatus [25]*.

Early life stages of fish, the egg and larval stages were generally the more sensitive to metal toxicity. Therefore, the ability of fish to tolerate toxicity of different metals in an aquatic medium would be different. A particular fish that is highly susceptible to toxicity of one metal may be less or non-susceptible to the toxicity of another metal at the same concentration of that metal. Similarly, the metal which is highly toxic to one organism at low concentration may be less or non toxic to other organism at the same or even higher concentration. The marked differences between two fish species has been reported for Zinc sensitivity, the order Perciformes being most resistant group and Clupeiformes the most sensitive [26]. At water hardness of 100 mg/l, carp fry and fingerlings (*Cyprinuscarpio*) have the 96-hr LC_{50} of cadmium as 4.30 and 17.10 mg/l [27]. It has been putfoth thatage, body size, feeding habits and sex are also important characteristics associated with variable LC_{50} of metals for different species of fish [28].

The acute toxicity of iron, zinc, lead, nickel and manganese to the fish *Catlacatla* was scrutinized and it was reported that fish showed significantly highest tolerance (determined as LC_{50}) against iron, followed by that of manganese, lead, zinc and nickel. LC_{50} values of iron, zinc, lead, nickel and manganese of 90 days fish age were 114.67, 25.88, 26.85, 18.99 and 67.71 mg/l, respectively [29]. Higher concentrations of iron (up to 52.90 mg L-1) can reduce visibility in the water and cause impaired food perception to fry and juvenile stages, resulting in prolonged stress and reduced growth [30] (Smith et al. 1973). After 30 days of exposure of mayfly (*Leptophlebiamarginata*) to iron at 10, 20 or 50 mg L^{-1} Fe as $FeSO_4$ decreased food consumption at all the tested concentration was observed [31]. Hypoxic conditions, temperature and acidification usually render the fish more susceptible to intoxication while increase in minerals contents (hardness and salinity) reduced metal toxicity [32]. The pH dependent accumulation of lead in *Labeorohita* was highest at pH 5.5 compared and 8.5 revealing that lead were more available as free ions at lower pH compared to higher pH due to weakly bounded form [33]. Therefore, bioavailability is strongly enhanced at decreasing water pH [34] and hence increasing the toxicity of heavy metal to the fish. Behavioural alterations have been recognized as sensitive indicators of chemically induced stress in aquatic organisms [35-37].

When heavy metal iron in the form of ferrous sulphate is introduced in the water in aquarium containing fish, the fish show abnormalities in activities which were followed by mortality. The lower concentration of iron however did not show any behavioural changes but when the fish was exposed to higher concentrations of iron, the behaviour changes in fish included hyperactivity loss of balance, vertical, opercular movement and downward swimming patters etc. The fish tried to avoid stress condition by reducing exposure to external body surface through mucus production. The extent of behavioural abnormality appeared to be directly proportional to the heavy metal iron concentration. Mucus production increased considerably with increments in toxicant concentration and time period. These vertical and downward swimming movements of fish may be due to the loss of equilibrium at high intoxication which makes the fish to turn upside down. Thus swimming performance is considered one of the measures which could serve as possible sensitive indicator of sublethal toxic exposure. Similar behavioural pattern with frequent surfacing, irregular opercula movement and loss of equilibrium in *Tilapia mossambica* has been reported when exposed to cadmium [38]. Similarly, hyperactivity, erratic swimming, and loss of equilibrium in brook trout, *Salvalinusfontinalis*, in response to lead treatment have been reported [39].

Behavioural measurements may be useful indicator of sub-lethal contamination due to concentrations even being lower than those that affect growth [40]. These behavioural changes usually occur much earlier than mortality. Several factors have been attributed to behavioural changes/abnormalities in fish exposed to heavy metals like Cd, Hg and Pb [41]. These include nervous impairment due to blockage of nervous transmission between the nervous system and various effectors sites, paralysis and depression of respiratory centre due to enzyme dysfunction and alteration of energy pathway which results in energy depletion [42].

The effect of heavy metal iron on the behaviour of *Catlacatla* was also studied and it was found that the same caused severe pathological symptoms like loss of equilibrium, changes in opercula movement, change of orientation and erratic swimming [43]. Erratic swimming, restlessness and surfacing observed during present study may be an avoiding reaction to the heavy metals as also observed by various workers [44-47]. Loss of balance during swimming may be also due to some neurological impairment in central nervous system as evident by inhibition of e. g ACHE by heavy metals [48-50]. The increased gulping activity by the exposed fish may be reflection of an attempt by the fish to extract more oxygen to meet the increased energy demand to withstand the iron toxicity. During the present work, it has been observed that the precipitation of ferric hydroxide directly attacks the gill tissue. The clogging action of ferric hydroxide causes injury in gill tissue which directly affects the respiratory system of fish [51]. The 96-h LC_{50} bioassays of brown trout (*Salmotrutta*) was evaluated to examine the toxicity of a commercial iron (III) sulphate liquor used for treating reservoirs to reduce algal growth and the analytical grade of iron sulphate [52]. They found that the 96-hr LC_{50} for the iron sulphate liquor was 28 mg/L total iron (0.05 mg soluble iron) and the 96-hr LC_{50} for the analytical grade iron sulphate was 47 mg/L total iron (0.24 mg/L soluble iron). They felt that the mechanism of toxicity to the fish was through respiratory disruption due to physical clogging of the gills.

CONCLUSION

The present studies can be concluded that iron contamination is hazardous to aquatic ecosystems and the toxicity of tested heavy metals to *Labeorohita* affect respiratory and nervous system of the animal resulting into death and this fact should be taken into consideration when this heavy metal is used in agriculture or in industrial purposes.

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