Effect of Acute and Sublethal Toxicity of Nitrite to Fresh Water Fish Cirrhinus Mrigala

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ABSTRACT: The effects of nitrite toxicity in freshwater fish Cirrhinus mrigala is mainly focused on acute and sublethal toxicity. During acute toxicity, poisoning, impaired oxygen carrying capacity of blood together with other physiological changes (disruption of ion regulatory, cardiovascular, and excretory processes) leads to death of fish. The fish showed various behavioural changes such as restlessness, abnormal swimming, behaviour, loss of balance, copious secretion of mucous and spreading of excess mucous all over the body surface, hyper excitability, loss of scales, floating upside down with abdomen directed towards, rapid opercular movement, gulping of air, asphyxiation and finally jerky movement before the death. Long-term exposure to sub-lethal concentrations of nitrite have considerable influence on fish health and physiological functions, namely a reduction of assimilation efficiency and subsequent decrease and suppression of growth, hemoglobin concentration, hematocrit values and decrease in leukocyte count and glucose concentration in the blood plasma. The result of these studies indicate that the toxicity of nitrite varies widely. This may be influenced by inter-species in the efficacy of nitrite transport and exclusion across gill epithelium. The median lethal concentration (LC50) of nitrite to an Indian major carp, Cirrhinus mrigala for 24h and 96h were found to be 28.31 and 19.95 mg/L respectively. 1/10 of the value for 24h (2.831) was taken as sublethal concentration.

Keywords : Cirrhinus mrigala. Toxicity of Nitrite

INTRODUCTION

Nitrite is a natural component of nitrogen cycle in ecosystems, and its presence in the environment is a potential problem due to its well documented toxicity to animals (Lewis and Morris, 1986; Jensen, 2003). Aquatic animals are at higher risk of nitrite intoxication. Since nitrite in the ambient water can be actively taken up and can accumulate to very high concentrations in the body fluids. Studies on fish revealed that nitrite induced a large variety of physiological disturbances many of which contribute to toxicity (Jensen, 1995, 2003). Nitrite normally occurring in nature is no harmful to the environment, because they play an essential role in tissue metabolism and growth of plants and animals. The action of nitrite (NO_2) in animal biology.is highly toxic when present in the body at medium concentrations, whereas it exerts beneficial effects at low concentrations. Nitrite is endogenously produced as an oxidative metabolite of messenger molecule nitric oxide (NO) and is naturally present at concentrations in most tissues of vertebrates (Kleinbongard et al., 2003; Bryan et al., 2005, Hansen and Jensen, 2010). Toxicological hazards measured by bioassay procedures are more realistic than predicted from chemical analysis and the available information on the toxicity of the compounds detected (Genjutulin, 1990). Bioassay tests can be used to establish the maximum acceptable concentration of a pollutant in a given environment without deliberate application of the chemical causing any unfavourable biological consequences (Cairns, 1982). Bioassay studies are accepted as standard methods for assessing toxicity of chemicals (APHA et al., 1985). The aim of using bioassay in monitoring of pollutant is to establish a relationship between toxicity and concentration of pollutant being studied in the biotope, the toxic effects can be divided into categories viz., effects that occur very quickly after a brief exposure to a chemical agent (Acute) and those that appear only after repetitive exposure to the substance (Chronic) (Ramade, 1987; Nagel, 1993). For biomonitoring of aquatic pollution, the organisms in the given aquatic systems are sampled for the analysis of various biological responses to chemical exposures. Suitable bioindicators usually give great help to the biomonitoring (Zhou et al., 2008).An important tool for understanding and evaluating the potential hazard of chemicals to aquatic organisms is the chronic or sublethal toxicity tests. A chronic toxicity test can indicate concentration of a chemical that will interfere with normal growth, development and attainment of reproductive potential of an aquatic organism. Chronic toxicity test

provide more sensitive measure of chemical toxicity. Biomonitoring using chronic toxicity assay may sensitively indicate the pollution stress posed by the pollutants at sublethal levels (Zhou et al., 2008). Sublethal affects in fish allows us to define toxicity of the environment and understand the potential danger of pollutants imputs (Oliva et al., 2009). Exposure of animals to sublethal levels of pollutant may inflict stress on mechanism required for maintaining a healthy physiological state, these changes may result in physiological, biochemical and behavioral processes, which will show more accurate prediction of acceptable levels of pollutants in the environment (Koeman and Strike, 1975; Waterpaugh and Beitinger, 1985). Sublethal effects, such as reproductive and endocrine end points, are gaining prominence in chronic ecotoxicity testing regimes (Hutchinson et al., 2003). According to Sprague (1969) acute toxicity is measured as median lethal concentration (LC50) which kills 50 percent of test organisms in a fixed time. The LC50 value is determined after 24 hours or 48 hours of exposure in tests of acute toxicity and in some cases after 96 hours. As such, guidelines for aquatic pollutants in natural ecosystems have been traditionally based on acute lethality test such as the 96 hours LC50 (CCME., 1999: USEPA, 2001), although impacts on development, growth and reproduction have also been considered (Rand and Petrocelli, 1985). Acute toxicity of nitrite has been investigated in number of fish species (Armstrong 1981; Handy and Poxton, 1993: Grosell and Jensen, 2000; Martinez and Seuza, 2002; Das et al., 2004). Acute toxicity tests are more widely used toxicity tests (Bechmann, 1994). Acute and short term chronic toxicity has been widely applied for decades. They have been used as tools in the evaluation and monitoring of environmental toxicity remediation procedures to identify toxic waste waters with in industrial plant as well as to; control and compare the effectiveness of water quality improvement technologies (Ferreira da costa et al., 2004). Among Indian major carps, Cirrhinus mrigala a well developed species to Indian climates is mostly consumed by common people in India. In aquaculture, nitrite is used as preservatives. Moreover the impacts of nitrite on Indian major carp are scanty. Hence in the present study an attempt was made to study the acute and sublethal toxicity of nitrite in Indian major carp, Cirrhinus mrigala.

MATERIALS AND METHODS

Nitrite (NO_2) was used as a toxicant in the present investigation. Analytical grade of sodium nitrite was obtained from (CAS no. 7632, purity > 99%) Loba Chemie Pvt. Ltd., Mumbai, India. The fish Cirrhinus mrigala is the most common among Indian carps of Indo-Gangetic foot plains of Bangladesh, India, Pakistan. The changes in physico-chemical characteristics, such as temperature, pH, Dissolved oxygen, alkalinity, hardness, salinity, calcium and magnesium of experimental water were recorded throughout the experimental period. Freshwater fish Cirrhinus mrigala, weighing 5.0-6.0 gm and measuring 7-8 cm were collected from Tamilnadu fisheries development corporation limited, Aliyar fish farm, Aliyar, Tamilnadu, India. Fish of same age and size which hatched from the same lot of eggs were collected, the age of fish being 2 to 3 months old. They were safely brought to the laboratory in well *packed* polythene bags containing aerated water and stocked in a large cement tanks (36'x18'x19'). Fish were acclimatized for about 20 days before the commencement of the experiment. During acclimatization period, fish were fed with ad libitum, with rice bran and ground nut oil cake in the form of dough once in daily. Water replaced every 24h after feeding in order to maintain a healthy environment for the fish. This ensures sufficient oxygen supply for the fish and the environment is devoid of any accumulated metabolic waste. The feeding was withheld for 24h before the commencement of the experiment and to keep the specimens in the same metabolic state. The fish were introduced into glass aquarium (26'x18'x18.5') cm which was washed thoroughly and maintained in the laboratory. Separate circular plastic tubs of 50 litres of water capacity were taken and different concentrations of nitrite were added. 10 healthy fishes were introduced into each tub. A control tub (no toxicant) with 50 litres of water and 10 fishes were also maintained. Three replicates were maintained for each concentration groups. The mortality/ survival of fish in control and nitrite treated tubs was recorded after 24h and the concentration at which 50% mortality of fish occurred was taken as the median lethal concentration (LC50) for 24h which was found to be 28.31 ppm. A similar experimental set up was also maintained to determine the lethal concentration of sodium nitrite to fish Cirrhinus mrigala for 96h. The test water was renewed at the end of 24h and freshly prepared solution was added to maintain the concentration of sodium nitrite at a constant

level. The median lethal concentration (LC50) of sodium nitrite for 96h was found to be 19.952 ppm. The median lethal concentration of nitrite was calculated by probit analysis method (Finney, 1978). In the present study the homogenicity of the fish population was tested using chi-square test (Busvine, 1971).Death was indicated by failure of the fish to respond to the gentle prodding with a glass rod and cessation of the opercular movement.

CACULATION OF REGRESSION LINE

The critical concentration of an organism to any toxicant can be estimated with sufficient accuracy from probit/log concentration graph (Busvine, 1971). In the present study, the two variables are plotted on a plain paper on logarithmic/probability paper and then a straight line is fitted by eye. The critical concentrations determined graphically are often remarkably close to calculated result. Based on the above method the regression line is calculated for the present study.

RESULTS

Many physico-chemical parameters have a considerable influence on the toxicity of chemicals. Among these parameters, temperature, dissolved oxygen, hardness, alkalinity and pH of the test solution or water are the important environmental factors and fish size is an important biological factor. In the present study, all the physical and chemical parameters of water used for experiment were always below the maximum permissible limits (Table 1).

In the present study, during acute treatment, marked behavioural changes were noticed. The fish showed behavioural changes such as restlessness, abnormal swimming, behaviour, loss of balance, copious secretion of mucous and spreading of excess mucous all over the body surface, hyper excitability, loss of scales, floating upside down with abdomen directed towards, rapid opercula movement, gulping of air, asphyxiation and finally jerky movement before the death. Long-term exposure to sub-lethal concentrations of nitrite have considerable influence on fish health and physiological functions, namely a reduction of assimilation efficiency and subsequent decrease and suppression of growth, hemoglobin concentration, hematocrit values and decrease in leukocyte count and glucose concentration in the blood plasma.

The data on the log concentration or probit regression of the fish Cirrhinus mrigala when treated with different concentration of nitrite for 24h and 96h were given in Table 2 and 3 and Fig 1 and 2. The median lethal concentration for 24h and 96h were found to be 28.31 and 19.952 ppm. For sublethal studies, 1/10 of the LC50 of 24h value (2.831 ppm) was taken. The Chi-square test on the toxicity data clearly indicates that the fish population used for the experiment was homogenous.

Table 1. Physico-chemical parameters of water used for the present investigation

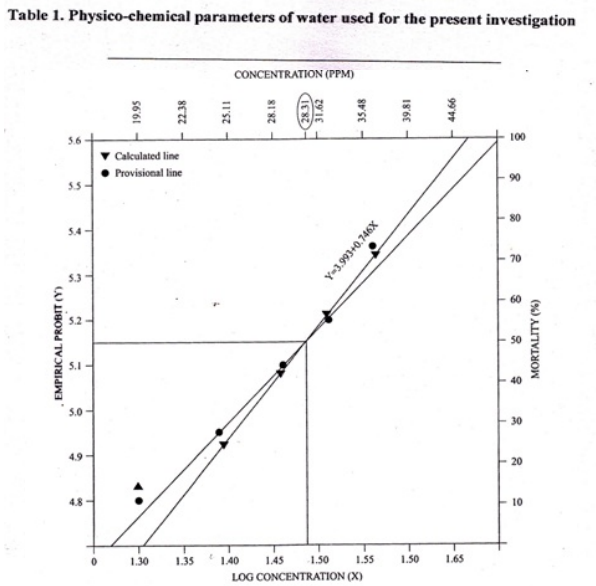

Fig 1. Log concentration of sodium nitrite Vs. per cent mortality of fish *Cirrhinus mrigala* and determination of LC50 (24h) value calculated from Table. 2

Table 2. Calculation of log concentration / probit regression line for 24h experiment in which fish *Cirrhinus mrigala* were exposed to different concentration of nitrite

Si. No.	Conc. in ppm of nitrite	No. of fish used	% Dead	Log conc.	Empirical probit	Expected probit	Working probit	Weight co-efficient	Weight					
				X		Y	y		W	WX	Wy	WX^2	Wy^2	WXy
1	20	10	42	1.301	4.80	4.86	4.79	0.627	6.27	8.157	30.03	10.612	143.85	39.073
2	25	10	48	1.397	4.95	4.95	4.94	0.634	6.34	8.856	31.31	12.373	154.71	43.753
3	29	10	54	1.462	5.10	5.10	5.10	0.634	6.34	9.269	32.33	13.551	164.90	47.272
4	33	10	58	1.518	5.20	5.20	5.20	0.627	6.27	9.517	32.60	14.448	169.54	49.492
5	37	10	64	1.568	5.36	5.30	5.35	0.616	6.16	9.658	32.95	15.145	176.31	51.192
									31.38	45.457	159.22	66.129	809.31	230.78
									SW	SWX	SWy	SWX^2	SWy^2	SWXy

$\bar{X}$ = 1.448
$\bar{Y}$ = 5.073
b = 0.746
Variance 'V' = 0.0572
LC50 24h value = 28.31ppm
Values are means of five individual observations

χ^2 = 1.419
Fiducial limits at 5% level
Lower limit m_1 = 0.984
Upper limit m_2 = 1.92

Regression equation Y = 3.993 + 0.746X

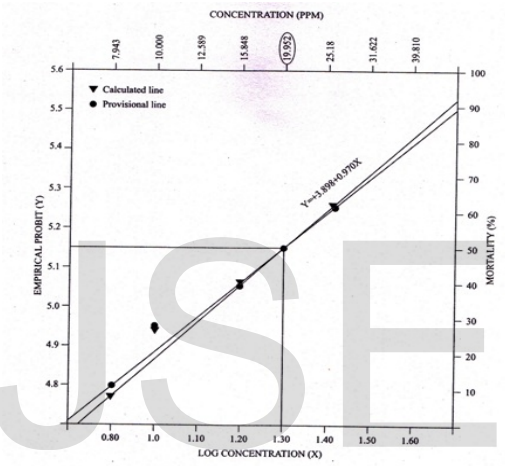

Fig 2. Log concentration of sodium nitrite Vs. per cent mortality of fish *Cirrhinus mrigala* and determination of LC50 (96 h) value calculated from Table.3

Table 3. Calculation of log concentration / probit regression line for 96h experiment in which fish *Cirrhinus mrigala* were exposed to different concentration of nitrite

Si. No.	Conc. in ppm of nitrite	No. of fish used	% Dead	Log conc.	Empirical probit	Expected probit	Working probit	Weight co-efficient	Weight					
				X		Y	y		W	WX	Wy	WX^2	Wy^2	WXy
1	8	10	42	0.903	4.80	4.80	4.795	0.627	6.27	5.661	30.064	5.112	144.159	27.148
2	12	10	48	1.079	4.95	4.95	4.949	0.634	6.34	6.840	31.376	7.381	155.283	33.855
3	16	10	52	1.204	5.05	5.06	5.055	0.637	6.37	7.669	32.200	9.234	162.772	38.769
4	20	10	56	1.301	5.15	5.15	5.151	0.634	6.34	8.248	32.657	10.731	168.217	42.487
5	26	10	60	1.41	5.25	5.25	5.256	0.627	6.27	8.840	32.955	12.465	173.212	46.466
									31.59	37.258	159.252	44.923	803.643	188.725
									SW	SWX	SWy	SWX^2	SWy^2	SWXy

$\bar{X}$ = 1.179
$\bar{Y}$ = 5.041
b = 0.970
Variance 'V' = 2.576
LC50 96 h value = 19.952 ppm
Values are means of five individual observations

χ^2 = -0.083
Fiducial limits at 5% level
Lower limit m_1 = -1.843
Upper limit m_2 = 4.443

Regression equation Y = +3.898+0.970X

DISCUSSION

Nitrite toxicity to fish depends on large number of factors, the most important of which is water chemistry, especially chloride concentration (Lewis and Morris, 1986; Svobodova et al., 2005b). Nitrite is toxic to fish, but toxic concentrations vary among species (Doblander and Lackner, 1997). It has been shown that the severity of NO_2 accumulation also varies among with varying metabolic profiles (Jensen, 2003). Nitrite toxicity to fish depends on the rate of nitrite accumulation, temperature, duration or exposure and ability of detoxification (Jensen, 2003; Madison and Wang, 2006). The differences in **NO_2** toxicity among fish species have been attributed to the dissimilar ability of fish to prevent the increase of NO_2 concentration in their plasma. It is well known that water quality affects the toxicity of NH3 and **NO_2** to fishes because **Cl^-** competes with NO_2 for the same ion channel; elevated concentrations of particularly Cl- have the potential to reduce the toxicity of NO_2 (Meinelt et al., 1997). Lewis and Morris (1986) suggested that the observed low LC50 value may be due to higher amount of chloride in the water, since chloride along with other anions in water provides protective action against NO_2 in active branchial uptake (Williams and Eddy, 1986; Stormer et al., 1996; Jensen, 2003) or might also be due to the species specific response of mrigala to nitrite (Das et al., 2004a). In general, nitrite is more toxic in freshwater compared to seawater (Grossel and Jensen, 1999; Jensen, 2003) and a low LC50 is expected in freshwater fishes.

In the present study the LC50 value exposed to nitrite for 24 and 96h were found to be 28.31 and 19.95ppm. Hence the comparison of toxicity values of different studies is difficult as suggested by Das et al. (2004). Hong et al. (2009) reported that the estimated 24, 48, 72, and 96h LC50 values and 95% confident intervals of sodium nitrite for E. sinensis were 48.87, 40.24, 38.87 and 38.87mg **$NaNO_2$ L-1.**The 96h LC50 for mrigala was **10**.4mg/**L-1** in common carp (Solbe, 1981), **20mg-1** in rainbow trout (Thurston et al., 1977) and **0.54 mg-1** nitrite **N-NO_2** in Tambaqui Colossama macropomum and in Neotropical fish (Costa et al., 2009). The uptake, toxicity and effects of nitrite vary among fishes depending on the test conditions. Both inter and intra-specific response to nitrite have been reported in fishes (Williams and Eddy, 1988; Stormer et al., 1996; Jensen, 2003). The main toxic action of nitrite on aquatic animals is due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen, causing hypoxia and ultimate death (Camargo and Alonso, 2006). Nitrite is taken up at the gills against an electrochemical gradient through the active uptake mechanism (Williams and Eddy, 1988). The continual uptake of nitrite across the gills of nitrite exposed fish leads to a time-dependent increase in internal nitrite concentrations that induce a large variety of physiological disturbances (Jensen, 2003). A great deal of research has characterized physiological mechanisms of toxicity in animals exposed to contaminants. Behavioural changes as a result of stress accepted as the most sensitive indication of potential toxic effects (Farah et al., 2004). In the present study during acute treatment the fish showed various behavioural changes such as restlessness, abnormal swimming, loss of balance, copious secretion of mucous and spreading of excess mucous all over the body surface, hyper excitability, loss of scales, floating upside down with abdomen directed towards, rapid opercular movement, gulping of air, asphyxiation and finally jerky movement before the death. Long-term exposure to sub-lethal concentrations of nitrite have considerable influence on fish health and physiological functions, namely a reduction of assimilation efficiency and subsequent decrease and suppression of growth, hemoglobin concentration, hematocrit values and decrease in leukocyte count and glucose concentration in the blood plasma.

It is concluded that in the present investigation, the bioassay study shows that nitrite is highly toxic to fish and the toxicity is inversely propotional to exposure time and it also depicts the mortality percentage is dose dependent.

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