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Effects of ammonia, nitrite and nitrate on hemoglobin content and oxygen consumption of freshwater fish, *Cyprinus carpio* (Linnaeus)

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Abstract: *Lethal effects of nitrogenous compounds ammonia, nitrite and nitrate on freshwater fish Cyprinus carpio were studied and the static LC_{50} values obtained for these 3 toxicants for 24 hr were 0.80 ppb, 171.36 ppm; 1075.10 ppm and continuous flowthrough LC_{50} values for 24 hr were 0.72 ppb, 154.31 ppm; 967.63 ppm respectively. The fish were exposed to lethal concentrations to study the changes in hematological parameters and the rate of oxygen consumption. During the period of exposure general decline in the content of hemoglobin was observed. Methemoglobin content increased in case of nitrite exposure consequently the hemoglobin levels decreased drastically. It is also observed that rate of oxygen consumption decreased progressively with the increase of toxicant concentration and duration of the exposure.*

Key words: *Cyprinus carpio, Ammonia, Nitrite, Nitrate, LC_{50} values, Methemoglobin, Oxygen consumption*

Introduction

Nitrogen compounds have been identified as major metabolic products in fish culture. Nitrite may reach toxic concentrations in high density aquaculture systems and in flowing waters due to industrial contamination and fertilizer use. It is an intermediate product in the bacterial oxidation of ammonia to nitrate in conditioned aquaculture systems (Collins, 1975). This nitrogen compound is highly toxic to aquatic organisms and posses a potential threat to cultured fish. Respiratory blood pigment hemoglobin manifests the transport of oxygen. Nitrite an intermediate product of ammonia nitrification, may reach toxic concentration in aquaculture systems when imbalances occur among species of nitrifying bacteria. Nitrite is present at unusually high concentrations in lakes (McCoy, 1972). One physiological response to nitrite is an increase in methemoglobin. The hemoglobin becomes oxidized *i.e.*, the ferrous ion (Fe^{++}) is oxidized to ferric ion (Fe^{+++}) and unable to bind and carry molecules of oxygen. Hence, the toxicity of nitrite to fish received much attention in recent years (Russo and Thurston, 1977).

Fish with elevated levels of methemoglobin may suffer from anoxia. (Huey *et al.,* 1980; Tomasso, 1981). When the methemoglobin content of the blood exceeds 70 to 80 % of the total hemoglobin, fish become torpid, unresponsive and disoriented (Klinger, 1957). The present work is an attempt to study the effects of ammonia, nitrite and nitrate on hemoglobin of *Cyprinus carpio.*

Determination of oxygen consumption by the fish is useful for assessment of lethal effects and is one of the important indicators which reflect physiological state of animal. In aquatic body toxicants present above the normal level *i.e.*, at lethal concentrations bring about mortality of fish and also increase the rate of oxygen consumption in survived fish.

Materials and Methods

To carry out the toxicity experiments and for hematological studies *Cyprinus carpio* of size 6 to 8 cm length and weight 5 to 6 g were brought from a fish farm at Nandivelugu and acclimatized to the laboratory conditions for 96 hr. Such acclimatized fish were used for toxicity tests. By following the APHA (1998) protocols, the toxicity tests were conducted. The fish were exposed to lethal concentrations of ammonia, nitrite and nitrate for 24 hr. Later the surviving fish were collected for using in the hematological studies that is for total hemoglobin and methemoglobin determination. The method of probit analysis (Finney, 1971) was used to calculate the LC_{50} values.

Fish measuring 20 $\pm$ ½ cm, in length and 200 $\pm$ ½ g in weight were used to carry out the experiment of whole animal respiration.

The dissolved oxygen content in the initial and final water samples were estimated by Winkler's modified method (Golterman and Clymo, 1969). The difference in the dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish.

Results and Discussion

The calculated LC_{50} values are presented in Table 1. The total content of hemoglobin estimated in the blood of *Cyprinus carpio* is 13.6 gl^{-1}. When the common carp was exposed to lethal concentrations of ammonia, nitrite and nitrate, a decrease in total haemoglobin and an increase in methemoglobin content was observed. Among the aforesaid three toxicants, ammonia and nitrate exposures did not show significant difference in total hemoglobin (Fig.1). Where as under nitrite exposure, the haemoglobin content decreased and methemoglobin content increased.

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Table - 1: LC_{50} (24 hr) static and continuous flow through values of ammonia, nitrite and nitrate to *Cyprinus carpio*

Toxicant	Static	Continuous flow through
Ammonia	0.80 (ppb)	0.72 (ppb)
Nitrite	171.36 (ppm)	154.31 (ppm)
Nitrate	1075.10 (ppm)	967.63 (ppm)

The toxicity of the three toxicants ammonia, nitrite and nitrate to the fish *Cyprinus carpio* is in the order: $NH_3^+ > NO_2^- > NO_3^-$

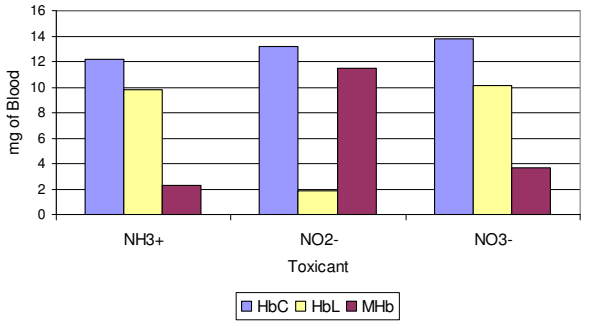

Fig.1: Changes observed in the hemoglobin in the blood of fish *Cyprinus carpio* exposed to lethal concentrations of NH_3^+, NO_2^- and NO_3^-

Decrement in haemoglobin and increment in methemoglobin has affected the oxygen carrying capacity of the blood. During ammonia and nitrate exposure haemoglobin levels decreased gradually whereas under nitrite exposure haemoglobin content showed a sudden decrease, correspondingly the oxygen consumption levels have fallen.

The results of whole animal respiration experiment (Fig. 2, 3 and 4) show that the rate of decrease in oxygen consumption increases with the duration of the exposure, probably due to the absorption of more toxicant through the gills. During experimentation the control fish demanded more oxygen in the early hours, later got settled and maintained a constant uptake of oxygen, while the exposed fish demanded more oxygen in the early hours and later showed a decrease in the oxygen demand. The increase in the intake of oxygen by the fish may be due to stress caused by the toxicants ammonia, nitrite and nitrate and decrement in the oxygen consumption was caused not only due to the damage of the gills but also due to the decrease in the hemoglobin content. The amount of hemoglobin that is reduced is transformed into methemoglobin.

When compared to the control fish, the exposed fish consumed more oxygen. The values obtained in the whole animal respiration *i.e.*, the intake of oxygen can be correlated to the hematological changes. When the hemoglobin content decreases its capacity to combine with oxygen also decreases. Hence it leads to a decrement in the rate of oxygen consumption. Decrease

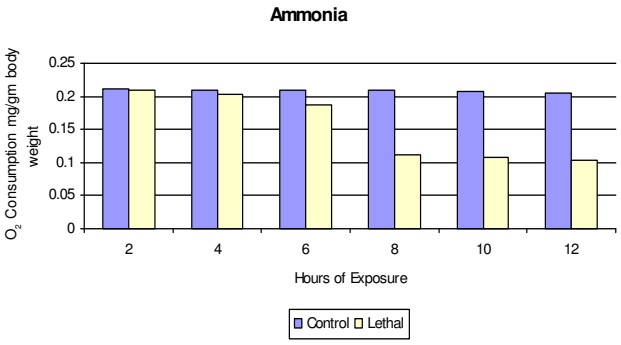

Fig. 2: Changes in oxygen consumption of the fish *Cyprinus carpio* exposed to lethal concentration of ammonia

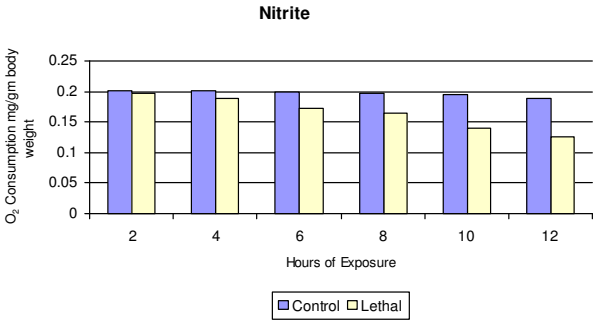

Fig. 3: Changes in oxygen consumption of the fish *Cyprinus carpio* exposed to lethal concentration of nitrite

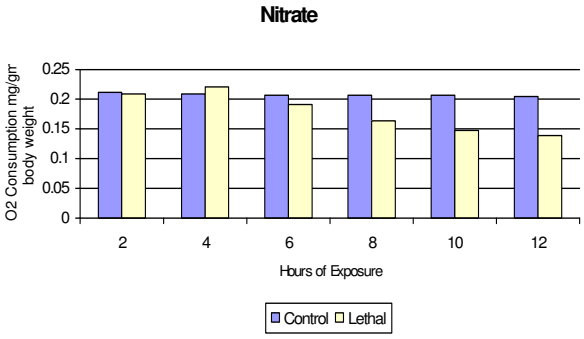

Fig. 4: Changes in oxygen consumption of the fish *Cyprinus carpio* exposed to lethal concentration of nitrate

in oxygen consumption has been reported for many pesticides (Veeraiah and Durga Prasad, 1998).

A gradual decrease in the intake of oxygen from initial stages to final stages was observed in the test fish, under the exposure of the toxicants $NH_3^+ > NO_2^- > NO_3^-$. The slight decrement in the intake of oxygen in control fish in the final stages of exposure can be attributed to the decrease in the oxygen levels of water in the container (this decrement is due to oxygen consumed by the test fish). The decrement is higher in ammonia exposure than the nitrite and nitrate. So ammonia is more toxic than the other two nitrogenous compounds. This is in agreement with the hematological changes (Fig. 1).

Nitrite enters the fish by way of the gills and passes into the circulatory system (Perrone, 1977). Toxic effects of nitrite include oxidation of hemoglobin to methemoglobin, a form incapable of binding molecular oxygen (Brown and McLey, 1975).

Fish with methemoglobin can be detected by the colour of the blood and also by brown colour of the gills. As nitrite rises, the fraction of methemoglobin in the blood reduces the oxygen carrying capacity of the blood (Cameron, 1971). Fish with elevated levels of methemoglobin may suffer from anoxia (Tomasso, 1981).

Nitrite can be converted to HNO_2 and subsequently to N_2O_2 under acid conditions. N_2O_2 and other oxides can react with nitrogenous compounds *i.e.*, amines to yield nitroso compounds, which are mutagenic and carcinogenic. Hotchkiss *et al*., (1992) have observed that the base substitutions are impaired during DNA replication exerting mutagenic effect. The potential role of nitroso compounds in the toxicity of nitrite in aquatic animals warrants investigation on the NO_2 compounds, which reach definitely high concentrations in fish farms due to fertilization and artificial feeding.

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