

Bioaccumulation and Toxicity of Iron Salt on Shingi Fish *Heteropneustes fossilis* (Bloch) and its Possible Impacts on Human Health

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Abstract

The freshwater Stinging catfish locally known as Shingi (*Heteropneustes fossilis*) is exposed to various concentrations of analytical grade $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The LC_{50} values for Fe^{2+} were found to be 109, 68 and 45 mg/l at 24, 48 and 72 hrs, respectively indicating that the toxicity increased with time. Gills appear to be the first target organ for iron toxicity followed by the liver and muscle. Moisture contents in fish tissue increased due to release of the toxicant by biological way. Protein and lipid contents are decreased and ash content is increased with the increase of exposure time. In contrast, iron is relatively nontoxic to Shingi fish at low dose, but long time exposure has adverse effects in fish tissue. Thus, exposure to iron salt at toxic level resulted in accumulation of iron in fish tissue. Hence, the consumption of this type of contaminated fish might have detrimental effect on human health. However, this needs extensive study to make any final conclusion.

Key words: Bioaccumulation, Iron toxicity, Shingi Fish, *Heteropneustes fossilis*

Introduction

Iron is the fourth most abundant element of the earth's crust and the second most abundant metal and is absolutely essential for almost all living organisms. This is due to its requirement for proteins and enzymes involved in a great number of key metabolic roles, among the most important of which are ribonucleotide reductases, which convert ribonucleotides to deoxyribonucleotides (Sanvisens *et al.*, 2011). The main biological function of iron is to transport oxygen from respiratory organs to the cells and carbon dioxide from the cells to the respiratory organs. In addition to these features, the iron in the form of certain enzymes is involved in a variety of portable electronic processes and the oxidation and reduction of the corresponding systems. An adult man absorbs about 5 mg of iron a day from his food, while the woman absorbs slightly more to compensate the losses during menstruation or pregnancy (Forth and Rummel, 1973). The absorption of iron is larger in children, exceeding 10 to 15 mg a day. There are several inorganic sources like ferrous salts, as the ferrous sulfate, that are quite effective in the anemia treatment due

to the deficiency of iron (Alleyne *et al.*, 2008) besides organic heme forms like liver, meat etc.

Rapid industrialization and economic development in a country like Bangladesh has resulted in increased water pollution. Metals like iron could enter food chain through direct consumption of water or aquatic organisms that are exposed to, like wise seen in cases of heavy metals (Paquin *et al.*, 2003). The significant health hazard from metals is known (Anim *et al.*, 2011; Ciesielski *et al.*, 2010) but the impact of iron exposed fish as well as human consumption is not known from Bangladesh. Since no mechanism exists for excreting iron, toxicity depends on the amount of iron already in the body and its consumption by food or absorption from environment. In all animals, oral doses between 100 and 200 mg/kg b.w. are potentially lethal. Fishes are often at the top of the aquatic food chain and have been used as a bio-indicator in monitoring aquatic ecosystems (Harrison and Klaverkamp, 1990; Kock and Triendl, 1996). Uptake of iron into aquatic animals occurs from two sources: food and water. Fe^{2+} is considered to be more toxic to aquatic animals than Fe^{3+} (Sykora *et al.*, 1972) suggested that

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ferric iron was not particularly dangerous to fish, although the soluble iron species may have been considerably more toxic. Billard and Roubaud (1985) found that dissolved iron at a concentration of 0.005 mg/l had an unfavourable effect on rainbow trout (*Salmo gairdneri*) spermatozoa when they were highly diluted with water (one part per hundred) and at 0.73 mg/l at a dilution of one part per ten (Billard and Roubaud, 1985).

Gills are the main site of iron toxicity (Dalzell and Mac Farlane, 1999). Several authors reported the deposition of iron flocs on to the gill epithelium, resulting in gill clogging and damage (Larson and Olsen, 1950; Kinne and Rosenthal, 1967; Brenner et al., 1976, Dalzell and Mac Farlane, 1999). From the study of Dalzell and Mac Farlane (1999) it appears that the commercial grade iron sulphate liquor was more toxic compared to an analytic grade iron preparation; however as the toxicity values obtained were relatively similar, the majority of toxicity appeared to be due to iron.

The objectives of the present research were to measure the extent of the toxicity of iron on the Shingi fish *Heteropneustes fossilis* (Bloch, 1794). The investigation included determination of LC₅₀ values for FeSO₄.7H₂O in Shingi fish and measure the effects of the iron exposure to its tissue.

Materials and Methods

Water bottom living *Heteropneustes fossilis* (Bloch, 1794) a common indigenous fish (local name: Shingi) of Bangladesh were collected from the same pond having similar size and weight. Analytical grade FeSO₄.7H₂O (Merck, Germany) were used to study the toxicological effects of iron on the fish.

Determination of LC₅₀: The procedure used by Olaifa et al. (2004) and El-Naga et al. (2005) were followed for lethal concentration (LC₅₀) determination. The fish were exposed to iron of different concentrations for 24 h, 48 h and 72 h and the cumulative mortality rate was observed. The water quality maintained in the experiment were good for aquatic animal as in average pH 6.4, dissolved oxygen 5.75 mg/l, hardness 100 mg/l and temperature 27.5 °C.

Determination of iron intake: Different organs (gills, liver and muscles) were collected in small glass tube and preserved in the refrigerator for analysis. To determine the iron uptake small amount of different sample was

digested separately by using a mixture of HNO₃ and HClO₄ in 2:1 ratio. The procedure used by Prester et al. (1998) was followed to prepare sample for AAS. The digested solutions were analyzed by using atomic absorption spectroscopy (AAS) (Varian AA 240). The total iron intake was calculated by subtracting the concentration of iron in the control fish from that of experimental fish.

Determination of variation of some biological parameters: The mass of the fish tissue was recorded before and after the heating in an oven using aluminum foil at a temperature of 110 °C to measure the moisture content. Protein content in fish tissue was determined by Kjeldahl method demonstrated by Mendham et al. (2000). After extraction the lipid content was determined by following the procedure used by Bligh and Dyer (1959) rapid method of total lipid extraction and purification. The dry ash content was measured by heating a known amount of dry fish in an open flame using a crucible and then cooled and weighed. Heating was continued until constant weight was obtained varying from 500 to 600 °C. Subtraction of dry ash content from the taken weight of dry sample measures the organic contents of the fish tissue.

Results and Discussion

In this study, the fish exposed to Fe²⁺ were observed to be highly irritable and displayed frenzied swimming when approached. Morbid fish swam upside down, their bodies were covered with thick mucus and finally died with mouth opened. The LC₅₀ values for FeSO₄.7H₂O were found to be 543 mg/l (109 mg/l as Fe²⁺), 342 mg/l (68 mg/l as Fe²⁺), and 222 mg/l (45 mg/l as Fe²⁺) at 24, 48 and 72 hrs, respectively. This finding indicates that iron is relatively non-toxic compared to other trace metals i.e. 72 hrs LC₅₀ is 21.21 mg/l Cr³⁺ in Shingi fish (Hannan et al., 2014), 34 mg/l Cd²⁺ in steelhead trout (Cusimano et al., 1986) 20 mg/l Cu²⁺ (Howarth and Sprague, 1978) in rainbow trout (*Oncorhynchus mykiss*) but exposure of low amount of iron for a long period of time has the potential to be a big threat to fresh water animals and ecosystems. Chemical analysis of tissues as fish exposed to FeSO₄.7H₂O contained more iron than control. Accumulations of iron in different organs of fish are given in Table 1.

Data from Table 1 indicates that the accumulation of iron in gills increases up to 48 h and then decreases signifying a possibility of transfer of iron to other organs. At 72 hrs concentration of iron increases to the internal organs, that indicates a possibility of transfer of iron from gills to liver and muscle. Accumulation of iron in the liver and muscle is maximum at 24 hrs and then decreases at 48 h might be due to the fact that the fish after prolonged exposure to a toxicant reduce the amount by excreting out through its excretory system.

Table 1. Iron accumulation in different organs of the fish.

Time of exposure (Hours)	Total iron (mg/l)			Accumulation of iron (mg/l)		
	Gills	Liver	Muscle	Gills	Liver	Muscle
Control	0.433	1.359	1.095			
24 h LC ₅₀	1.957	2.841	1.770	1.524	1.482	0.675
48 h LC ₅₀	2.277	2.079	1.081	1.844	0.720	0.014
72 h LC ₅₀	0.593	2.413	1.160	0.160	1.054	0.065

Table 2. Variation in some biological parameters of fish tissue due to iron toxicity.

Time of exposure (hrs)	Parameters (mg/l)			
	Moisture content (%)	Protein content (%)	Lipid content (%)	Dry ash content (%)
Control	72.08	51.31	24.47	7.87
24 h LC ₅₀	73.86	48.89	23.53	8.05
48 h LC ₅₀	75.49	48.24	19.86	8.98
72 h LC ₅₀	76.15	47.18	17.44	9.68

From the data shown on the Table 2 it is clear that both protein and lipid content went down as the fish was exposed to iron, consistent with the previous works. According to Dalzell and Mac Farlane (1999), the reduction in protein is due to decrease in enzyme activity into inhibitory effect of metal. The responsible enzymes of protein-carbohydrate metabolism (aspartate and alanine transaminases) showed low levels than control group value due to its sharing in transforming proteins to glycogen when exposed to sub-lethal level of iron concentration. The decrease in the amount of lipid suggests that the fish were severely stressed and metabolism of lipid was impaired by over activity of the fish due to toxic effect. The ash content is found to increase with increased toxicity is due to increase of inorganic residue indicates the accumulation of iron in fish tissue.

The moisture content (Table 2) at LC₅₀ concentration increases from 72.08% (control) to 73.86, 75.49 and 76.15% at 24, 48 and 72 hrs, respectively. This increases in moisture content might be due to the exposure to the toxic level of iron and due to the increase urge to release the toxicant by vomiting and other biological way. As a result, the fish becomes weak and excess fluid is accumulated in the body. In addition the increased level of moisture may be due to the kidney failure in the fish.

Conclusion

The present results support the general hypothesis that iron is relatively non-toxic to fish compared with other trace metals with regard to direct acute toxicity. But the high LC₅₀ values with time indicate that the over dose amount of iron can be toxic to the fish if exposed for a longer time. Thus, exposure to iron at toxic level resulted in accumulation of the metal in fish tissue. Rapid industrialization in Bangladesh has resulted in increased water pollution that has higher iron level. Fishes living in this type of water may have higher level of iron in their tissue. Therefore, intake of this type of fishes might have adverse effects on human health. However, the assessment of oral level in human health issues due to consumption of iron overloaded fish need to be explored.

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