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Effects of Water Borne Iron on Spawn of Indian Major Carps (*Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.))

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Abstract Effects of water-borne iron on Indian major carps spawn were evaluated in the present study. Ferrous sulphate was used to prepare different test iron concentrations. Mrigal had the lowest 96 h LC_{50} value of 0.30 ± 0.06mg L^{-1} while rohu had the highest value of 0.73 ± 0.06mg L^{-1} of iron. Accumulation of iron in mrigal spawn was highest whereas it was lowest in catla. Abnormal behaviour and reduced growth were observed in chronic toxicity. Application factors were calculated to establish acceptable ranges and safe levels.

Keywords Iron toxicity · Indian Major Carps

Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (More et al. 2003). Among several metals, iron plays an important role in the biology of living organisms. It forms complexes with molecular oxygen in hemoglobin and myoglobin, thereby acting as common oxygen transport proteins in vertebrates. Iron is also used at the active site of many important redox enzymes dealing with cellular respiration, oxidation and reduction in plants and animals. Iron is a vital micronutrient for teleost fish, an integral component of protein involved in cellular respiration and oxygen transfer, and is essential for certain enzymes that drive the body's chemical reactions and maintains good health (Bury and Grosell 2003).

Excess iron is toxic and acts as a catalyst in the Fenton reaction. This generates free radicals which are detrimental to health (Crichton et al. 2002). A possible mechanism for dissolved iron toxicity is disruption of sodium balance. Several pathological changes like 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 (Peuranen et al. 1994).

The iron content of water in north eastern region of India, particularly in the state of Tripura is higher than the desirable level. Maximum desirable limit of iron (as Fe) in drinking water should be 0.3 mg L^{-1} according to Environmental Protection Agency, USA (EPA 1986) and Bureau of Indian Standards (BIS 1991). On the other hand, the maximum total iron concentration should not exceed 1.0 mg L^{-1} to protect aquatic systems from detrimental effects of iron (EPA 1986). The desirable iron concentration for aquaculture practice is less than 0.01 mg L^{-1} (Meade 1989). The high iron content of aquaculture water (0.14–3.96 mg L^{-1}) in different parts of Tripura has been one of the major reasons for heavy mortality in the carp hatchery as well in spawn rearing nursery ponds (Bhattacharya and Saha 1991).

The present communication describes the deleterious effects of excess water-borne iron on spawn of Indian major carps (IMCs), catla, *Catla catla* (Ham.); rohu, *Labeo rohita* (Ham.) and mrigal, *Chirrhinus mrigala* (Ham.). The objectives of the study were to (1) study the acute and chronic toxicity of water-borne iron on spawns of IMCs; (2) find out the LC_{50} values of water-borne iron on spawn of IMCs; (3) study the bioaccumulation of water-borne iron in the body of IMCs spawn during toxicity tests; and (4) find out the application factor (AF) or safe level of iron concentration for IMCs spawn rearing.

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Materials and Methods

Four days old IMCs spawns (7–8 mm) were collected from the carp hatchery of College of Fisheries, Lembucherra, Tripura, India. The spawns were acclimated in glass aquaria with proper aeration for 48 h. Water were exchanged daily up to 50% to remove faecal materials. Deep tube well water was stored in 1,000 L Fibre Reinforced Plastic (FRP) tanks for preparing different iron concentrations and dilution water. The stored water was treated with lime (@ 20 mg L^{-1}) with vigorous aeration to reduce the iron level. The physiochemical water parameters like temperature, pH, dissolved oxygen, free carbon dioxide, electrical conductivity and Fe concentration of the untreated deep tube well water were 28.5 ± 0.1°C, 6.5 ± 0.025, 3.09 ± 0.05 mg L^{-1}, 126.0 ± 1.528 μS cm^{-1} and 10 ± 1.02 mg L^{-1} respectively. The iron stock solution was prepared by dissolving ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), extra pure AR (SRL, Mumbai with 99.5% purity) in distilled water. Freshly prepared stock solution was used to prepare different iron test concentration for the experiments.

Range finding experiment was conducted (APHA 2005) to determine the concentration range of iron to be included in definitive short-term test. An iron (as $FeSO_4$) concentration series of 0.01, 0.1, 1, 10 and 100 mg L^{-1} was used. All the tests were conducted in triplicate for each concentration with 15 spawns.

Short-term definitive test for 96 h was conducted (APHA 2005) in 1L glass beaker with mild aeration to maintain optimum dissolved oxygen (DO). A concentration series between 0.1 and 2 mg L^{-1} was selected for the test. Mortality was recorded in every 6 h interval. No feeding was done during the experiment. The iron concentration (as $FeSO_4$) of test water was maintained throughout the experiment by measuring it using atomic absorption spectrophotometer, AAS (AAnalyst 200, Perkin-elmer) following the method described by Saha (2010). Replenishment of the test water with freshly prepared $FeSO_4 \cdot 7H_2O$ solution was done, if needed, to maintain the concentration. Various toxic factors such as 'no observed effect concentration' (NOEC), 'lowest observed effect concentration' (LOEC), 'maximum acceptable toxicant concentration' (MATC) and 'application factor' (AF) was determined to conduct short term toxicity test. Lethal concentration, the lowest concentration of a substance in an environmental medium which kills 100% of test organisms or species under defined conditions in a definite period of time was determined. For calculation of MATC and AF following formula were used:

$$\mathrm{MATC} = (\mathrm{LOEC} \times \mathrm{NOEC})^{1/2}$$

$$\mathrm{AF} = \mathrm{MATC}/\mathrm{LC}_{50}$$

For short-term partial life cycle test, lower concentration series with around one-third of LC_{50} value was selected. A concentration series of 0.1, 0.2, 0.4, 0.6 and 0.8 mg L^{-1} was used for evaluating the chronic toxicity of iron. The test was conducted for a period of 7 days with continuous monitoring of the environmental parameters, feeding habit, growth and mortality. Alike Short-term definitive test for 96 h, the iron concentration (as $FeSO_4$) of test water was also maintained throughout the experiment. The environmental parameters such as DO, pH, temperature and electrical conductivity were maintained in normal range. At the end of the test bioaccumulation of iron was measured by Flame-AAS (AOAC 2000). In this method whole body of spawns were dissolved in 0.1 M HNO_3 after dry ashing and digestion with 6 M HCl.

Test water was collected and analysed for various environmental parameters such as temperature, pH, DO and electrical conductivity using a water analyser (Multi 340i/SET). Turbidity, CO_2, alkalinity and hardness were analysed using standard methods (APHA 2005). Iron concentration was measured by Flame-AAS (Saha 2010).

Statistical analysis of the experimental data was done by using SPSS-15.0 for Windows (SPSS Inc., Chicago, IL, USA) and BioStat 2009 Professional 5.8.4 (AnalystSoft Inc., Vancouver, BC V6H 4E4, Canada) software. One-way and two-way analysis of variance (ANOVA) was used for comparison of mean values. Probit analysis was done for calculation of LC_{50} values. Exploratory data analysis was performed using correlation and regression to find out possible relationship between different parameters. Results are presented as mean ± standard deviation or standard error. Probability levels of 0.05 were used to find out the significance in all cases. Chi-square (χ^2) test was done to analyze the independence between observed and expected frequencies of mortality.

Results and Discussion

Iron plays a key physiological role in all aspects of animal life. However, it causes deleterious effects on living organisms at supra-optimal concentrations (Davies 1991; Misra and Mani 1992). Ferrous iron is considered to be the most toxic form of iron to aquatic animals, in part; because it is the most readily bioavailable form under most circumstances (Ilavazhahan et al. 2010). IMCs are the most important fresh water fish species cultured in north eastern India and it is essential to protect them against metal toxicity. Among the five concentrations of Fe (0.01, 0.1, 1, 10 and 100 mg L^{-1}) used, mortalities of spawn were observed in all the cases except 0.01 mg L^{-1}. Hundred percent mortality was

observed in the iron concentration of 10 mg l^{-1} and above. From the result of the range finding test a concentration range between 0.1 and 10 mg L^{-1} was selected for the short term–definitive test for all the three IMCs spawns.

Various LC_{50} values and toxic factors of 24, 48, 72 and 96 h are presented in Table 1. The LC_{50} is a very widely used measure for all the biological population relating to toxicity. It can be described as a concentration of some toxic material in a medium which can cause death of 50% of the population within a definite period of time. The ferric iron is not particularly dangerous to fish, although the soluble iron species may be considerably toxic (Sykora et al. 1972). In the present acute toxicity study a significant ($p < 0.05$) difference of mortality of spawn was found in different concentrations of iron as compared to that of control. Hundred percent mortality was observed for all the three species at 2 mg L^{-1} Fe concentration (Fig. 1). The 96 h LC_{50} values of Fe for catla, rohu and mrigal spawn was 0.59 ± 0.07 mg L^{-1}, 0.73 ± 0.06 mg L^{-1} and 0.30 ± 0.06 mg L^{-1}, respectively.

Chi-square test did not show any significant difference between predicted and experimental value obtained during the determination of LC_{50} for all IMCs spawn. The results obtained in this study showed that the toxicity of iron varied significantly among the three fish species. Among the three species, rohu was the most iron tolerant species followed by catla and mrigal (Table 1). The reason for less tolerance of mrigal to iron might be due to their inherent susceptibility to iron. As a transition metal, iron atom has an incomplete d-sub cell. Addition of iron containing compound in water leads to formation of free cations (Fe^{2+}) which have high affinity towards other anion. In presence of oxygen, Fe^{2+} can react with hydroxyl radical (OH^{-}) of water to give a precipitate of ferric hydroxide. Iron is readily oxidised by dissolved oxygen to the ferric form in the neutral to slightly acidic pH range (Heath 1995). The most common dissolved inorganic form of iron is Fe $(OH)_2^+$ (Dave 1984). It has been observed that ferric hydroxide greatly diminishes total biomass of benthic organisms and limits fish populations in streams with survivable pH levels (Koryak et al. 1972).

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 (Smith et al. 1973). After 30 days of exposure of mayfly (*Leptophlebia marginata*) to iron at 10, 20 or 50 mg L^{-1} Fe as $FeSO_4$ decreased food consumption at all the tested concentration was observed (Gerhardt 1992). In short-term partial life cycle test of present study a decrease in growth rate was observed in spawns of IMC's along with some behavioural changes (Table 2). The study showed a negative correlation between iron concentration and weight of spawn (Table 3). Among

Table 1 Comparisons of LC_{50} values and various toxic factors during 24, 48, 72, 96 h

Toxic Factor Fe (as $FeSO_4$) in mg L^{-1}	Catla				Rohu				Mrigal			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
LC50 (Mean ± SE)	1.22 ± 0.07	1.04 ± 0.08	0.8 ± 0.07	0.59 ± 0.07	1.27 ± 0.07	0.95 ± 0.06	0.75 ± 0.06	0.75 ± 0.06	1.21 ± 0.09	0.69 ± 0.06	0.44 ± 0.06	0.30 ± 0.06
Lower limit	1.09	0.89	0.65	0.46	1.13	0.83	0.60	0.61	1.04	0.57	0.32	0.19
Upper limit	1.35	1.19	0.94	0.72	1.42	1.07	0.84	0.84	1.38	0.81	0.55	0.41
Lethal Concentration	2.01	2.00	1.95	1.66	1.99	1.71	1.61	1.53	2.57	1.68	1.36	1.21
LOEC	0.32	0.27	0.25	0.21	0.52	0.29	0.20	0.18	0.26	0.22	0.21	0.17
NOEC	0.20	0.11	0.04	0.02	0.21	0.09	0.08	0.06	0.10	0.04	0.02	0.02
MATC	0.25	0.17	0.10	0.07	0.33	0.16	0.14	0.10	0.16	0.09	0.06	0.05
AF	0.207	0.17	0.13	0.12	0.26	0.17	0.19	0.14	0.13	0.13	0.15	0.17

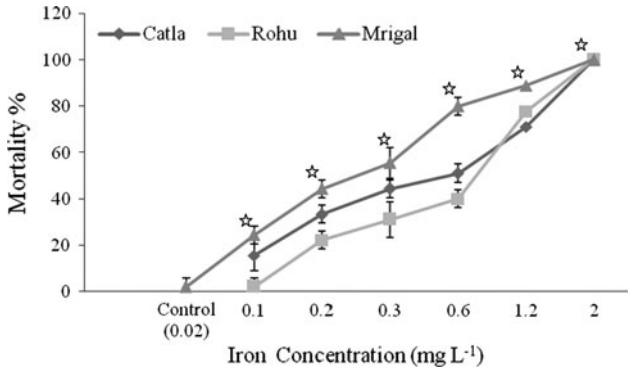

Fig. 1 Comparative mortality against concentration of Fe of catla, rohu and mrigal spawn. *mortality was significantly ($p < 0.05$) higher in all the test concentrations compared to the control for all species

IMCs, growth of mrigal was affected mostly by the iron concentration followed by rohu and catla. Sykora et al. (1972) found that brook trout had retarded growth at 12 mg L^{-1} suspended iron and higher, and fish exposed to levels exceeding 6 mg L^{-1} were more susceptible to injury and disease.

The accumulation of iron in spawn body was significantly higher as compared to the control group. Higher amounts of accumulation of iron were observed in higher concentrations groups (Fig. 2). However, when all the concentrations and corresponding accumulations were considered as a whole, a positive correlation between iron concentration and iron accumulation was observed in all the spawns. The correlation coefficient values indicated that the iron accumulation was highest in mrigal followed by rohu and catla (Table 4).

The iron toxicity in IMCs spawns might be due to the accumulation of iron in different parts of the body, especially gills of the spawns which might have altered respiration and osmoregulation, reduced feeding rate and hampered the growth of fishes and ultimately caused mortality.

From the present study we can conclude that mrigal spawns were most sensitive to iron toxicity whereas rohu spawns were most resistant among the three species. The LC_{50} value for mrigal was lowest as compared to catla and rohu. Comparatively high amount of iron accumulation was observed in mrigal compared to rohu and catla.

In short-term partial life cycle test reduction in feeding rate, behavioural changes and reduced growth was observed.

Table 3 Correlation between Fe concentration and weight of spawns during short-term partial life cycle test (n = 18)

Test organism	Iron concentration versus spawn weight
Catla spawn	−0.867**
Rohu spawn	–0.905**
Mrigal spawn	−0.914*

* correlation is significant at the 0.05 level; ** correlation is significant at the 0.01 level

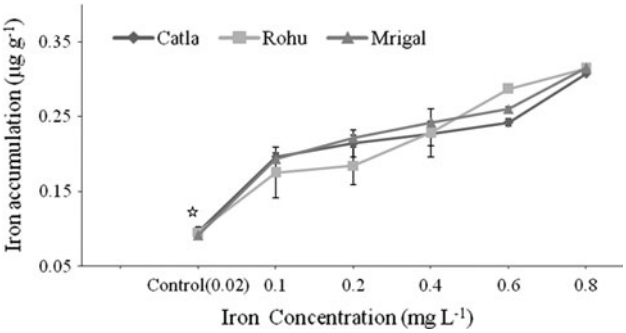

Fig. 2 Iron accumulations in whole body of catla, rohu and mrigal spawn during short-term partial life cycle test. *accumulation of iron was significantly ($p < 0.05$) higher in all the test concentration as compared to control for all species

Table 4 Correlation between iron concentration and iron accumulation of catla, rohu and mrigal spawn during short term partial life cycle test (n = 18)

Test organism	Iron concentration versus iron accumulation
Catla spawn	0.692*
Rohu spawn	0.885*
Mrigal spawn	0.899*

* correlation is significant at the 0.05 level

Table 2 Behavioural and weight change after short-term exposure of iron

Fe (as $FeSO_4$) in mg L^{-1}	Catla		Rohu		Mrigal	
	Behaviour	Weight (mg) ± SD	Behaviour	Weight (mg) ± SD	Behaviour	Weight (mg) ± SD
0.1	Normal	1.15 ± 0.022*	Normal	1.24 ± 0.000*	Normal	1.18 ± 0.000*
0.2	Normal	1.15 ± 0.049*	Normal	1.10 ± 0.092*	Near Normal	1.17 ± 0.006*
0.4	Near Normal	1.14 ± 0.020*	Near Normal	1.14 ± 0.046*	Lethargy	1.15 ± 0.000*
0.6	Lethargy	1.13 ± 0.036*	Lethargy	1.15 ± 0.079*	Lethargy	1.10 ± 0.005*
0.8	Lethargy	1.13 ± 0.015*	Lethargy	1.10 ± 0.015*	Lethargy	0.96 ± 0.005*
Control (0.02)	Normal	1.162 ± 0.065	Normal	1.54 ± 0.034	Normal	1.34 ± 0.006

* indicates statistically significant differences ($p < 0.05$) when compared with control

Finally, the application factor or safe level range of Fe (as $FeSO_4$) concentrations of water was calculated as 0.12–0.17 mg L^{-1} for IMC spawn rearing.

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References

AOAC (2000) Official methods, 999.11. determination of cadmium, copper, iron and zinc in foods. Atomic Absorption Spectrophotometry after dry ashing. Chapter-9, pp 19–22

APHA (2005) Standard methods for the examination of water and wastewater, 21st (ed.), APHA-AWWA-WEF, Pub: APHA, Washington, DC 20001-3710

Bhattacharya T, Saha RK (1991) Water quality of Agartala, final report, Tripura State Pollution Control Board. 43 pp +24 figs

BIS (1991) Bureau of Indian Standards, Indian standard drinking water specification (BIS 10500:1991)

Bury N, Grosell M (2003) Iron acquisition by teleost fish. Comp Biochem Physiol Part C 135:97–105

Crichton RR, Wilmet S, Legsyer R, Ward RJ (2002) Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. J Inorg Biochem 91:9–18

Dave G (1984) Effects of waterborne iron on growth, reproduction, survival and haemoglobin in *Daphnia magna*. Comp Biochem Physiol 78(C):433–438

Davies FG (1991) A hand book of environmental health and pollution hazards. University of California Press, Oxford

EPA (1986) U.S. Environmental Protection Agency. Quality criteria for water 1986. Office of Water Regulations Standards Washington, DC 20460. Water, EPA 440/5-84-001

Gerhardt A (1992) Effects of sub-acute doses of iron (Fe) on *Leptpphlebia marginata* (Insecta: Ephemeroptera). Freshw Biol 27:79–84

Heath AG (1995) Uptake, accumulation, biotransformation, and excretion of xenobiotics. Water pollution and fish physiology. CRC Press, Boca Raton, p 79

Ilavazhahan M, Tamil Selvi R, Jayaraj SS (2010) Determination of LC_{50} of the bacterial pathogen, pesticide and heavy metal for the fingerling of freshwater fish *Catla catla*. Glob J Environ Res 4(2): 76–82

Koryak M, Shapiro MA, Sykora JL (1972) Riffle zoobenthos in streams receiving acid mine drainage. Water Res 6:1239–1247

Meade JW (1989) Aquaculture management. Van Nostrarid Reinhold, New York

Misra SG, Mani DM (1992) Metallic pollution (1st edn.). Asish Publishing Inc. India

More TG, Rajput RA, Bandela NN (2003) Impact of heavy metals on DNA content in the whole body of freshwater bivalve (*Lamelleiden marginalis)*. Environ Sci Pollut Res 22:605–616

Peuranen S, Vuorinen PJ, Vuorinen M, Hollender A (1994) The effects of iron, humic acids and low pH on the gills and physiology of brown trout (*Salmo trutta*). Ann Zool Fennici 31:389–396

Saha RK (2010) Soil and water quality management for sustainable aquaculture, 1st edn. Narendra Publishing House, Delhi, pp 33–186

Smith EJ, Sykora JL, Shapiro MA (1973) Effect of lime neutralized iron hydroxide suspensions on survival, growth, and reproduction of the fathead minnow (*Pimephales promelas)*. J Fish Res Board Can 30:1147–1153

Sykora JL, Smith EJ, Synak M (1972) Effect of lime neutralized iron hydroxide suspensions on juvenile brook trout (*Salvelinus fontinalis*, Mitchell). Water Res 6:935–950