Journal of Experimental Sciences Vol. 2, Issue 2, Pages 27-31 [2011]
www.jexpsciences.com

Regular Article

Free Cyanide Induced Physiological Changes in the Freshwater Fish, *Poecilia Reticulata*

M.S. Prashanth[1*], H.A. Sayeswara[2] and Mahesh Anand Goudar[3]

[1]Department of Zoology, V.K. Chavan-Patil Arts, Commerce and Science College, Shivaji University, KARAVE-416 507, Chandgad (Tal), Kolhapur (Dist.), Maharashtra India; [2]Sahyadri Science College (Autonomous), Kuvempu University, Shivamogga-577201 Karnataka, India; [3]D.V.S.College of Arts and Science, Kuvempu University, Shivamogga-577201 Karnataka, India

ABSTRACT: Static renewal bioassay test were conducted to determine the toxicity of free cyanide (sodium cyanide) to the freshwater fish, *Poecilia reticulate.* The fish were exposed to different concentrations of sodium cyanide and rate of mortality was recorded for 96 hr. The LC50 value of sodium cyanide for 96 hr was found out to be 9.13 µg/L. Behavioural changes were observed when fish exposed to sodium cyanide showed increased opercular movement, increased surface behaviour, loss of equilibrium, increased secretion of mucus, irregular swimming activity and aggressiveness. The swimming behaviour was in a cork-screw pattern, rotating along horizontal axis was more prominent in lethal concentration. In sub lethal treatment, the schooling behaviour of the fish was slowly disrupted, and the ventilation rate increased. The fish at 15th day of exposure exhibited balanced swimming and active feeding and behaved in normal way. Decreased level of oxygen consumption was recorded in lethal concentration for 1st to 4th day (-36.12 to -70.93%), but in sub lethal concentration, it was improved and reached normal level at 15th day (-16.40 to -7.16%). The decrement may be due to the respiratory distress as a consequence of the impairment of oxidative metabolism.

Key words: Cyanide, Toxicity, Behaviour, LC50, *Poecilia reticulate*, Oxygen consumption *fossilis*

Introduction

Impact of pollution on ecosystem and human health is a vital and international issue since there are an ever-increasing number of examples of environmental disturbances likely to affect the biota and humans. Chemical pollution originates from factories, farms, lawns, or almost anywhere there are people. Environmental pollutants such as metals, pesticides, cyanides and other organics pose serious risks to many aquatic organisms. Accordingly, a great deal of previous research revealed information on physiological mechanisms of toxicity in animals exposed to contaminants (Scott and Sloman, 2004).

The contamination of inland & surface waters and land/soil, due to the release of variety of chemicals may prove toxic to all groups of living organisms. One such dangerous and toxic chemical known group known as "cyanide". The extent of cyanide toxicity in fish depends upon the rate of its intoxification in vivo (Cheng et al., 2001). Acute cyanide poisoning in man and animals results in non coordination of movements, convulsions, coma and respiratory arrest. But due to effective detoxification of cyanide, cumulative effects of cyanide are rarely seen, but even sublethal doses may at times alter the physiological parameters when administered over a period of time (Mathangi and Namasivayam, 2000). Knowledge of acute toxicity of a xenobiotic often can be very helpful in predicting and preventing acute damage to aquatic life in receiving waters as well as in regulating toxic waste discharges (APHA, 2005). Cyanide compounds are useful to society in terms of their key role in synthetic and industrial processes, for certain fumigation and agricultural uses and for some therapeutic applications. Cyanide serves no useful purpose in the human body, yet the substance is present in food, air, and water.

Although known for its potent toxic nature, cyanides 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, generated from metal mining, electroplating, printed circuit board manufacturing, etc, often contain toxic heavy metals (viz. copper, nickel, iron, zinc, cadmium, chromium, silver, gold, etc.), which readily bind with sodium cyanide resulting in the formation of metal-cyanide complexes (M_xCN - where M_x stands for metal moiety) of variable stability and toxicity (Patil, 1999). These industries consequently, discharge large quantities of cyanide and metal-cyanide containing wastes. 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 are able to kill some sensitive animals species present in waters (Blaha, 1976); and fish is one such type of sensitive species. Poecilia reticulate is one of the freshwater fish, widely used in mosquito control. In the present paper, the authors described the impact of sodium cyanide on behavioural changes and oxygen consumption by the said species.

Materials and Methods

Freshwater fish, Poecilia reticulate *(length 3±1 cm; weight 2±1 g) were obtained from in and around Chandgad and Kolhapur local ponds, Maharashtra, India and reared in large cement tank in laboratory condition. During acclimatization, the 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.*

The physico-chemical characterisation of the water used for fish bioassay was carried out according to the methods described in Standard Methods (APHA, 2005). 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 percent saturation 57 mg/L (as $CaCO_3$) and specific gravity 1.00374.

Sodium cyanide (NaCN) was used as a toxicant (commonly know as free cyanide). The stock solution of cyanide (1000 mg/L) was prepared according to the method prescribed in the Standard Methods (APHA, 2005). 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 hr exposure. For this, the experimental fish were divided into batches of ten each, and were exposed to different concentrations of sodium cyanide ranging from 0 µg/L to 16 µg/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 hr. A batch of fish was also maintained simultaneously in freshwater medium without cyanide, which served as negative control. All the experiments were performed induplicate 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).

In order to understand the influence of time over toxicity the effect of lethal concentration of sodium cyanide on *Poecilia reticulate* was

* Corresponding Author, Email: drmsp@rediffmail.com, drmsprashant@gmail.com

ISSN: 2218-1768

studied at different periods of exposure. Before experimentation, the healthy fish were collected from the tank, acclimatized to laboratory conditions in glass troughs for fifteen days. Each trough contained 15 L of water with approximately uniform sized fish. They were fed with commercial fish food pallets during acclimatization. The fish were divided into two groups. One group without toxicant served as control and the other group was exposed to lethal and sub lethal concentration of sodium cyanide for 1, 2, 3 and 4 and 1,5,10 and 15 days were chosen to observe the short-term and long term exposure respectively. For sub lethal concentration 1/5th of LC50 was selected. During this experiment the behavioural changes were observed and recorded.

Results

Toxicity of Test Animal

The percentage mortality of Poecilia reticulate was observed to be 0% and 100% at sodium cyanide concentration of 0 µg/L and 16 µg/L, respectively (Table 1). The LC50 value obtained through sigmoid curve was 9.6 µg/L and linear curve was found to be 8.6 µg/L. The LC50 value obtained were verified using Dragstedt and Behren's equation and was found to be 9.2 µg/L. Thus the average LC50 for 96 hr was found to be 9.13 µg/L.

Behavioural Studies

Normal fish*:* Control fishes maintained a fairly compact school, covering about one third of the bottom during the first five days of the total 15 days of the experiment. By five day, the school became less compact covering up to two-third of the tank area. Fishes were observed to scrap 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 little less response to form a dense school towards the end of the study.

Treated fish: when fish exposed to lethal concentration of sodium cyanide, they migrated immediately to the bottom of the tank. The schooling behaviour was observed to be disrupted on the first day itself and 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. This was followed by irregular, erratic and darting movements with imbalanced swimming activity and non-stop movement of pectoral fins (fanning). The fish exhibited peculiar behaviour of trying to leap out from the toxic medium, which can be viewed as an escape phenomenon. The frequency of surfacing phenomenon was greater on the second day of exposure wherein the fish frequently come to the water surface. Respiratory disruption was observed in the normal ventilating cycle (Cough, Yawn) with a more rapid, repeated opening and closing of the mouth and opercular coverings. Partially extended fins and single wide opening of the mouth and opercular coverings accompanied by hyperextension of all fins were found and the fish was in a state of excitement on the third day. The swimming behaviour was in a corck screw pattern rotating along horizontal axis and followed by 'S' jerk, partial jerk, sudden, rapid, nondirected spurt of forward movement (Burst swimming); Nudge and Nip phenomenon, i.e., the movement of fish towards another fish (Nudge) and biting (Nip). The fish progressively showed signs of tiredness and lost positive rheotaxis characterised by weakness and apathy. On the 4 day they lost their equilibrium and response, to external stimuli such as touch and light followed by drowning to the bottom. 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 and they settled at the bottom. 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 5 and 10 days. Further, the fish at 15 days of exposure exhibited balanced swimming and active feeding. The fish behaved in normal way and they were active with their coordinated movements.

Oxygen consumption

The rate of whole animal oxygen consumption of normal and sodium cyanide treated fishes are presented in Table 2. The data indicates that in fish exposed to lethal and sub lethal levels of sodium cyanide, oxygen consumption was affected. The whole animal O_2 consumption is reduced by about 36.12 per cent on day one and reached maximum reduction of 70.93 per cent by d 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 16.40 per cent, which reached 48.56 by day 5. Subsequently, there was an improvement in O_2 consumption as by day 10 it was reduced to 28.95 per cent and day 15 it show minimal decreases which was 7.16 percent. All the results are significant at 0.05, levels.

Discussion

The bio-assessment of the toxicity of chemical with reference to aquatic biota is playing a crucial role in establishing the toxicity evaluation with reference to aquatic fauna. It is a complex one because the synergistic and antagonistic interaction with the chemicals modulates the toxic effect on biota. In other words, the hydrominerology and flow dynamics of water play a crucial role in the assessment of toxicity of chemicals Prashanth (2003). The assessment of toxicity of sodium cyanide with reference to aquatic biota, especially fish is crucial in establishing the toxicity evaluation. The LC50 96 hr value of sodium cyanide to *Poecilia reticulata* was found to be 9.13 µg/L (Table 1). This data clearly indicates that sodium cyanide is also toxic to *Poecilia reticulata* as to other fish species mentioned in the table, Sodium cyanide is known to be readily taken up by aquatic organisms and varied levels of bioconcentration, bioaccumulation are reported. In laboratory tests sodium cyanide was reported to be highly toxic for aquatic organisms with LC50 values ranging from 0.04 µg/L to 1 µg/L for newly hatched shrimp (Patil and Paknikar, 2000).

The migration of the fish to the bottom of the tank following the addition of sodium cyanide, clearly indicates the avoidance behaviour of the fish as observed in trout which was reported by Murthy (1987). Prashanth and Patil have also observed the avoidance nature by *Catla catla* on exposure to sodium cyanide. Disruption of schooling behaviour of the fish, due to the lethal and sub lethal stress of the toxicant, results in increased swimming activity, and entails increased expenditure of energy (Murthy, 1987). The disturbance of the schooling behaviour of the fish in treated media indicates that the group hydrodynamic effect of fish which would help them to swim within the school has been lost.

Disruption of schooling behaviour of the fish, due to the lethal and sub lethal stress of the toxicant, results in increased swimming activity, and entails increased expenditure of energy (Murthy, 1987). The disturbance of the schooling behaviour of the fish in treated media indicates that the group hydrodynamic effect of fish which would help them to swim within the school has been lost. (Zuyer and Balyayer, 1970). A change in the normal physiological and bio-chemical aspects in the treated fish in the present study could be attributed to the disruption of the schooling behaviour of the fish, which in turn leads to higher activities as suggested by (Murthy, 1987). Prashanth & Patil, (2006) have reported that sodium cyanide has a marked effect on the schooling behaviour of the *Catla catla*. Loss of such behaviour following toxicant exposures has been observed by many workers (Drummond *et al*., 1951). 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, convalusion, 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 (2007) in *C. mrigala* Prashanth *et al.,* (2010) in *C. catla* respectively.

The difference in toxicity response might be due to either potency of toxicant or difference in the test conditions. The mean LC_{50} value of sodium cyanide estimated in the present investigation was 9.13 µg/L (Table 1). The unusual behaviour of the fish *Poecilia reticulata* under 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 on gills. The toxic stress of pesticides has direct bearing on tissue composition (Tilak and Yacobu, 2002). This was also reported by David, (1995) and Muniyan and Veeraragahavan, (1999), and 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 sodium cyanide is not safe to non-target organisms like fishes. In order to protect whole aquatic ecosystem, awareness must be developed among the farmers to control the agricultural pests by biological methods.

The respiratory disruption in the normal ventilating cycle (Cough, Yawn) with a more rapid, repeated opening and closing of the mouth and opercular coverings 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 and Balaji, 2000; Prashanth and Patil, 2006). 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 and it was suggested by Prashanth *et al.,* (2005).

The 'S' jerk, partial jerk, sudden, rapid nondirected spurt of forward movement (Burst swimming) and 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 and Patil, 2006; Prashanth *et al.,* 2010). The erratic swimming, jerky movements and convalusion before death were evident and the serenity varied with pesticide concentration. It indicates the signs of asphyxiation as indicated by engulphing of air when fish, *Sarotherodan mossambicus* exposed dimethorate (Kalavathy *et al.,* 2001).

The and 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. 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 (Prashanth & Patil, 2006).

The increase of hyperactivity, excitement, hyperventilation and hyperexcitability of the fish invariably in the lethal and sub lethal exposure to sodium cyanide may probably be due to the hindrance in the functioning of the enzyme AChE in relation to nervous system as suggested by earlier workers in pesticides and cyanides (Deva Prakasa Raiu, 2000; Prashanth *et al.,* 2010). It leads to accumulation of acetylcholine, which is likely to cause prolonged excitory post synaptic potential. This may lead to stimulation and later cause a block in the cholinergic system. David, (1995) has observed hyperactivity, in *Labeo rohita* exposed to fenvalerate to affect central nervous system.

The accumulation and increased secretion of mucus in the fish exposed to sodium 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 statement also falls in line with present observation**.** In the present study as evidenced by the results the abnormal changes in the fish exposed to lethal concentration sodium cyanide is time dependent.

Murthy (1987), behavioural changes of the fish under toxicants stress may have deleterious effects of making the fish fall an easy prey in their natural habitat and may affect the stability of the population. Behavioural characteristics are obviously sensitive indicators of toxicant effect. It is necessary, however, to select behavioural indices for monitoring that relation 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 systems. Toxicants from the environment mainly enter fish by means of their respiratory systems. 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.

The rate of whole animal oxygen consumption presented in the Table 2, it is clearly evident that sodium cyanide affects the oxygen consumption of the fish, *Poecilia reticulata* under lethal and sub lethal concentrations. It may be due to 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 pesticides (Kabeer Ahmed, 1981; Rangaswamy, 1984; Deva Prakasa Raju, 2000; David *et al.,* 2002; Prashanth *et al.,* 2010). It is 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.

Discharge of mucus layer over the gill lamellae has been observed during sodium 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 sodium cyanide. This toxic substance appears to alter the metabolic cycle at sub-cellular level.

Larger decline in the rate of oxygen utilization 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.

Decreasing of oxygen consumption of fish in the sub lethal concentration of sodium cyanide may be attributed to lowering down of energy requirements and if so, such lowering of maintenance energy of 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 sodium cyanide. If so, the lowering of the oxidative metabolism in *Poecilia reticulata* might have been compensated at least by some degree of glycolysis.

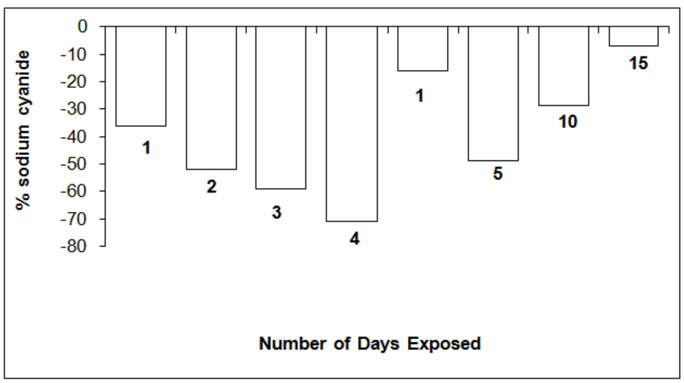
Table 1: Toxicity of Sodium cyanide on *Poecilia reticulate*

Sl. No.	Conc. of toxicant (µg/L)	Log conc. of toxicant	Number fish exposed			% Mortality	Probit Mortality
			Exposed	Alive	Dead		
1	0	0.0000	10	10	0	00	0.00
2	2	0.3010	10	9	1	10	3.72
3	4	0.6021	10	7	3	30	4.48
4	6	0.7782	10	6	4	40	4.75
5	8	0.9031	10	5	5	50	5.00
6	10	1.9542	10	4	6	60	5.25
7	12	1.0792	10	2	8	80	5.84
8	14	1.1461	10	1	9	90	6.28
9	16	1.2041	10	0	10	100	8.09

Table 2: Oxygen consumption of *Poecilia reticulata* exposed to sodium cyanide (mg/L)

	Control	1	2	3	4	1	5	10	15
Mean	0.55	0.35	0.26	0.22	0.16	0.46	0.28	0.39	0.51
% change		-36.12	-52.24	-59.37	-70.93	-16.40	-48.56	-28.95	-7.16

Graph 1: Oxygen consumption of *Poecilia reticulata* exposed to sodium cyanide



Conclusion

It is concluded that sodium cyanide (free cyanide) is innately toxic to fish, *Poecilia reticulata*, but many factors influence the degree of toxicity. 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 pesticides 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. Thus, the present study clearly indicates the significant changes in the *Poecilia reticulata* when exposed to low quantity of sodium cyanide.

References

ANNON. 1972: EPA bans most DDT uses, readies lead action. Environ Sci Technol 6 : 675.

APHA. 2005: Standard methods for examination of water and wastewater. 21st Edn., American Public Health Association (APHA), Washington DC, USA.

BLAHA J. 1976: Mathematical analysis of the chemical system cyanide heavy meals in water determination of components and toxicity of the system-I. The theoretical solution. Water Research. 10:815-819.

CARPENTER P. 1982: Immunology and Serology, 3rd ed., W. B. Saunders Co., Philadelphia. USA.

CHAUDHARY VARSLIA RATHOD and VANKHEDE G. N. 2001: Effect of water extract of the bark of *Buchanania langan* (Linn) On behaviour and Chromatophores of a freshwater fish, *Labeo rohita*. J. Enrion. Bio. 22 (3): 229-331.

CHENG YIH-DIH, LIU TSUNG-YUN, and LIN SHAN-YANG. 2001: Cyanide-induced alterations to the biophysical conformations of the isolated fish liver. Ecotoxicology. 10: 71-77.

DAVID M.1995: Effect of fenvalerate on behavioral, physiological and biochemical aspects of freshwater fish, *Labeo rohita* (Hamalton) Ph.D. Thesis, S.K. University. Anantapur, A.P. India

DAVID M., MUSHIGERI S.B. and PRASHANTH M.S. 2002. Toxicity of fenvalerate to the freshwater fish, *Labeo rohita*. Geobios, 29: 25-28.

DEVA PRAKASA RAJU B. (2000): Malathion induced changes in the protein metabolism of freshwater fish, *Tilapia mossambica*. Ph.D Thesis. S.K.University, Anantapur, Andhra Pradesh. India.

DOUDOROFF P., ANDERSON B.G., URCHVICK G.E., GALTSOLF B.S., HART N.B., PARTRICK R., STRONG E.R., SURBER E.W. and VANFFORN W.H.1951: Bio-assay method for the evaluation of acute toxicity of industrial wastes to fish sewage. Ind Wastes. 23:1380-1397.

FINNEY D.T.1971: Probit Analysis, 3rd ed. *Cambridge University Press.* London.

KABEER AHMED, JAGANNATHA RAO K.S. and RAMANA RAO K.V. 1981: Effect of malathion exposure on some physiological parameters of whole blood and on tissue cations of teleost, *Tilapia mossambica*. J. Biol. Sci. 3: 17-21.

KALAVATHY, K., SIVAKUMARI, A.A. and RASHMI CHANDRAN. 2000: Toxic effect of the pesticide dimethoate on the fish, *Sarotherodon mossambicus.* J. Eco. Res. Bicon. 2(152):27-32.

MAGARE, S.R., AND PATIL, H.T. 2000: Effect of pesticides on oxygen consumption. Red blood cell count and metabolites of fish, *Puntius ticto*. Environ. and Eco. 18(4): 891-894.

MATHANGI D.C AND NAMASIVAYAM A. 2000. Effect of Chronic Sublethal Cyanide Administration on Brain Neurotransmitters and Behaviour in Rats. J Occup Health .42: 88–90.

MUNIYAN M. AND VEERARAGAHAVAN, K.1999: Acute toxicity of ethofenprox to the fresh water fish, *Oreochromis mossmabicus* (Perers). J. Environ. Biol. 20(1): 153-155.

MURTHY A.S.1987: Sub lethal effects of pesticides on fish. Toxicity of pesticide to fish. *C R S Press Boca Roton* F. I. USA, Vol. II, Ch 4 55-100.

OPPERHUIZEN A., VAN DER VELDE E.W., GOBAS F.A.P.C., LIEM. D.A.K. and Van DER STEEN J.M.D.1985: Relationship between bio-concentration in fish and steric factor of hydrophobic chemicals. Chemosphere, 14(11/12): 1871-1896.

PATIL Y.B. 1999: Studies on biological detoxification of metal-cyanides containing industrial effluents, Ph.D., Thesis, University of Pune, Pune.

PATIL, Y.B. and PAKNIKAR, K.M. 2000. Development of a process for detoxification of metal-cyanides from wastewaters. Process Biochemistry, 35: 1139-1151.

PRASHANTH M.S. 2003. Cypermenthrin induced physiological, biochemical and histrophalogical changes in freshwater fish, *Cirrhinus mrigala*. Ph.D., thesis, Karnataka University, Dharwad. India

PRASHANTH M.S., DAVID M. AND MATHAD S.G. 2005: Behavioural Changes in freshwater fish, *Cirrhinus mrigala* (Hamilton) exposed to cypermethrin. J. of Environ. Bio. 26(13): 141-144.

PRASHANTH M.S. AND YOGESH B PATIL.2006: Behavioural surveillance of Indian major carp *Catla catla* (Hamilton) exposed to free cyanide. J. of Cur. Sci. 9(1): 313-318 .

PRASHANTH M.S. AND NEELGUND, S.E. 2007: Free cyanide-induced Biochemical changes in Nitrogen metabolism of the Indian major carp, *Cirrhinus mrigala*. *Journal of Basic and Clinical Physiology and Pharmacology*. 8(4):77-287.

PRASHANTH M.S., HA SAYESWARA AND H S RAVIKUMAR PATI. 2010. Impact of copper cyanide on behavioural changes and oxygen consumption in Indian major carp *Catla catla* (Hamilton)', Electronic Journal of Environmental, Agricultural and Food Chemistry, 9(9): 1433-1442,

RADHAIAH V. and JAYANTHA RAO K. 1988: Behavioural response of fish, *Tilapia mossambica* exposed to fenvalerate. Environ. Ecol. 6(2): 2-23.

RANGASWAMY C.P. 1984: Impact of endosulfan toxicity on some physiological properties of the blood and aspects of energy metabolism of a fresh water fish, *Tilapia mossambica*. Ph.D thesis, S.V. University Tirurpathi, India .

RAVINDER V. 1988: *In vivo* effects of decis on certain aspects of metabolism of freshwater fish, *Clarias batrachus*. Ph.D., Thesis, Osmania University, Hyderabad, India (1988).

SANTHAKUMAR M. AND BALAJI M. 2000: Acute toxicity of an organophosporus insecticide monocrotophas and its effects on behaviour of an air-breathing fish, *Anabas testudineus* (Bloch). J. Environ. Biol. 21(2), 121-123.

SARKAR, S.K.1999: Effects of heavy metals (Copper sulphate and Cadmium sulphate) on the O_2 consumption of fish, *Cyprinus carpio.* U.P. J. Zool. 19(1): 13-16.

SCOTT G.R AND SLOMAN K.A. 2004: The effects of environmental pollutants on complex fish behaviour: integrating behavioral and physiological indicators of toxicity. Aquat. Toxicol. 68: 369-392.

SKIDMORE J.F. 1964: Toxicity of Zinc compounds to aquatic animals, with special reference to fish. Quarterly Rev. Bio., 39(3), 227-248.

THORAT S.R. 2001: Chronic effect of endosulfan on freshwater fish, *Catla catla*. J. Ecotoxicol. Envion. Moint. 11(4): 221-223.

TILAK K S and YACOBU K. 2002: Toxicity and effect of fenvalerate on fish, *Ctenopharyngodon idellus*, J Ecotoxi Environ Moint. 12(1): 9-15.

ZUYER, G.V. and V.V.BELYAYEN. 1970. An experimental study of the swimming of fish in groups as exemp lifed by the horse mackerel Trachurus *Mediterraneus panticus. J. Icthyol*. 10: 545.