Adverse Affect of Nitrite in Hemoglobin Content to Freshwater Fish "*Cirrhinus mrigala*"

Dr. Y. Thangam

Assistant Professor, J. K. K. Nataraja College of Arts and Science, Komarapalayam, Namakkal Dt, Tamilnadu, India

Abstract: ***Pollution from toxic chemicals affects human health, environment, and ecology. Contamination of natural waters form anthropogenic sources of nitrogen is a widespread problem. In the aquatic environment, the most common ionic forms of inorganic nitrogen are nitrite (NO_2^-). These ions present naturally in aquatic ecosystems as a result of atmospheric deposition, surface and groundwater runoff, dissolution of nitrogen-rich geological deposits. This study represents the changes of nitrite ions in hemoglobin level to fresh water fish Cirrhinus mrigala. Table1. Represent the data on changes in the hemoglobin content of fish Cirrhinus mrigala exposed to sublethal concentration of nitrite for 35 days. On 7th day, the hemoglobin content in the nitrite exposed fish was found to be slightly increased showing a minimum percent of 2.26. However, after 7th day, a decrease in hemoglobin level was observed in the rest of the study period showing a minimum percent decrease of 5.44 at the end of 14th day and a maximum percent decrease of 9.86 at the end of 35th day. There were significant ($P < 0.05$) variation among the treatments (F_1, 40 = 232.02; $P < 0.05$), periods (F_4, 40 = 14.54 $P < 0.05$) and their interactions (F_4, 40 = 35.08; $P < 0.05$).***

Keywords: Nitrite, Fish *Cirrhinus mrigala*, Hemoglobin, Cyanmethemoglobin.

1. Introduction

Aquatic organisms are at higher risk of nitrite intoxication. Nitrite an important toxicant to freshwater fish even when it is present in the environment in low concentration. It also cause fuel algal blooms that results in high levels of cyanobacteria and other toxic microorganisms leading to dead zones, unusable recreational waterways, and commercial fish kills. In water it receives excess nitrogenous waste, an imbalance between bacterial nitrification and denitrification occurs, eventually leading to nitrite accumulation. Exposure of nitrite to the fish *Cirrhinus mrigala* by hematological parameters especially through hemoglobin causes lysis and shrinkening of RBC. Based on the accumulation of nitrite content in fish the toxicity hemoglobin is assessed.

Aquatic ecosystems are equipped with a variety of physico-chemical and biological mechanism to eliminate or reduce adverse effects of toxic substances, toxicants may evoke changes in developments, growth, reproduction and behavior causes death of freshwater organisms (Offem and Ayotunde, 2008). A great deal of research has been conducted to understand the effects of toxicants on the physiology and survival of animals especially in fish. High levels of nutrients (i.e., ammonia, nitrite, nitrate, phosphate, and potassium) discharged from various anthropogenic sources, including agricultural fertilizer, livestock waste, urban runoff, and sewage leaks. Among the major nutrients, more attention has been made to nitrite likely because of their perceived higher toxicity. Kroupova *et al.* (2008) reported that nitrite, an intermediate compound of the nitrification process, does not usually predominate among other nitrogenous substances in natural waters. Aquatic animals are in general, better adapted to relatively low levels of inorganic nitrogen since natural ecosystems often are not N saturated. Therefore, high levels of ammonia, nitrite and nitrate, derived from human activities, can impair the ability of aquatic animals to survive, grow and reproduce, resulting in direct (acute or chronic) toxicity of these inorganic nitrogenous compounds (Philips *et al.*, 2002).

Adhikari *et al.* (2004) reported that blood is the patho-physiological reflector of whole body, so blood parameters are important in diagnosing the structural and functional status of the animal exposed to toxicant. Oliveira Ribeiro *et al.* (2006) reported that physiological stress indicators such as hematological and blood parameters could be useful to evaluate the effects of contaminants in fish. Nitrite induces a large variety of physiological disturbances in fish and the toxicity may result from a combination of effects, nevertheless, the most studied is the formation of methemoglobin (MetHb). Nitrite are actively transported into blood stream through the gills in freshwater fish by means of chloride cells, which posses a mechanism to actively transport chloride towards internal medium. The principal effect of such nitrite loading is a progressive oxidation of hemoglobin to methemoglobin, but several other physiological changes occur (Jensen, 2003). The physiological disturbance like depression, suppression of hemopoietic activity of the kidney in addition to the increased removal of dysfunctional RBC from blood (Stormer *et al.*, 1996).

Estimation of hemoglobin can be used as an index of anemia and fluid volume disturbance. Matkovics *et al.* (1987) observed a significant decrease in hemoglobin content of *Cyprinus carpio* when exposed to paraquet toxicity. Significant decrease in hemoglobin level was noted in *Asian swamp eel Monopterus albus*, exposed to endosulfan (Hii *et al.*, 2007) and in European catfish, *Silurus glanis L.* exposed to cypermethin (Adhikari *et al.*, 2004). Similarly, significant decrease in hemoglobin content was noted by exposure to metals like cadmium in *Perca fluvitalis* (Sjobeck *et al.*, 1984), lead in *Salvelinus fontinalis* (Christensen *et al.*, 1977), nitrite in *Catla catla* (Das *et al.*, 2004), copper in *Prochilodus scrofa* (Caravalho and Fernandes, 2006). In contrast to above observations, significant increase in hemoglobin level was noticed in *Rhamdia quelen* due to sub-lethal toxicity of cypermethrin (Borges *et al*., 2007). Similarly Lal *et al*., (1986) observed a significant increase in hemoglobin content of catfish *Heteropneustes fossils* when exposed to malathion toxicity. Likewise, hemoglobin content was increased in selenium exposed fish *Clarias gariepinus* Abdel Tawwab *et al*. (2007a), in methyl mercury exposed fish *Hoplias malabaricus* (Olivera *et al*., 2006) and in copper exposed fish *Prochildus scrofa* (Drastichova *et al*., 2004). In the present study nitrite were exposed to fish *Cirrhinus mrigala* and the hemoglobin values were noted. Significant increase and decrease in hemoglobin content were observed throughout the study period. This may be due to the decrease in nonspecific immunity of fish which may be attributed to the reduction in the leucopoiesis due to stress.

2. Materials and Methods

Hemoglobin content of blood was estimated by Cyanmethemoglobin method (Drabkin, 1987).

3. Results

Table 1. Represent the data on changes in the hemoglobin content of fish *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days. On 7th day, the hemoglobin content in the nitrite exposed fish was found to be slightly increased showing a minimum percent of 2.26. However, after 7th day, a decrease in hemoglobin level was observed in the rest of the study period showing a minimum percent decrease of 5.44 at the end of 14th day, and maximum percent decrease of 9.86 at the end of 35th day. There were significant ($P < 0.05$) variation among the treatments (F_1, 40 = 232.02; $P < 0.05$), periods (F_4, 40 = 14.54 $P < 0.05$) and their interactions (F_4, 40 = 35.08; $P < 0.05$).

Table 1: Changes in the hemoglobin content of *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days

Exposure period (in days)	Control	Experiment	Percent change
7	13.6 ± 0.276 b	13.99 ± 0.010a	+2.26
14	14.15 ± 0.084a	13.38 ± 0.115b	-5.44
21	14.33 ± 0.061a	13.06 ± 0.037c	-8.86
28	14.00 ± 0.052a	12.69 ± 0.034d	-9.36
35	14.00 ± 0.049a	12.62 ± 0.082e	-9.86

Values are means± S.E. of five individual observations. (+) Denotes percent increase over control. (-) Denotes percent decrease over control.** Significant at 5% level. Means in a column bearing same letter(s) are significantly different according to DMRT (P> 0.05).

4. Discussion

Water pollution monitoring becomes a crucial problem as more and more contaminants enter the aquatic and marine environment every year. The current trend is prediction of the toxicity level using various measurable attributes of the aquatic environment for biomonitoring (Pace, 2001). Studies on fish have revealed that nitrite induces a large variety of physiological disturbances, many of which contribute to toxicity. Nitrite compounds exert environmental stress on fish and affect oxygen consumption, ammonia excretion, and haemolymph. Mevel and Charmroux (1981) reported that when nitrite reached 0.043 mmol L – 1, the death rate increased sharply by 32% in fish. Fish virtually found everywhere in the aquatic environment they play a major ecological role in the aquatic food-webs because of their function as a carrier of energy from lower to higher tropic levels (Beyer *et al*., 1996). Fish has been proposed as a good animal model for disclosing the physiology that underlies the toxicology of nitrite (Jensen, 2003). The reduction in the Hb content may be the result of the prevailing anoxic condition, since depression and exhaustion in the hemopoietic potential occurs under such conditions (Das *et al*., 2004).

The progressive reduction in hemoglobin content may be attributed to the nitrite causing met hemoglobinemia and depression/ exhaustion for reduction in hemoglobin content. The increase and greater reduction in the hemoglobin content in the higher concentrations of nitrite may be attributed mainly to the suppression of hemopoietic activity of the kidney in addition to the increased removal of dysfunctional RBC from blood (Stormer *et al*., 1996). Decrease in hemoglobin content in fingerlings when exposed to higher nitrite concentration may be due to passage of nitrite into blood stream which causes lysis of anemia. In the present study during the sublethal treatment the hemoglobin values in fish *Cirrhinus mrigala* were decreased and increased. This may be due to destruction of blood cells or defect in Hb molecules and affect of Methaemoglobinaemia (MetHb) during nitrite toxicity. Further nitrite might have affected the hematopoietic system and biochemical pathway of heme formation. Hemoglobin level of *Cirrhinus mrigala* during sublethal nitrite exposure may be a consequence of red blood shrinkage, hemolysis and/or the reduction of the red blood life cycle. Red blood cell shrinkage is followed by loss of hemoglobin solubility, resulting in Hb crystals and structural damage to erythrocytes. Furthermore, the high activity of the MetHb-reductase system convert the Hb into MetHb during nitrite stress, this results in high metabolic change to the red blood cells, shortening the normal life span of the cells.

References

[1] Offem, B.O., Ayotunde, E. O. 2008. Toxicity of lead to freshwater invertebrates. (water flea: *Daphnia magha and Cyclop sp*) in fish ponds in a tropical wood plain. *Water. Air. Soil Pollut.* 192, 39-46.
[2] Kroupova, H *et al*. Effects of subchronic nitrite exposure on rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicol. Environ. Saf.* **71**, 813-820.
[3] Philips, S., Laanbroek, H. J., Verstraete, W. 2002. Origin causes and effects of increased nitrite concentration in aquatic environments. *Rev Environ. Sci. Biotechnol*. 1.
[4] Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C. T., Ayyappan, S. 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost; *Labeo rohita* (Hamilton). *Ecotoxicol. Environ. Saf.* 58, 220-226.

[5] Oliveira Ribeiro,C.A . 2006. Hematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead andtributylinchloride.*Environ.Res.*101, 74-80.
[6] Jensen, F. B.2003. Nitrite disrupts multiple physiological functions in aquatic animals. *Comp. Biochem. Physiol. B*. 157, 533-541.
[7] Matkovics, B., Witas, H., Gabrielak, T., Szabo. 1987. Paraquat as an affecting antioxidant enzymes of common carp erythrocytes. *Comp. Biochem. Physiol. C.* 87, 217-219.
[8] Hii, Y. S., Lee, M. Y., Tse, S. C. 2007. Acute toxicity of organachlorine insecticide endosulfan and its effect on behavior and some hematological parameters of *Asian swamp eel* (*Monopterus albus, Zuiew*). *Pestic. Biochem. Physiol.* 89, 46-53.
[9] Sjobeck, M. L., Haux, C., Larsson, A., Lithner, G. 1984. Biochemical and hematological studies on perch, *Perca fluviatilis*, from the cadmium-contaminated river Eman. *Ecotoxicol. Environ. Saf.* 8, 303-312.
[10] Christensen, G.M., Hund, E., Fiandt, J. 1977. The effect of methyl mercury chloride, cadmium chloride, and lead nitrite on six biochemical factors of the brook trout, *Salvelinus fontinalis*. *Toxicol. Appl. Pharmacol*. 42, 523-530.
[11] Das, P. C., Ayyappan, S., Jena, J. K., Das, B. K. 2004. Acute toxicity of ammonia and its sublethal effects on selected hematological and enzymatic parameter of mrigala, *Cirrhinus mrigala*. (Hamilton). *Aquacult. Res*. 35, 134-143.
[12] Caravalho, C. S., Fernandes, M. N. 2006. Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scorfa* at liw and high pH. *Aquacult*. 251, 109-117.
[13] Borges, L. V 2007. Changes in hematological and serum biochemical values in jundia Rhamdia quelen due to sublethal toxicity of cypermethrin. Chemosphere, 69, 920-924.
[14] Lal, A. S., Kumari, A., Sinha, N. 1986. Biochemical and hematological changes following malathion treatment in the freshwater catfish, Heteropneustes fossilis (Bloch). Environ. Pollut. A. Ecol. Biol. 42(2), 151-156.
[15] Abdel-Tawwab, M., Mousa, M., A., A. Ahmad, M., H., Sakr S., F., M. 2007. The use of calcium pre-exposure as a protective agent against environmental copper toxicity for *juvenile Nile tilapia*, *Oreochromis niloticus* (L.). *Aquacult*. 264, 236–246.
[16] Drabkin, D. L. 1987. Spectrometric studies, XIV- The crystallographic and optimal properties of the hemoglobin of man in comparison with those of other species. *J. Biol. Chem.* 164, 742-781.
[17] Pace, M. 2001. Prediction and the aquatic sciences. *Can. J. Fish. Aquat. Sci*. 58(1), 63-72.
[18] Mevel, G., Chamroux, G. A study on nitrification in the presence of prawns (*Penaeus japonicas*) in marine enclosed systems. *Aquacult*. 23, 29-43 (1981).
[19] Beyer, J. 1996. Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus L.*) and Atlantic cod (*Gadus morhua L.*) exposed by caging to polluted sediments in Sorfjorden, Norway. *Aquat. Toxicol*. 36, 75-98.
[20] Stormer, J., Jensen, F. B., Rankin, J. C. 1996. Uptake of nitrite, nitrate and bromide in rainbow trout, *Oncorhynchus mykiss* effects on ionic balance. *Can. J. Fish. Aquat. Sci*. 53, 1943-1950.