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EFFECT OF SODIUM CYANIDE ON BEHAVIOUR AND RESPIRATORY SURVEILLANCE IN FRESHWATER FISH, *LABEO ROHITA* (HAMILTON)

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Abstract

The toxicity of sodium cyanide (free cyanide) to the freshwater fish, *Labeo rohita* was studied using static bioassay method. The LC50 in 96 h was found to be 33 μg/L. Behavioural changes when exposed to lethal concentration of sodium cyanide showed increased opercular movement, increased surface behaviour, loss of equilibrium, change in body colour, increased secretion of mucus, irregular swimming activity, rapid jerk movement, partial jerk and aggressiveness. The swimming behaviour was in a cork-screw palter, rotating along horizontal axis. In sub lethal treatment, the schooling behaviour of the fish was slowly disrupted, the ventilation rate was increased. The fish at 21st days of exposure exhibited balanced swimming and active feeding and behaved in normal way. Oxygen consumption was decreased in lethal concentration (-22.64 to -70.13%), but in sub lethal concentration decreased trend was improved and reached normal level at 21st day (-25.10 to -2.19). Alterations in oxygen consumption may be due to respiratory distress as a consequence of impairment in oxidative metabolism. Fish in sub lethal concentration were found under stress, but that was not fatal.

Keywords: Cyanide, Toxicity, Behaviour, LC_{50}, *Labeo rohita*, Oxygen consumption

Introduction

Development of industries with rapid pace during the recent times has taken its toll by causing environmental pollution of air, water and soil. The contamination of inland & surface waters and land/soil, due to the release of variety of chemicals may prove toxic to all classes of living organisms. One such dangerous and toxic chemical known is "cyanide". Although known for its potent toxic nature, cyanide have large applications in variety of industrial processes like metal mining (mainly gold and silver), electroplating, steel, automobiles, carbonisation, printed circuit board manufacturing and chemical industries, etc. Consequently, these industries emanate huge quantity of cyanide (HCN/CN^-) containing effluents. Wastewaters especially generated from industries like metal mining, electroplating, printed circuit board manufacturing, etc. Often contains toxic and precious heavy metals (viz. copper, nickel, iron, zinc, cadmium, chromium, silver, gold, etc.), which readily binds with free cyanide resulting in the formation of metal-cyanide complexes (Eq. 1) (M_xCN - where M_x stands for metal moiety) of variable stability and toxicity (Sharpe, 1976).

$$4NaCN + CuSO_4 \longrightarrow Na_2Cu(CN)_4 + Na_2SO_4 \quad (1)$$

Sodium Cyanide — Copper Sulphate — Sodium copper cyanide — Sodium sulphate

$$2Na^+ + Cu(CN)_4^{-2}$$

The M_xCN formed can occur in the waters and wastewaters in various forms depending upon the concentration of metal and cyanide. For example, copper-cyanide (Eq. 1) can occur in the water in various anionic forms such as $Cu(CN)_2^-$, $Cu(CN)_3^{2-}$, $Cu(CN)_4^{2-}$, etc. The amount of effluent discharged daily by cyanide user industries is in the range of 200 to 1000 litres for small-scale industries and 1-20 cubic metre or more for large-scale industries. The total CN^- and metal content of these effluents are in the range of 0.5-100 and 1-250 mg/L, respectively, as against to their permissible (discharge) limits of 0.2 and 0.01-5 mg/L, respectively. Clearly, M_xCN containing effluents cannot be discharged in the environment without giving proper treatment (Patil 1999). Several physical-chemical methods are employed for the treatment and the levels of total cyanides in discharged liquid wastes are brought down to 0.2 mg/L (200 μg/L). But even 0.2 mg/L of cyanide concentration in aqueous systems is toxic for most of the life forms. There are reports that even cyanide concentration as low as 0.01- 0.1 mg/L

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are able to kill some sensitive animals species present in waters (Blaha, 1976); and fish is one such type of sensitive species. *Labeo rohita*, one of the Indian major carp is an edible freshwater fish of great economic importance. In the present paper, the authors describe the impact of sodium cyanide on behavioural changes and oxygen consumption of the said species.

Materials and Methods

Procurement and maintenance of fish: Freshwater fish, Labeo rohita (length 7±1 cm; weight 5±2 g) were obtained from Fisheries bund breeding center, State Fisheries Department Fish Farms, B.R. Project, Shivamogga District, Karnataka, India and reared in large cement tank. During acclimatization, fish were fed with rice bran and oil cake in the ratio of 2:1 on alternate days. Water of the tank was changed daily to avoid any fungal and bacterial contamination.

Physico-chemical characterization of water

The physico-chemical characterization of the water used for fish bioassay was carried out according to the methods described in Standard Methods (APHA-AWWA-WEF, 1998). The water quality parameters were as follows: temperature 28±1°C, salinity 198 mg/L, pH 8, dissolved oxygen 6.7 to 7.2 mg/L, chloride 46.3 mg/L, sodium 1.22 mg/L, potassium 30.5 mg/L, calcium 17.04 mg/L, magnesium 1 mg/L, carbon dioxide 9.0 mg/L, Hardness 115 mg/L, oxygen per cent<R> saturation 57 mg/L (as $CaCO_3$) and specific gravity 1.00374.

Toxicant selected and preparation of stock solution

The present study, sodium cyanide (NaCN) was used as a toxicant. The stock solution of cyanide (1000 mg/L) was prepared according to the method prescribed in the Standard Methods (APHA-AWWA-WEF, 1998). For experimental purpose, the required cyanide concentration was drawn from the prepared stock solution.

Toxicity evaluation

The percent mortality of fish in different concentrations of sodium cyanide was determined at 96 h exposure. For this, the experimental fish were divided into batches of ten each, and were exposed to different concentrations of capper cyanide ranging from 10 μg/L to 55 μg/L (i.e. 0.010 to 0.05 mg/L). This range was obtained on trial and error basis. Toxicity evaluation was carried out in static water (Doudoroff *et al.,* 1951) and mortality rate was observed and recorded for all the concentration after 96 hours. A batch of fish was also maintained simultaneously in freshwater medium without cyanide, which served as negative control. All the experiments were performed in duplicates and repeated thrice to confirm the results. The mean values were derived following the method of Finney Probit Kill Theory (1971) and Dragstedt and Behren's equation (Carpenter, 1982). For long-term effect one tenth of LC50 sodium cyanide was taken in the present study sodium cyanide.

Fixation of exposure periods

In order to understand the influence of time over toxicity effect of lethal concentration of sodium cyanide on *Labeo rohita* was studied at different periods of exposure. Before experimentation, healthy fishes were collected from the large cement tank with the help of nylon net and hand net. They were acclimatized to laboratory conditions in glass troughs for fifteen days. Each trough contained 15 l of water with uniform sized fish (length 7 cm; weight 5 g). They were fed with commercial fish food pellets during acclimation. After 15 days, if fishes were in normal behavioural activity and good health conditions, those species were selected for experiment purpose. The fish were divided into two groups. One group without cyanide served as control and the other group was exposed to lethal concentration of free cyanide for 1, 2, 3 and 4 days were chosen to observe the short-term effects and for sub lethal concentration of sodium cyanide for 1, 7, 14 and 21 days were chosen to observe the long-term effects. During this experiment the behavioural changes were critically observed.

Results

Toxicity Studies: The percentage mortality of *Labeo rohita* was observed to be 0% and 100% at sodium cyanide concentration of 10μg/L and 55 μg/L, (Table 1). The LC50 value obtained through sigmoid curve was 33 μg/L and linear curve was found to be 32 μg/L. The LC50 value obtained were verified using Dragstedt and Behren's equation and was found to be 34 μg/L. Thus the average LC50 for 96 h was found to be33μg/L.

Table 1: Toxicity of sodium cyanide on *Labeo rohita*

Sl. No.	Conc. of toxicant	Log conc. of toxicant	Number fish exposed			% Mortality	Probit Mortality
			Exposed	Alive	Dead		
1	10	1.0000	10	10	0	0	0.00
2	15	1.1761	10	9	1	10	3.72
3	20	1.3010	10	8	2	20	4.16
4	25	1.3979	10	7	3	30	4.48
5	30	1.4771	10	6	4	40	4.75
6	35	1.5441	10	4	6	60	5.25
7	40	1.6021	10	3	7	70	5.52
8	45	1.6532	10	2	8	80	5.84
9	50	1.6990	10	1	9	90	6.28
10	55	1.7719	10	0	10	100	8.09

Behavioural observations

Control fish

Normal fish maintained a fairly compact school, covering about one third of the bottom during the first seven days of the 21 days experiment. By seventh day, the school became less compact covering up to two-third of the tank area. Fishes were observed to scrap the bottom surface. When startled, they instantly formed a tight school that was maintained briefly. They were sensitive to light and moved to the bottom of the tank when light was passed into the tank. Except a less response to form a dense school towards the end of the study, no other extraordinary behaviour was observed.

Exposed fish

The behavioural changes were observed when exposed to sodium cyanide. The fish showed erratic swimming, hyper and hypoactive, imbalance in posture, increase in surfacing activity, opecular movement, gradual loss in equilibrium and spreading of excess of mucus all over the surface of the body. The fish occupied twice the area than that of the control group. They were spread out and appeared to be swimming independent of one another. The swimming behaviour was in a cork-screw palter, rotating along horizontal axis and followed by 'S' jerk, partial jerk, sudden rapid, non directed spurt of forward movement. The fish eventually died with their mouth and opecular wide opened. A change in colour of gill lamellae from reddish to light brown with coagulation of mucus on the gill lamellae was seen in dead fish.

In sub lethal treatment, the schooling behaviour of the fish was slowly disrupted during the first day. The ventilation rate was increased, hyperactivity, excitement, hyperventilation etc, were not much influenced on exposure to the sub lethal concentration of Sodium cynide at 7 and 14 days. Further, the fish at 21 days of exposure exhibited balanced swimming and active feeding. The fish behaved in normal way.

Oxygen consumption

The rate of whole animal oxygen consumption of control and sodium cyanide treated fishes are presented in Table 2. The data indicates that in fish exposed to lethal and sub lethal concentrations of sodium cyanide, oxygen consumption was reduced. The whole animal O_2 consumption is reduced by about 22.64 per cent on day one and reached maximum reduction of 70.13 per cent by day 4. Even during day 2 and 3 there was high rate of decrement as seen in Table 2. The decrement was a sudden reduction from day one to day two, from then it was gradual reduction. However in sub lethal concentrations, the day 1 showed high rate of decrease of 25.10 per cent, which reached 19.22 by day 7. Subsequently, there was an improvement in O_2 consumption as by day 14 it was reduced to 12.92 per cent and day 21 showed minimal decrease which was recorded at 2.19 per cent. All the results are significant at 0.05, levels.

Table 2: Oxygen consumption of *Labeo rohita* exposed to Sodium cyanide

	Control	1	2	3	4	1	7	14	21
Mean	0.8234	0.6534	0.5557	0.4433	0.2459	0.6167	0.6651	0.7170	0.8053
% change		- 20.6440	-32.5123	-46.1677	-70.1309	- 25.1067	-19.2232	-12.9288	- 2.1979
SD	0.0030	0.0011	0.0018	0.0011	0.0013	0.0018	0.0020	0.0010	0.0042

Discussion

In the science of aquatic toxicology, fish play an important role in toxicity testing and hazard evaluation, as do the white rat and guinea pig in mammalian toxicology (Annon, 1972). The assessment of toxicity of capper cyanide with reference to aquatic biota, especially fish is crucial in establishing the toxicity evaluation. The LC_{50} 96 hrs value of sodium cyanide to *Labeo rohita* was found to be 33 µg/1 (Table 1). This data clearly indicates that sodium cyanide is also toxic to *Labeo rohita* as to other fish species mentioned in the table, sodium cyanide is known to be readily taken up by aquatic organisms and bioconcentration factors ranged from 130 to 4,900 for various organisms (Snail, Daphnia and Fish). In laboratory tests capper cyanide was reported to be highly toxic for aquatic organisms with LC_{50} values ranging from 0.02 µg/l to 1 µg/1 for newly hatched shrimp.

Various symptoms of poisoning can be observed from studies involving the determination of LC_{50}. In the present study, the fish maintained in normal freshwater behaved in usual manner i.e., they were very active with their well coordinated movements. They were alert at slightest disturbance. But at the sub lethal concentrations of capper cyanide they became irritable and hyper-excited. Jumping movements as well as restlessness were observed and finally the fish turned upside down. Mucus secretion and loss of equilibrium were also observed. They slowly became sluggish with short jerky movements, surfacing and gulping of air and erratic circular movements. Finally they settled down at the bottom with loss of equilibrium and rolling of the body, convulsions prior to death. The fish very often come to the surface in order to avoid toxic environment. Moreover, examination of the gill of dead fish revealed that the gill lamellae colour was changed from red to brown. Tilapia exposed to lethal and sub lethal concentration of endosulfun and lidane exhibited abnormal behaviour at lethal concentration; a sudden heavy stress on the fish showed erratic swimming, convulsion, spiralling, treamors, jerky movements and rapid opereular movements. The fish struggled hard for breathing, often moved to the surface to engulf atmospheric air and tried to escape the toxic aquatic medium. After a few hours, equilibrium was lost and the fishes spiralled and slowly moved upward in a vertical position. Finally they lost equilibrium completely and were flat at the bottom (Thorat, 2001). Similar symptoms were also observed by Deva Prakasa Raju, 2000 in *L. rohita* and Prashanth and Neelgund 2008 in *C. mrigala* respectively.

Some workers have reported the toxicity evaluation of cyanides in the freshwater fish, *Catla catla* (Prashanth and Patil 2006). The difference might be due to either potency of toxicant or difference in the test conditions. The mean LC_{50} value of capper cyanide estimated in the present investigation was 33 µg/l (Table 1). The unusual behaviour of the fish, *Labeo rohita* in stress condition may be due to obstructed functions of neurotransmitters. The gill opercular movements increased initially to support enhanced physiological activities in stressful habitat and later decreased may be due to mucus accumulation of gill. The toxic stress of pesticides has direct bearing on tissue chemical compounds (Tilak and Yacobu, 2002). This was also reported by David, 1995 and Muniyan and Veeraragahavan, 1999, Chaudhary *et al*., 2001. The excessive secretion of mucus over the gills may inhibit the diffusion of oxygen during the process of gaseous exchange. It suggests that the copper cyanide is not safe to non-target organisms like fishes. In order to protect whole aquatic ecosystem, awareness must be given to the farmers to control the agricultural pests by biological methods.

The behavioural changes are the manifestation of motivational, biochemical, physiological and environmentally influenced state of the organism. The migration of fish to the bottom of the tank following the addition of sodium cyanide clearly indicates the avoidance behaviour of the fish, which was reported by Murthy (1987) in trout. The opecular movement of the fish ceases immediately following exposure to cyanide. The increase in opecular movement and corresponding increase in frequency of surfacing of fish clearly indicates that fish adaptively shifts towards aerial respiration (by obtaining atmospheric oxygen surfacing) and the fish tries to avoid contact with the cyanide through gill chamber (Santhakumar and Balaji, 2000; Prashanth & Patil, 2006). The increased ventilation rate by rapid, repeated opening and closing of mouth and opercular coverings accompanied by partially extended fins (caughing) was observed in the present study. This could be due to clearance of the accumulated mucus debris in the gill region for proper breathing, which was suggested by Prashanth *et al.,* 2005.

The erratic swimming of the treated fish indicates loss of equilibrium. It is likely that the region in the brain, which is associated with the maintenance of equilibrium, should have been affected (Deva Parkasa Raju, 2000; Prashanth *et al.,* 2005; Prashanth & Patil, 2006). The erratic swimming, jerky movements and convulsions before death were evident and the serenity varied with pesticide concentration. It indicates the sings of esphyxiation as indicated by gasping to death when fish, *Sarotherodan mossambicus* exposed dimethorate (Kalavathy *et al.,* 2001).

The surfacing phenomenon of fish observed under cyanide exposure might either be due to hypoxic condition of the fish as reported by Radhaiah and Jayantha Rao (1988). This fact was clearly evidenced in the present study. The observation on the metabolic

shift from aerobic to an anaerobic condition involving glycolytic oxidation with enormous amount of lactic acid accumulation were also seen. Chronic exposure of finfish to aroclor was found to induce surfacing phenomenon of fish as pointed out by Drummond *et al*., 1986.

Aggressive behaviour such as nudge and nip were increased following exposure to the toxic material. Orientation and locomotor patterns were found to be involved in most aspects of fish behaviour such as migration, mating, courtship and feeding, which were altered under stress conditions of environmental toxicants (David, 1995; Madhab Prasad *et al.,* 2002; Prashanth *et al.,* 2005; Prashanth & Patil, 2006).

The hyperexcitability of the fish invariably in the lethal exposure to free cyanide/pesticides may probably be due to the hindrance in the functioning of the enzyme AChE in relation to nervous system as suggested by many authors (Deva Prakasa Raiu, 2000; Prashanth, 2003). It leads to accumulation of acetylcholine, which is likely to cause prolonged excitatory post synaptic potential. This may first lead to stimulation and later cause a block in the cholinergic system.

The accumulation and increased secretion of mucus in the fish exposed to fee cyanide may be adaptive responses perhaps providing additional protection against corrosive nature of the pesticide and to avoid the absorptions of the toxicant by the general body surface. This agrees to the earlier findings done by David (1995). In the present study as evidenced by the results the abnormal changes in the fish exposed to lethal concentration free cyanide is time dependent.

Since most fish breathe water in which they live, changes in the chemical properties there of, may be reflected in the animal's ventilator activity, particularly if the environment affects respiratory gas exchange (Sellers *et al*., 1975). Toxicants from the environment mainly enter fish by means of their respiratory systems (Tovell *et al*., 1975). A mechanism of toxicant uptake through gills probably occurs through pores by simple diffusion and is then absorbed through cell membranes (Opperhuizen *et al*., 1985). Studies on the course of oxygen consumption in lethal and sub lethal concentration indicate the sequence of the type of compensatory mechanism, if any, which operates within the animal to overcome the load of toxic stress.

From the results, presented in the table 2, it is clearly evident that capper cyanide affects the oxygen consumption of the fish, *Labeo rohita* under lethal and sub lethal concentrations. The observed decrease in oxygen consumption by the whole animal may be due to the respiratory distress as a consequence of the impairment of oxidative metabolism. Several authors reported similar decline in whole animal oxygen consumption in different species of fishes exposed to toxicants (Kabeer *et al.,* 1981; Rangaswamy, 1984; Deva Prakasa Raju, 2000; David *et al.,* 2002). Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gill for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress (Radhaiah and Jayantha Rao, 1988; Esther, *et al*., 2001). In consonance with this, he also reported that the depletion in O_2 consumption was due to the disorganization of the respiratory function caused by rupture in the respiratory epithelium of the gill. It is also due to the disturbance in mitochondrial integrity and decreased activities of some mitochondrial enzymes (Ravinder, 1988). Magare and Patil (2000) reported a decrease in the rate of O_2 consumption in *Puntus ticto* exposed to endosulfan. In addition to gill damage decrease in haemaglobin content and decrease in tissues respiration (Sarkar, 1999) may also interfere with respiratory process resulting in respiratory failure.

The decrement can also be attributed to the induction of hypoxic conditions within the animal due to the in-time contact of the respiratory surface with the polluted water resulting in the alteration of normal respiratory area of the animal. The secretion of mucus layer over the gill lamellae has been observed during copper cyanide stress. Excessive secretion of mucus over the gills may inhibit the diffusion of oxygen during the process of gaseous exchange (Muniyan and Veeragahavan, 1999; David *et al*., 2002). The coagulation of mucus on the gills caused demolition of various important processes such as gas exchange, nitrogen excretion, salt balance and circulation of blood (Skidmore, 1964). The alternative reason for the decrease in the oxygen consumption would be due to the internal action of copper cyanide. This toxic substance appears to alter the metabolic cycle at sub-cellular level.

Greater decrease in the rate of O_2 consumption of the fish, exposed to lethal concentration than the sub lethal concentration, may be due to the considerable damage to the gill structure and also due to the greater precipitation of mucus upon gill filaments leading to the clogging of gills. Probably suffocation imposed by the coagulated mucus film and necrosis on the epithelial and inter lamellar cells of gills is one of the reasons for the death of animal in lethal concentration. Greater decrease in the rate of O_2 consumption of the fish in lethal concentration may also be due to the greater damage caused to R.B.C. as evident by the drastic decrease in the number of these cells (Venkataramana, 1987).

Lowering of O_2 consumption of fish in the sub lethal concentration of copper cyanide may be mostly due to the lowering down of energy requirements and if so, such lowering of maintenance energy requirement is to be considered adaptive and even strategic. This lowering of maintenance energy requirements may be

achieved by reducing osmotic gradient through the lowering of electrolyte levels in the body fluids. Further, there is evidence for a considerable metabolic reorganization and increased utilization of anaerobic metabolism in fishes exposed to sub lethal concentration of copper cyanide. If so, the lowering of the oxidative metabolism in *Labeo rohita* might have been compensated at least by some degree of glycolysis.

Behavioural characteristics are obviously sensitive indicators of toxicant effect. It is necessary, however, to select behavioural indices of monitoring that relate to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural system, should be considered for species forming social organizational. If social interactions are not considered, only a certain portion of a population may be protected, and the toxicity of contaminant may be underestimated. And also primarily influence modulations in the level of oxygen consumption. This has led to the imbalance in cellular homeostasis. The inhibition of oxygen consumption is explained by this manner in which sodium cyanide is incorporated into this fish system for energy.

It is concluded that sodium cyanide is innately toxic to fish, *Labeo rohita*, but many factors influence the degree of hazard. The above-analogy also warrants for an indispensable need to evaluate more toxicity data for wide range of animal groups of the eco-web in order to understand the broad spectrum of sodium cyanide in comparison to other toxicants available. This also provides a platform to establish tolerable limits and safe levels of toxic agents for the biota of aquatic environment and to save the residue imbalance in aquatic bio-ecological cycles, which help in involving bio-detector monitoring.

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