

Impact of Sodium Cyanide on Behaviour and Oxygen Consumption in an Air Breathing Fish *Channa gachua* (Hamilton)

Qaisur Rahman^{1*} and Shamim Akhter Choudhary²

¹Department of Zoology, Vinoba Bhave University, Hazaribag-825301, Jharkhand, India

²Department of Zoology, Government College for Women, Gandhi Nagar-180004, J&K, India

*Corresponding author: qaisur.rahman@gmail.com and drshamimch05@gmail.com

Abstract

Pollution of water bodies affects all the biotic communities including fish. The chemical fertilizers and pesticides are the main sources of water pollution. The general effects of water pollution on fish may be considered as physical effects, oxidation effects, toxic chemical effects etc. As fish is excellent indicator of water quality and parameters such as fish population size, growth rate, condition factor and diversity are also indicative of the total health of water. Toxic chemicals generated through man's industrial agricultural and domestic activities eventually reach aquatic environment and cause a major threat to the inhabiting organisms. Since the aquatic environment is fragile and hence sensitive to the toxic effects of chemical pollutants including hydrophobic pollutants. Pollutants such as pesticides are known to alter the behavioural pattern, growth and reproductive potential and resistance to disease of aquatic organisms by effect on a variety of biochemical and physiological mechanisms. The toxicity of sodium cyanide (free cyanide) to the fresh water fish, *C. gachua* was studied using static bioassay method. The LC₅₀ in 96 hours was found to be 33µgL⁻¹. 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 *C. gachua* at 21st days of exposure exhibited balanced swimming and active feeding and behaved in normal way. Hence, oxygen consumption was decreased in lethal concentration (-22.64 -70.13%), but in sub lethal concentration decreased trend was improved and reached normal level at 21st day (-25.10-2.19). 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.

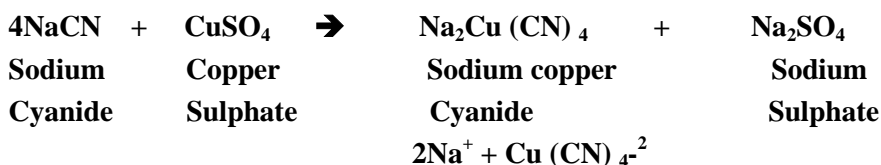
Keywords: Sodium cyanide, toxicity, behaviour, oxygen consumption, *C. gachua*.

Introduction

Most of the water bodies are polluted by heavy load of industrial effluents, sewage and chemicals of industries in metropolitan cities. Water is essential for all living organisms to perform all life functions but if aquatic organisms are exposed to toxic water contents then their physiology and biochemical

processes are affected badly. All these chemicals interact in various ways with cells and they can be detected by chemical analysis only. Water quality i.e. hardness, oxygen, temperature, pH etc. has a marked influence on the expression of toxicity (Qaisur and Shamim, 2014). Besides algae macrophytes and benthic macro invertebrates, fish are considered excellent indicators of the water quality. Fish population size, growth rate, condition factor and diversity are indicative of health of water and prevailing environmental conditions. Environmental pollution and its effect on the health of aquatic ecosystems is a great problem that has been studied intensely. Development of industries with rapid pace during the recent times has taken toll by causing environmental pollution of air, water and soil. The contamination of inland and surface waters and land or 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. Waste waters especially generated from industries like metal mining, electroplating, printed circuit board manufacturing, etc. often contains toxic heavy metals such as copper, nickel, iron, zinc, cadmium, chromium, silver, gold, etc. which readily binds with free cyanide resulting in the formation of metal cyanide complexes by Qaisur and Shamim (2013). The MxCN where Mx stands for metal moiety of variable stability and toxicity respectively (Sharpe, 1976).

Equation. 1



The MxCN formed can occur in the waters and wastewaters in various forms depending upon the concentration of metal and cyanide. For example, copper-cyanide (**Equation. 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, MxCN containing effluents cannot be discharged in the environment without giving proper treatment. Several physicochemical 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 are able to kill some sensitive animals species present in waters (Blaha, 1976) and fish is one such type of sensitive species. *C. gachua*, one of the Indian major carp is an edible freshwater fish of great economic importance. In the present investigation an attempt has been made to describe the impact of sodium cyanide on behavioural changes and oxygen consumption of *C. gachua*.

Materials and Methods

Live specimens of *C. gachua* were procured from local fish dealers at Hazaribag (Latitude 25° 59'N and Longitude 85° 22'E) and maintained in large glass aquaria size (90x60x60cm) with continuous flow of water. The specimens were fed on chopped goat liver daily during a minimum acclimation period of 15 days in the laboratory. Freshwater fish *C. gachua* (length 8.0 ± 1.6 cm and weight 75.0 ± 2.3 g) were used in the present investigation. Water of the tank was changed daily to avoid any fungal and bacterial contamination. The physico chemical characterization of the water used for fish bioassay was carried out according to the methods described in Standard Methods of APHA (1998). The water quality parameters were as follows temperature $28 \pm 1^\circ\text{C}$, 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 saturation 57 mg/L (as CaCO_3) and specific gravity 1.00374 mg/L. In 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, 1998). For experimental purpose the required cyanide concentration was drawn from the prepared stock solution. The percent mortality of fish in different concentrations of sodium cyanide was determined at 96 hours exposure. For this the experimental fish were divided into batches of ten each, and were exposed to different concentrations of copper cyanide ranging from 10 µg/L to 55 µg/L (i.e. 0.010 to 0.05 mg/L) respectively. This range was obtained on trial and error basis. Toxicity evaluation was carried out in static water (Doudroff *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 fresh water 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 (1971) and Dragstedt and Behren's equation (Carpenter, 1975). In order to understand the influence of time over toxicity, effect of lethal concentration of sodium cyanide on *C. gachua* was studied at different periods of exposure. After 15 days fishes were in normal behavioural activity and good health conditions. 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

The percent mortality of *C. gachua* was observed to be 0% and 100% at sodium cyanide concentration of 10 μ g/L and 55 μ g/L (**Table 1**). The LC₅₀ value obtained through sigmoid curve was 33 μ g/L and linear curve was found to be 32 μ g/L. The LC₅₀ value obtained were verified using Dragstedt and Behren's equation and was found to be 34 μ g/L. Thus the average LC₅₀ for 96 hours was found to be 33 μ g/L. 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 scarp the bottom surface. When started, 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.

The behavioural changes were observed when exposed to sodium cyanide. The fish *C. gachua* 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 behavior 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 open. 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 cyanide 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. The rate of whole animal oxygen consumption of control and sodium cyanide treated fishe 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 oxygen consumption was reduced by about 22.64% on day one and reached maximum reduction of 70.13% by day 4. Even during day 2 and 3 there was high rate of decrement as seen. The decrement was a sudden reduction from day one today two, from then it was gradual reduction. However in sub lethal concentrations, the day 1 showed high rate of decrease of 25.10 %, which reached 19. 22% by day 7 respectively. Subsequently, there was an improvement in oxygen consumption as by day 14 it was reduced to 12.92% and day 21 showed minimal decrease which was recorded at 2.19 %. All the results are significant at 0.05 levels.

Table 1: Showing the impact of toxicity of sodium cyanide in fish *C. gachua*.

Serial Number	Conc. Toxicant	Log. Conc.	Number of Exposed			% mortality	Probit mortality
			Exposed	Alive	Dead		
1.	10	1.000	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

Table 2: Showing oxygen consumption in *C. gachua* exposed to sodium cyanide.

	Control	1 Day	2 Days	3 Days	4		7		14		21	
					Days	1 Day	Days	Days	Days	Days		
Mean	0.8234	0.6534	0.5557	0.4433	0.2459	0.6169	0.6651	0.7170	0.8053			
%change	-----	-20.64	-32.51	-46.18	-70.13	-25.10	-19.22	-12.92	-2.20			
S.D.	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 in mammalian toxicology (Annon, 1972). The assessment of toxicity of copper cyanide with reference to aquatic biota, fish is crucial in establishing the toxicity evaluation. The LC₅₀ for 96 hours value of sodium cyanide to *C. gachua* was found to be 33µg/L. This data clearly indicates that sodium cyanide is highly toxic to *C. gachua* as to other fish species mentioned. Sodium cyanide is known to be readily taken up by aquatic organisms and bio concentration factors ranged from 130 to 4,900 for various organisms (snail, daphnia and fish). In laboratory tests copper cyanide was reported to be highly toxic for aquatic organisms with LC₅₀ values ranging from 0.02 µg/L to 1 µg/L for newly hatched shrimp. Various symptoms of poisoning can be observed from studies involving the determination of LC₅₀ respectively. In the present study, the fish maintained in normal fresh water 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 copper cyanide they became irritable and hyper excited. Jumping movements as well as rest-lessness 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 (David *et al.*, 2003). *Tilapia* exposed to lethal and sub lethal concentration of endosulphan and lindane exhibited abnormal behaviour at lethal concentration a sudden heavy stress on the fish showed erratic swimming, convulsion, spiralling, tremors, jerky movements and rapid opercular movements. The fish *C. gachua* 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 in *Labeo rohita* and in *C. mrigala* (Prashanth and Neelgund 2008). Some workers have reported the toxicity evaluation of cyanides in the fresh water fish, *Catla catla* (Prashanth and Patil, 2006) Qaisur and Shamim (2013) in *C. gachua*. The difference might be due to either potency of toxicant or difference in the test conditions. The mean LC₅₀ value of copper cyanide estimated in the present investigation was 33µg/ L. The unusual behaviour of the fish, *C. gachua* 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; Muniyan and Veerarahavan, 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 opercular movement of the fish ceases immediately following exposure to cyanide. The increase in opercular 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 *et al.*, 2000). The increased ventilation rate by rapid repeated opening and closing of mouth and opercular coverings accompanied by partially extended fins (coughing) 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 (Prashanth and Patil 2006; Qaisur and Shamim, 2013). The erratic swimming, jerky movements and convulsions before death were evident and the serenity varied with pesticide concentration. It indicates the signs of asphyxiation as indicated

by gasping to death when fish, *Sarotherodan mossambicus* exposed dimethoate (Kalavathy *et al.*, 2001; Qaisur and Shamim, 2015). The surfacing phenomenon of fish observed under cyanide exposure might either be due to hypoxic condition of the fish (Radhaiah and Jayantha, 1988). 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 (Drummond *et al.*, 1986; Malla and Mohideen, 1988). 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 and Madhab *et al.*, 2002). The hyper excitability of the fish invariably in the lethal exposure to free cyanide or 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. 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 free 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 Vineetkumar and David (2008). 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 in water in which they live, and changes in the chemical properties 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 and Sanjoy *et al.*, (2009). 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 which operates within the animal to overcome the load of toxic stress. It is clearly evident that copper cyanide affects the oxygen consumption of the fish *C. gachua* 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; 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, 1988; Esther, *et al.*, 2001; Sakr and Jamal (2005). In consonance with this, he also reported that the depletion in oxygen 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. Since Magare and Patil (2000) reported a decrease in the rate of oxygen consumption in *Puntius ticto* exposed to endosulfan. In addition to gill damage decrease in haemoglobin content and decrease in tissues respiration may also interfere with respiratory process resulting in respiratory failure respectively (Sarkar, 1999; Manoharan and Subbiah, (1982).

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 oxygen 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 oxygen 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 (Qaisur *et al.*, 2015).

Lowering of oxygen 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 *C. gachua* 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 under estimated. *C. gachua* reported that this has led to the imbalance in cellular homeostasis (Qaisur and Shamim, 2013). 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, *C. gachua* 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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